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Title Page

The *APOE* ϵ 4 allele modulates brain white matter integrity in healthy adults

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Abstract

The Apolipoprotein E (*APOE*) $\epsilon 4$ allele is the best-established genetic risk factor for sporadic Alzheimer's disease (AD) and is also associated with structural gray matter (GM) and functional brain changes in healthy young, middle-aged and elderly subjects. Because *APOE* is implicated in brain mechanisms associated with white matter (WM) development and repair, we investigated the potential role played by the *APOE* polymorphism on WM structure in healthy younger (age range: 20-35 years) and older (aged 50-78 years) adults using diffusion tensor imaging (DTI). General reduction of fractional anisotropy (FA) and increase in mean diffusivity (MD) values was found in carriers of the *APOE* $\epsilon 4$ allele relative to non-carriers. No significant interactions between genotype and age were observed, suggesting that differences in WM structure between *APOE* $\epsilon 4$ -carriers and non-carriers do not undergo significant differential changes with age. This result was not explained by differences in brain morphology or cognitive measures. The *APOE* $\epsilon 4$ allele modulates brain WM structure before any clinical or neurophysiological expression of impending disease.

Keywords

APOE, diffusion tensor imaging, neuroimaging, white matter, healthy subjects, aging

Introduction

Apolipoprotein E (apoE, protein; *APOE*, gene) is a very-low-density lipoprotein that has a key role in coordinating the mobilization and redistribution of cholesterol, phospholipids, and fatty acids.¹ In the central nervous system, apoE is implicated in mechanisms such as neuronal development, brain plasticity, and repair functions.^{2,3} The human *APOE* gene has 3 allelic variants ($\epsilon 2$, $\epsilon 3$, and $\epsilon 4$). The $\epsilon 4$ allele is associated with higher risk of developing both early-onset⁴ and late-onset⁵ Alzheimer's disease (AD), poor outcome from traumatic brain injury⁶ and age-related cognitive impairment.⁷

Neuroimaging studies have shown that the *APOE* $\epsilon 4$ allele is associated with modification of brain function and gray matter (GM) structure, both in AD patients^{8,9,10} and in healthy subjects.^{11,12,13,14,15} GM volume reduction in AD patients carrying the $\epsilon 4$ allele relative to non-carriers has been found mainly in the hippocampus and entorhinal cortex.^{8,10,16,17} Similarly, reductions in MTL volumes have been reported among healthy middle-aged/ elderly,^{18,19,20,21} and adolescent²² *APOE* $\epsilon 4$ -carriers, although not all studies replicate this finding.^{23,24,25} Positron emission tomography (PET) studies have consistently shown resting glucose metabolism reduction in healthy young and middle-aged *APOE* $\epsilon 4$ -carriers in brain regions known to be affected by AD pathology, such as the parietal, temporal and prefrontal cortices.^{12,13} Task-based functional magnetic resonance imaging (fMRI) studies have been less consistent, showing increased^{11,14,15}, decreased^{26,27} or even no difference²⁸ in task-related BOLD signal between healthy *APOE* $\epsilon 4$ -carriers and non-carriers. Methodological differences may explain inconsistencies in the studies.

Because of the link between *APOE* and AD, to date structural imaging studies have largely concentrated on GM changes, especially in the entorhinal cortex

and hippocampal regions, which are the first structures to show AD pathology.²⁹ Very few studies have investigated the potential effects of the *APOE* genotype on white matter (WM) structure. This is surprising considering that the major role of apoE in the brain is the transport of lipid components which contribute to building up the myelin sheath.^{30,31,32} Moreover, AD pathology not only involves GM atrophy but also changes in WM³³, which have been shown to correlate with disease severity.^{34,35} Diffusion tensor imaging (DTI) is an imaging technique that is increasingly used for WM analysis *in vivo*. DTI-derived measurements, such as fractional anisotropy (FA) and mean diffusivity (MD), have proved to be sensitive for detection of disease-related WM changes.³⁶ A recent meta-analysis of DTI measurements in AD and MCI reported widespread increases in MD and decreases in FA with large effect sizes.³⁷ To date studies using DTI to investigate the effects of *APOE* on FA and MD have focused on specific age groups³⁸ or were limited to preselected regions-of-interest.³⁹ The aim of this study is to investigate the relationship between *APOE* genotype, WM structure and age in healthy individuals using an unbiased whole-brain analysis (tract-based spatial statistics - TBSS⁴⁰) using DTI. This will allow us to investigate whether the $\epsilon 4$ allele selectively modulates well-defined WM pathways or rather has a more general effect. In particular, we will test whether the *APOE* $\epsilon 4$ allele alters WM integrity both in distinct groups of younger (age range = 20 to 35 years old) and older (age range = 50 to 78 years old) participants.

Based on previously reported studies showing age-related WM changes in healthy subjects^{41,42,43,44}, we hypothesize that older subjects will have increased MD and reduced FA. As the *APOE* $\epsilon 4$ allele is associated with an earlier onset of AD⁴ and cognitive decline at a younger age⁷, we expect that

the trajectories of WM changes with age will differ between *APOE* ϵ 4-carriers and non-carriers. Moreover, we will study GM volumes to investigate whether whole brain GM or WM analyses are more sensitive to changes associated with the *APOE* ϵ 4 allele. Based on previous studies²⁰, we hypothesize that GM volume will be reduced in older *APOE* ϵ 4-carriers. Lastly, we will compare the data between younger and older groups to study a possible interaction between age and genotype. We will test alternative hypotheses: either brain structure of *APOE* ϵ 4-carriers already differs from non-carriers in young adulthood, or structural modifications associated with the *APOE* ϵ 4 allele only manifest at a certain age, perhaps preceding AD development in healthy adults.

Materials and methods

Participants: Imaging data for 73 right-handed subjects (35 males) aged 20 to 78 years were acquired. Thirty-four subjects aged 20 to 35 years comprised the “younger” group (mean age 28.6 years \pm 4.20), and thirty-nine subjects aged 50 to 78 years (mean 64.9 years \pm 7.19) comprised the “older” group.

A priori exclusion criteria were: current or past history of neurological or psychiatric disorders, memory complaints, head injury, substance abuse (including alcohol), corticosteroid therapy, and youth diabetes therapy. *A posteriori* (after MRI scan) exclusion criteria included presence of brain vascular insults and two or more hyperintense lesions equal or larger than 10 mm diameter, or more than eight hyperintense lesions with a diameter from 5 to 9 mm⁴⁵, on a Fluid Attenuated Inversion Recovery (FLAIR) image. In order to exclude possible confounds due to cognitive complaints older subjects underwent a pre-screening cognitive test [Addenbrooke’s Cognitive Examination-revised version (ACE-R)⁴⁶].

Subjects were pre-screened for *APOE* genotype (using a cheek swab sample) and selected for the study on the basis of either having an *APOE* ϵ 4 allele (*APOE* ϵ 4-carrier) or being an ϵ 3 homozygote (non-carrier). Three *APOE* ϵ 4-carriers were homozygous for the ϵ 4 allele (one in the younger and two in the older group). The genotyping process was conducted at the Wellcome Trust Centre for Human Genetics in Oxford. DNA was extracted from cheek swab samples of subjects according to standard procedures to allow PCR for the characterization of *APOE* genotype. The study was approved by the local Ethics Committee, and written informed consent was signed by all participants.

Image Acquisition: Scans were performed at the University of Oxford Centre for Clinical Magnetic Resonance Research (OCMR) using a 3-T Siemens Trio scanner (Siemens AG, Erlangen, Germany) with a 12-channel head coil. The neuroimaging protocol included a 3D T1-weighted structural scan (TR = 2040 ms, TE = 4.7 ms, flip angle = 8°, field of view = 192 mm, voxel dimension = 1 mm isotropic, acquisition time = 12 min), diffusion weighted imaging (echo planar imaging, TR = 9300 ms, TE = 94 ms, field of view = 192 mm, voxel dimension = 2 mm isotropic, B-value = 1000, gradients applied = 60 isotropically distributed, acquisition time = 21 min) and fluid attenuated inversion recovery (FLAIR) imaging (TR = 9000 ms, TE = 89 ms, field of view = 220 mm, voxel dimension = 1.1x0.9x3 mm, acquisition time = 5 min 8 sec).

Image Analysis: Data analysis was carried out using FMRIB Software Library (FSL) tools (www.fmrib.ox.ac.uk/fsl).

Diffusion Tensor Imaging (DTI): Raw images were pre-processed using “Eddy Current correction”, in order to correct for distortions due to the gradient directions applied. Fractional anisotropy (FA) and mean diffusivity (MD) maps were generated using DTIFit, part of FMRIB’s Diffusion Toolbox (<http://www.fmrib.ox.ac.uk/fsl/fdt>), that fits a diffusion tensor model at each voxel⁴⁷. The magnitude and direction of tissue water mobility is measured in the three dimensions⁴⁸ and the diffusion tensor is modelled as an ellipsoid with symmetry across any one of its three axes. The largest axis or eigenvalue is denoted λ_1 or axial diffusivity. The two minor axes (λ_2 , λ_3) are usually averaged to compute radial diffusivity. The average of all three eigenvalues is called mean diffusivity (MD). Fractional anisotropy (FA) is a ratio of axial to radial diffusivity, and thus provides a measure of the directionality of diffusion. In axons, net intravoxel water diffusion perpendicular to the fibres is reduced

due to the myelin sheath but unrestricted parallel to the axon. This directionality of water diffusion gives rise to a higher FA in WM, whereas less restricted diffusion, e.g. in GM or cerebrospinal fluid leads to lower FA. GM and WM have similar MD values.

The FA output images were used as input for tract-based spatial statistics (TBSS), a voxelwise approach for analysis of FA data.⁴⁰ All subjects' FA data were aligned into a common space using the nonlinear registration tool FNIRT. The mean FA image was generated and thinned to create a mean FA skeleton, which represents the centres of all tracts common to the group. Each subject's aligned FA data was then projected onto this skeleton and the resulting data fed into voxelwise GLM cross-subject statistics. A voxel by voxel permutation nonparametric test (5000 permutations) was used to assess group-related differences using threshold-free cluster enhancement (TFCE), which avoids using an arbitrary threshold for the initial cluster-formation.⁴⁰ All results are shown at $p < 0.05$ corrected for multiple comparisons across space. In addition to FA data, mean diffusivity (MD), axial diffusivity (DA) and radial diffusivity (DR) were also compared using TBSS in an analogous fashion. Separate analyses were performed for the younger and older group, then data from both groups were pooled together. GM volume, WM volume and cognitive scores were added to the TBSS analysis as covariates of no interest.

Structural MRI: A voxel-based morphometry (VBM) approach was used to study brain morphology and to relate it to the *APOE* polymorphism. Total brain volume, GM and WM and cerebrospinal fluid (CSF) measurements were calculated using FMRIB's Automated Segmentation Tool (FAST).⁴⁹ Whole-brain analysis was carried out with FSL-VBM⁵⁰, using default settings. In brief,

brain extraction and tissue-type segmentation were performed, and resulting GM or WM partial volume images were aligned to standard space using FMRIB's Linear Image Registration Tool (FLIRT) and then nonlinear (FNIRT) registration tools. The resulting images were averaged, modulated, and smoothed with an isotropic Gaussian kernel of 3mm (~7 mm FWHM) to create a study-specific template. Finally, voxelwise general linear modelling (GLM) was applied using permutation nonparametric testing (5000 permutations) and $p < 0.05$ correcting for multiple comparisons across space. GM volume and total brain volume were used as covariates of no interest in the VBM analysis.

ROI analysis of hippocampal volume: Individual hippocampal measures were obtained using FMRIB'S Integrated Registration and Segmentation Tool (FIRST), an automatic subcortical segmentation program.⁵¹ Boundary correction was used for the classification of the boundary voxels. ROIs were visually inspected in the coronal plane to ensure accuracy.

White matter lesions: Measurement of WM lesions in the older group was manually performed on FLAIR by a trained neuroscientist (VH) blind to the genotypic profile using the Jim 4.0 software (Xinapse Medical Systems, Thorpe Waterville, UK). All axial slices of each subject were investigated for WM hyperintensities and periventricular WM lesions. After marking all hyperintensities and periventricular lesions as Regions-of-interest (ROIs), total ROI volume was calculated for each subject.

Statistics: Statistical analyses of non-imaging variables were carried out using SPSS software (SPSS Inc., Chicago, USA). T-tests comparison were used for continuous variables (sociodemographic, cognitive performance and brain volumes). Exact Fisher's continuity correction was used for categorical

variables (gender and family history of dementia). An alpha of $p < 0.05$ was considered significant.

Results

Participants: Seventy-three subjects completed the MRI protocol. Two of the older subjects (one *APOE* ε4-carrier, one non-carrier) were excluded because they showed an abnormally high volume of white matter lesions (more than 3 standard deviations from each group mean), leaving a total of 71 subjects (Table 1). There was no effect of genotype on white matter lesion volume ($F_{1,34} = 2.40$, $p = 0.13$). Within the younger and older group, *APOE* ε4-carriers and non-carriers did not differ for age, sex, years of education, number of individuals with a family history of dementia and ACE-r results. Similarly, memory performance and reaction times in an encoding memory task as described in Filippini et al.⁵² were not different between *APOE* ε4-carriers and non-carriers (Table 1).

Diffusion tensor imaging (DTI): In the younger group whole brain WM analysis with TBSS revealed decreased FA values in *APOE* ε4-carriers relative to non-carriers in widespread areas, including the cingulum, corona radiata, corpus callosum, external capsule, internal capsule and superior longitudinal fasciculus (Figure 1a). There were no regions in which FA values were higher in *APOE* ε4-carriers relative to non-carriers. The decreased FA in *APOE* ε4-carriers was not associated with significant differences in either MD, DA or DR.

In the older group, whole brain analysis with TBSS showed increased MD in *APOE* ε4-carriers relative to non-carriers in widespread areas including the cingulum, corona radiata, corpus callosum, external capsule, internal capsule and superior longitudinal fasciculus (Figure 1b) but there were no significant differences in FA, DR or DA. There were no regions in which MD values were lower in *APOE* ε4-carriers relative to non-carriers.

TBSS analysis with the data from both age groups combined was performed to study a possible interaction between *AGE* (younger or older) and *GENOTYPE* (*APOE* ϵ 4-carrier and non-carrier) factors. No significant interaction between the two factors was detected for FA or MD. However, a main effect of *GENOTYPE* was found for FA values in areas including the cingulum, corona radiata, corpus callosum, external capsule, internal capsule and superior longitudinal fasciculus (Figure 1c). Additionally, DR was significantly higher in *APOE* ϵ 4-carriers compared to non-carriers (Figure 1d) but there were no significant effects on MD or DA.

As we expected, a widespread main effect of *AGE* was observed for FA. FA was significantly increased in the younger compared to the older group in WM tracts including the cingulum, corona radiata, corpus callosum, external capsule, internal capsule and superior longitudinal fasciculus (Figure 4a). This was associated with significantly increased DA and DR in the older group (Figure 4c and d). The same structures showed significantly increased MD values in the older group (Figure 4b). There were no regions in which FA values were higher or MD, DA or DR values were lower in the older group compared to the younger group.

Structural MRI: No differences between *APOE* ϵ 4-carriers and non-carriers in both age groups were found for total brain volume, GM volume, WM volume, cerebrospinal fluid (CSF) and hippocampal volumes (Table 1). Moreover, no differences between *APOE* ϵ 4-carriers and non-carriers were observed in GM or WM using a whole brain Voxel-Based-Morphometry approach.

Discussion

We investigated the effect of the *APOE* genotype on WM structure in healthy individuals using Diffusion Tensor Imaging (DTI) and found that the *APOE* ϵ 4 allele affects WM integrity. In detail, we observed significantly reduced FA values in younger *APOE* ϵ 4-carriers and increased MD in older ϵ 4-carriers relative to matched non-carriers in widespread areas of the brain. Reduced FA values in *APOE* ϵ 4-carriers relative to non-carriers were also found when the data from both groups were combined. No significant interactions between *AGE* and *GENOTYPE* were observed, suggesting that *APOE* has an effect on WM that is evident even in early adulthood and remains relatively stable throughout adulthood. Increased MD and decreased FA are generally markers of pathology, thus our observation may provide clues to the basis of the vulnerability of *APOE* ϵ 4-carriers to late-life pathology.

EFFECT OF *APOE* GENOTYPE ON WM

This is the first study to describe an effect of the *APOE* ϵ 4 allele on WM structure in a group of healthy young adults. Whole-brain analysis showed that young *APOE* ϵ 4-carriers had reduced FA values in widespread brain areas. Therefore carrying the *APOE* ϵ 4 allele influences WM integrity even in young adulthood. A genotype effect on WM structure was also found in the older group. Although FA was not altered, widespread significant increases in MD were observed in *APOE* ϵ 4-carriers. This is in accordance with several other studies that reported effects of the *APOE* ϵ 4 allele on WM integrity in brain tracts such as parahippocampal WM^{38,53} and the corpus callosum^{39,54} whereas a recent study did not replicate these results⁵⁵.

Although the *APOE* genotype affects different measures of WM integrity in the two age groups, this is probably caused by age differences in FA and MD

measures. It is possible that FA is more sensitive to detect differences in younger adults because we found that FA values decrease with age independent of genotype. The opposite effect would be found for MD.

AD and amnesic MCI are associated with an increased volume of WM lesions compared to healthy controls^{56,57}, which predicts the rate of cognitive decline.⁵⁸ We did not find a significant effect of the *APOE* ϵ 4 allele and this is in concordance with two studies reporting an effect of *APOE* genotype on volume of WM lesions only for homozygous individuals and no gene-dose effect.^{59,60}

Although *APOE* genotype effects were observed within WM regions in both the younger and the older group, we found that GM structure did not differ between *APOE* ϵ 4-carriers and non-carriers. This was interesting because we expected that at least in the older group the higher risk of AD for *APOE* ϵ 4-carriers would show in GM changes, especially in regions affected early by AD⁶¹, as was shown in two studies^{20,53}. However, other studies also failed to observe *APOE*-related structural GM differences in healthy subjects.^{23,24,25} We therefore suggest that brain function^{11,12,13,14,15,52} and WM-related measurements are more sensitive than GM-related measurements to detect the physiological effects of carrying an *APOE* ϵ 4 allele.

EFFECT OF AGE ON WM INTEGRITY

As expected, we found age-related changes in FA and MD. FA was significantly lower in the older than in the younger group independent of *APOE* genotype. In addition, a widespread increase in MD was found in the older group. Several studies also observed reductions in FA with age mainly in prefrontal structures^{41,42,43} as well as changes in WM volume unevenly distributed across brain regions.⁴⁴ Therefore, our results are similar to age-

related changes in WM integrity observed by other groups and our unbiased whole-brain approach strengthens these results.

INTERPRETATION

The main goal of our study was to investigate whether differences in WM structure between *APOE* ϵ 4-carriers and non-carriers change across the lifespan. As TBSS revealed no significant *AGE* by *GENOTYPE* interactions for FA or MD we showed that genotype-dependent differences in WM structure are already present in young adulthood and do not undergo significant differential changes with age. Previously, we have shown functional differences between *APOE* ϵ 4-carriers and non-carriers in the same group of younger subjects.⁵² It is possible that WM differences might underlie these functional differences found in the default-mode network (DMN). Indeed, it has been shown that structural connectivity between the cingulum, superior frontal occipital fasciculus and genu of the corpus callosum determine the functional links of the DMN.⁶² We found that FA was significantly decreased in young *APOE* ϵ 4-carriers in the cingulum and genu of the corpus callosum. However, the relationship between structural and functional differences between *APOE* ϵ 4-carriers and non-carriers require further investigation.

The effect of APOE on brain structure and function and the associated risk of AD remains poorly understood. The major role of apoE in the brain is the transport of lipid components which contribute to building up the myelin sheath.^{30,31,32} *In-vitro* and *in-vivo* studies have shown that the apoE isoforms differentially modulate neuritic outgrowth, sprouting and branching^{63,64,65} and the apoE4 isoform has been specifically associated with microtubule depolymerisation⁶⁵ and axonal degeneration⁶⁶ thus potentially affecting WM integrity. Although these studies point to a differential role for the apoE

isoforms on white matter structure, the molecular mechanisms require further investigation.

LIMITATIONS AND FUTURE DIRECTIONS

Our cross-sectional study did not cover the entire lifespan but instead concentrated on distinct age groups of younger (20-35 years) and older (50-78 years) volunteers. The age-related changes in WM that we observed are unlikely to be caused by a cohort effect, but longitudinal studies would be required to rule this out. Given our observation of white matter changes even in young adulthood, it would be particularly interesting to investigate differences in brain development of children and adolescents carrying the *APOE* ϵ 4 allele.

It is important to note that the changes in WM that we observe in *APOE* ϵ 4-carriers may not relate to AD risk because there are other genes in linkage disequilibrium with *APOE* ϵ 4 that might affect WM structure.⁶⁷ For example *APOC1* and *TOMM40* have been associated with increased risk of AD development⁶⁸ or earlier age of AD onset.⁶⁹ Large population samples would be needed to study the contribution of other genes to WM structure and AD. The link between *APOE*, WM structure and AD risk would be strengthened if evidence of a gene-dose effect in WM integrity that mirrored the gene dose effect in AD risk could be found. WM changes might prove to be a useful imaging marker for early detection of subjects at increased risk of developing AD. The methods for including imaging markers in genome wide association studies are now being worked out.⁷⁰

CONCLUSION

In conclusion, this study demonstrated that individuals carrying the *APOE* ϵ 4 allelic variant have different WM-related measurements even at a young age but show no differences in GM structure. No significant interactions of genotype and age group were found indicating that differences in WM structure between *APOE* ϵ 4-carriers and non-carriers remain relatively stable during adulthood. The WM changes we observed in young adults occur decades before any impending cognitive or clinical manifestations of disease, and are thus probably not caused by preclinical AD pathology. Nevertheless, the changes we see mirror those found in early AD and MCI and may therefore inform our understanding of the increased risk of disease in *APOE* ϵ 4-carriers.

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Conflict of Interest

The authors declare no conflict of interest.

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Table 1 Sociodemographic features and general brain morphology of both age groups. Values denote mean (\pm Standard Deviation) or number of subjects, *Values are expressed as percentage of whole-brain volume; p-values refer to t-tests (parametric data) and chi-square tests (categorical data).

Younger group	<i>APOE</i> ϵ4 carriers (N = 17)	<i>APOE</i> ϵ4 non-carriers (N = 17)	P
Age [years]	28.88 (\pm 4.69)	28.24 (\pm 3.77)	0.66
Sex [Male / Female]	10 / 7	10 / 7	1.00
Education [years]	19.53 (\pm 2.35)	19.41 (\pm 1.91)	0.87
Family history of dementia	2	2	1.00
Encoding memory task (max 83)	70.88 (\pm 8.17)	69.24 (\pm 8.12)	0.56
Reaction time, familiar blocks [s]	0.78 (\pm 0.21)	0.76 (\pm 0.15)	0.70
Reaction time, novel blocks [s]	1.07 (\pm 0.42)	0.99 (\pm 0.27)	0.56
Whole brain volume [cm ³]	2038.99 (\pm 168.25)	2016.80 (\pm 205.80)	0.73
Gray Matter*	43.63 (\pm 0.54)	43.78 (\pm 0.93)	0.59
White Matter*	32.76 (\pm 1.11)	32.90 (\pm 0.86)	0.68
CSF*	23.61 (\pm 1.07)	23.32 (\pm 1.41)	0.51
Left Hippocampus*	0.19 (\pm 0.02)	0.20 (\pm 0.01)	0.24
Right Hippocampus*	0.22 (\pm 0.03)	0.23 (\pm 0.02)	0.24
Older group	<i>APOE</i> ϵ4 carriers (N = 16)	<i>APOE</i> ϵ4 non-carriers (N = 21)	P
Age [years]	65.13 (\pm 8.45)	64.85 (\pm 6.27)	0.92
Sex [Male / Female]	6 / 10	8 / 13	1.00
Education [years]	15.69 (\pm 3.79)	16.66 (\pm 3.54)	0.43
Family history of dementia	3	6	0.70
ACE-r results (max 100)	98.81 (\pm 0.98)	99.29 (\pm 1.27)	0.21
Encoding memory task (max 83)	68.20 (\pm 9.76)	68.90 (\pm 6.57)	0.81
Reaction time, familiar blocks [s]	0.75 (\pm 0.09)	0.72 (\pm 0.11)	0.44
Reaction time, novel blocks [s]	0.99 (\pm 0.15)	0.96 (\pm 0.16)	0.55
Whole brain volume [cm ³]	1900.07 (\pm 173.27)	1872.54 (\pm 184.07)	0.65
Gray Matter*	41.50 (\pm 1.05)	41.77 (\pm 0.55)	0.35
White Matter*	33.39 (\pm 1.27)	32.95 (\pm 1.18)	0.29
CSF*	25.12 (\pm 1.62)	25.28 (\pm 1.34)	0.74
Left Hippocampus*	0.19 (\pm 0.03)	0.20 (\pm 0.03)	0.37
Right Hippocampus*	0.20 (\pm 0.04)	0.21 (\pm 0.03)	0.35

Figure 1 Mean FA images with mean FA skeleton used for TBSS (green).

(a) The TBSS results show that FA was significantly decreased in carriers of the *APOE* ϵ 4 allele in the younger group (red) ($P < 0.05$). R: right, L: left.

(b) within the older group MD was significantly increased (blue) in *APOE* ϵ 4-carriers.

(c) significantly reduced FA (red) in *APOE* ϵ 4-carriers was found when data from both age group were pooled.

(d) DR was significantly increased (yellow) in *APOE* ϵ 4-carriers when data from both age groups were pooled.

Figure 2 Mean FA images with mean FA skeleton used for TBSS (green).

(a) FA was significantly higher in the younger compared to the older group independent of genotype (red) ($P < 0.05$). R: right, L: left.

MD (b), DA (c) and DR (d) were significantly increased in the older group.



