The ε4 genotype of apolipoprotein E and white matter integrity in Alzheimer's disease

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Abstract

Background: In this multicenter study, we investigated a possible association between the *APOE* ε4 allele and white matter (WM) integrity in Alzheimer's disease (AD) using diffusion tensor imaging (DTI).

Methods: We analyzed fractional anisotropy (FA) and mean diffusivity (MD) as indices of WM integrity in 70 AD patients (35 *APOE* ε4 carriers, 35 noncarriers) and 56 healthy control (HC) subjects (28 *APOE* ε4 carriers, 28 noncarriers). *APOE* ε4 carriers and noncarriers were matched for age and gender within each diagnostic group.

Results: We found significant effects of diagnosis ($P_{corrected} < .05$ [FWE]; i.e., smaller FA values and larger MD values in AD patients compared with HCs) and significant effects ($P < .001$) of *APOE* ε4 carrier status on MD in HCs but not in AD subjects.

Conclusions: The results indicate that *APOE* ε4 has a moderate effect on WM integrity in HCs, but no effect on WM integrity in manifest AD.

1. Introduction

More than one half of the patients diagnosed with Alzheimer's disease (AD) have white matter (WM) abnormalities [1]. Microstructural alterations in WM are detectable in vivo by diffusion tensor imaging (DTI), which allows examination of quantifiable indices of WM integrity, such as fractional anisotropy (FA) and mean diffusivity (MD). In general, DTI studies have shown a pattern of reduced FA and increased MD in AD patients compared with healthy controls (HCs), indicating WM deterioration.

WM abnormalities in AD have also been studied in relation to the apolipoprotein E 4 (*APOE* ε4) [2–5], a major genetic risk factor for the development of sporadic AD [6]. The exact mechanism by which *APOE* ε4 contributes to the disease remains unclear, but evidence suggests that increasing number of *APOE* ε4 alleles increases the risk of AD while reducing the mean age of onset [7]. Only a small number of in vivo studies so far investigated the effect of *APOE* ε4 on microstructural WM integrity in AD patients, showing incongruous results (e.g. [8,9]). In the present study,

we investigated the possibly deleterious effect of the ɛ4 allele on WM integrity in 70 AD patients and 56 cognitively healthy elderly subjects, focusing on FA and MD indices.

2. Methods

Data for the study were obtained retrospectively from the European Diffusion Tensor Imaging Study on Dementia (EDSD) database [10] and from the database of the DZNE Rostock. All participants or their legal representatives signed informed consent. The study was approved by the local ethics committees and has followed the Helsinki Declaration guidelines on conducting research involving human subjects.

2.1. Participants

We selected participants diagnosed with AD according to National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association criteria [11] who had mild to moderate dementia (Mini-Mental State Examination [MMSE] score ≥10). Only participants with no history of neurological, psychiatric, or medical condition (except AD for the patients' group) and no history of stroke, vascular dementia, depression, hypothyroidism, or substance abuse were included in the study. In addition, we chose only HCs who had no memory or other cognitive complaints and who had a normal score on the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) examination [12], allowing for scores within 1 standard deviation of the age- and education-adjusted norm. APOE genotyping was conducted according to the standard methods.

Data from 149 subjects were initially selected for the study. However, after matching APOE ɛ4 carriers (APOE ɛ4+) with noncarriers (APOE ɛ4−) on age and gender within each group, we obtained a sample of 126 subjects: 35 AD APOE ɛ4+, 35 AD APOE ɛ4−, 28 HC APOE ɛ4+, and 28 HC APOE ɛ4− subjects. Participants' demographic information, APOE status, and their scores on cognitive tests are summarized in Table 1.

2.2. Image acquisition

The participants were scanned in five different magnetic resonance imaging (MRI) scanners: two 1.5-T (Avanto, Sonata) and three 3-T scanners (Achieva, Trio, Verio). The number of gradients varied across centers, ranging from 6 to 20. Because of a possible effect of the scanner type on the data, centers were treated as covariates in the analysis. Diffusion-weighted images were collected in a single-shot echo planar imaging sequence in all centers. During the same session, anatomical scans were also obtained for each participant.

Table 1
Participants characteristics

Characteristics	AD APOE ɛ 4+	AD APOE ɛ 4−	HC APOE ɛ 4+	HC APOE ɛ 4−
Age*	74.1 ± 6.9	71.7 ± 7	67.4 ± 6.6	68 ± 5.3
Age range	54–84	52–84	56–85	58–83
Education*	11.6 ± 2.3	11.9 ± 2.3	13.8 ± 3.1	13.5 ± 3.1
Gender: male/female	19/16	19/16	16/12	16/12
MMSE*	22.3 ± 4.1	23.3 ± 3.8	29 ± 0.8	28.8 ± 0.9
Semantic fluency*	12.7 ± 4.7	13.7 ± 5.3	23.7 ± 6.7	23.9 ± 7.3
Boston Naming*	11.9 ± 2.9	11.7 ± 2.3	14.6 ± 0.56	14.7 ± 0.6

Abbreviations: AD, Alzheimer's disease; HC, healthy control.

NOTE. Data presented as mean ± SD unless otherwise indicated.

*Statistically significant differences ($P < .05$) between AD and HC groups overall. Age: AD vs HC (t (124) = 3466; $P < .001$); education: AD vs HC (t (124) = 3957; $P < .005$); Mini-Mental State Examination: AD vs HC: ($U = 45,000$; $P < .005$); semantic fluency: AD vs HC: ($U = 411,500$; $P < .005$); Boston Naming Test: AD vs HC: ($U = 439,000$; $P < .005$).

2.3. Image preprocessing

The DTI toolbox of FSL (4.1) (http://www.fmrib.ox.ac.uk/fsl/) was used for the DTI data preprocessing, which included correction for head motion and eddy currents, removal of non-brain voxels with the Brain Extraction Tool, and fitting of diffusion tensors to the data with DTIfit. SPM8 (http://www.fil.ion.ucl.ac.uk/spm/) implemented in MATLAB (R2011b) (http://www.mathworks.de/products/matlab/) was used for deformation-based analysis of MPRAGE data, FA, and MD maps. Using the B0 scans, the FA and MD maps were coregistered with the corresponding anatomical scans. Anatomical images were segmented into gray matter, WM, and cerebrospinal fluid and warped to the MNI space template specifically created for the EDSD data [10] using DARTEL within VBM8 implemented in SPM8. The deformation fields that were obtained in this step were applied to the spatially coregistered FA and MD maps. The normalized FA and MD maps were masked with a binary WM mask, which was created from the same sample of images used to create the DARTEL template.

2.4. Statistical analyses

We used SPM 8 to conduct 2 × 2 general linear model analysis of variance, inspecting for the main effects of diagnosis (AD vs HC), APOE ɛ4 status (APOE ɛ4+ vs APOE ɛ4−), and their interactions for FA and MD diffusion measures. Given the previous findings on the reduced FA and increased MD indices in AD patients, we tested whether these effects would be more pronounced in the APOE ɛ4+ than in APOE ɛ4− subjects. The post hoc t test contrasts were set accordingly. SPSS 20 (http://www-01.ibm.com/software/analytics/spss/) was used for statistical analyses outside of the imaging space. The anatomical location information was obtained with the Talairach Daemon software (http://www.talairach.org) after MNI to Talairach coordinates transformation (http://brainmap.org/icbm2tal). The
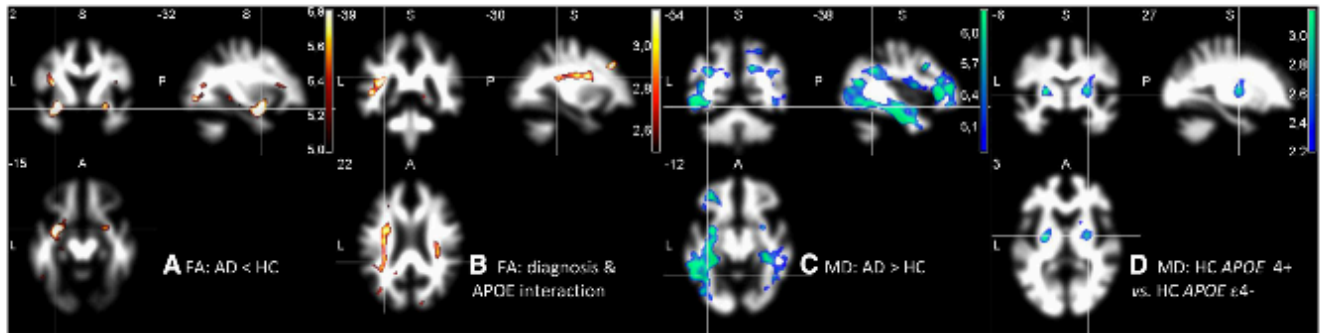
Fig. 1. Fractional anisotropy (FA) (A) and mean diffusivity (MD) (C) maps showing WM areas with statistically significant differences ($P < .05$ [FWE]) between AD patients and HC subjects. (B) Brain areas in which a larger effect of *APOE* ε4 on FA values was found in AD patients than in HC subjects (at $P < .001$, uncorrected). (D) Areas in which higher MD values in HC *APOE* ε4+ compared with HC *APOE* ε4− subjects were found (at $P < .001$, uncorrected).

results were verified by comparison to the Talairach and Tournoux atlas [13].

3. Results

The results show the main effect of diagnosis (i.e., significantly [$P_{corrected} < 0.05$ {familywise error rate (FWE)}] smaller FA values and larger MD values in AD patients in comparison to HCs). This effect was found consistently in analyses using images smoothed with 4-, 8-, and 12-mm full-width half maximum (FWHM) isotropic Gaussian kernels. Smaller FA values in AD patients compared with HCs were found in the parahippocampal gyrus WM and limbic area WM bilaterally, the occipitotemporal area WM (e.g., left fusiform gyrus WM), the left insular WM, and the left medial frontal gyrus WM (Fig. 1A). Larger MD values in AD patients compared with HCs were found bilaterally in the parahippocampal and limbic area WM, the precentral and middle frontal gyri WM, the angular and supramarginal gyri WM, the middle temporal gyrus WM, the insular WM, and the corpus callosum (Fig. 1C).

When the threshold for statistical significance was set at $P < .001$ uncorrected for multiple comparisons, while keeping the minimum cluster size threshold of 50 voxels, interaction between the diagnosis and *APOE* ε4 was found for FA (i.e., larger effect of *APOE* ε4 on FA values was found in patients [AD *APOE* ε4+ vs AD *APOE* ε4−] than in control subjects [HC *APOE* ε4+ vs HC *APOE* ε4−]) in WM underneath the middle frontal gyrus, insular WM, and superior temporal gyrus (Fig. 1B). The same effect was observed for MD in WM underneath the middle occipital gyrus. At the same threshold, a significant difference in MD between HC *APOE* ε4+ and HC *APOE* ε4− subjects was found in the vicinity of the left lentiform nucleus WM (Fig. 1D). When excluding DTI scans with <12 gradient directions, the results remained unchanged.

4. Discussion

We found a significant difference in MD values between HC *APOE* ε4+ and HC *APOE* ε4− subjects (at $P < .001$

uncorrected) in the vicinity of the left lentiform nucleus, closer to the putamen than the globus pallidus. This modest effect needs to be interpreted with caution because it is not clear why *APOE* ε4 appears to affect MD in HCs only in this particular area while sparing other areas that have been previously reported as more prominently implicated in aging and AD. Although the lentiform nucleus is not a typical area of vulnerability in AD, deficits associated with this structure have been observed in normal aging, such as significantly increased values of the apparent diffusion coefficient in this area in cognitively healthy elderly compared with young subjects [14] and significant shrinkage of this area in elderly compared with young subjects, with a larger volume reduction in the left than in the right hemisphere [15]. Volumetric reductions of the putamen have been found in AD patients [16]. It appears then that WM in the vicinity of the lentiform nucleus is sensitive to microstructural changes that are detectable by DTI, although future studies need to replicate this finding. The diagnosis*APOE ε4 status interaction revealed modest changes in FA and MD in fronto-temporal WM and middle occipital gyrus WM, respectively, indicating more WM changes in *APOE* ε4 carriers in the AD group than in *APOE* ε4 carriers in the HC group. However, because a main effect of *APOE* ε4 status was not found for FA or MD in AD patients, and it was observed only for MD in HCs, it is difficult to interpret this finding.

Wang et al [9] used DTI to investigate the parahippocampal WM in 17 AD patients with mild dementia, among who 10 were *APOE* ε4 carriers and 6 were noncarriers (*APOE* ε4 status for one patient was unknown). The *APOE* ε4 allele was found to significantly contribute to the volume loss and increase in MD values in this region across both groups, but not accounting for the effect of diagnosis (although *APOE* ε4 genotype was significantly more frequent in the AD sample of this previous study). Bagepally et al [8] focused only on FA and found significantly reduced FA values in the bilateral temporoparietal, limbic, and parahippocampal regions in AD patients compared with HCs as well as "a modest association" (p. 145) between *APOE* ε4 and WM integrity in the medial temporal (left in AD patients,

bilaterally in HCs) and limbic regions; however, this was at a very liberal level of significance of $P < .05$ uncorrected, increasing the chance of false-positive findings. In our study, we used several times the number of subjects compared with these previous studies to reduce the risk of chance findings.

The multicenter acquisition of our data may have added to interindividual variance, reducing the power of our approach to more robustly detect the effects of *APOE* ε4 genotype, although a previous physical and clinical phantom study and a study that was based on experimental and simulation data suggest a limited variability of DTI data given that some minimal standards of acquisition are met [17,18]. A possible limitation of our study is the lack of *APOE* ε4 dose subgroups, which would require much larger sample sizes. Another possible shortcoming is the potential effect of partial volume. To reduce this effect, we used high-dimensional warping to achieve high accuracy of spatial mapping. Nevertheless, a minor effect of partial volume cannot be excluded.

In conclusion, our data provide evidence for a modest effect of *APOE* ε4 genotype on MD in HCs in a large multicenter sample. These data support the notion that changes in cortical structural connectivity may be related to risk factors of AD in cognitively healthy elderly subjects.

RESEARCH IN CONTEXT

1. Systematic review: We searched *PubMed* to identify primary research studies that investigated *APOE* ε4 and WM in AD using DTI. References cited in the identified studies that fitted our search criteria were also consulted.

2. Interpretation: The results of our study show significant changes in indices of WM integrity between AD patients and HC subjects and are aligned with most findings on the topic. We also found a modest effect of *APOE* ε4 on MD in HCs but not in AD patients.

3. Future directions: Future studies on the relation of *APOE* ε4 and WM changes in AD need to include large samples with *APOE* ε4 dose subgroups and age subgroups, addressing issues such as how *APOE* affects the aging-disease progression interplay and whether the disease overcomes *APOE* effect as it progresses.

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