Disrupted white matter structural networks in healthy older adult *APOE ε4* carriers - An International Multicenter DTI Study

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Abstract

The *ε4* allelic variant of the Apolipoprotein E gene (*APOE ε4*) is the best-established genetic risk factor for late-onset Alzheimer's disease (AD). White matter (WM) microstructural damages measured with Diffusion Tensor Imaging (DTI) represent an early sign of fiber tract disconnection in AD. We examined the impact of *APOEε4 on* WM microstructure in elderly individuals from the multicenter European DTI Study on Dementia. Voxelwise statistical analysis of Fractional anisotropy (FA), mean diffusivity, radial and axial diffusivity (MD, radD and axD respectively) was carried out using Tract-Based Spatial Statistics. Seventy-four healthy elderly individuals – 31 *APOE ε4* carriers (*APOE ε4*+) and 43 *APOE ε4* non-carriers (*APOE ε4*-) –were considered for data analysis. All the results were corrected for scanner acquisition protocols, age, gender and for multiple comparisons. *APOE ε4*+ and *APOE ε4*- subjects were comparable regarding sociodemographic features and global cognition. A significant reduction of FA and increased radD was found in the *APOE ε4*+ compared to the *APOE ε4*- in the cingulum, in the corpus callosum, in the inferior fronto-occipital and in the inferior longitudinal fasciculi, internal and external capsule. *APOE ε4*+, compared to *APOE ε4*- showed higher MD in the genu, right internal capsule, superior longitudinal fasciculus and corona radiate. Comparisons stratified by center supported the results obtained on the whole sample. These findings support previous evidence in monocentric studies indicating a modulatory role of *APOE ε4* allele on WM microstructure in elderly individuals at risk for AD suggesting early vulnerability and/or reduced resilience of WM tracts involved in AD.

Keywords : Diffusion Tensor Imaging; Apolipoprotein E; multicenter study; White Matter Integrity; Aging

INTRODUCTION

The *APOE* gene, located on chromosome 19q13.2, encodes for the ApoE protein (Boyles et al., 1985, Nakai et al., 1996). ApoE participates in lipid metabolism, particularly in cholesterol transport and clearance. Moreover, its activity is associated with relevant components of brain WM such as myelin, of which cholesterol is a major constituent (Westlye et al., 2012). It is also implicated in neuronal growth and repair, nerve regeneration, immune response, and activation of lipolytic enzymes (Karch et al., 2014, Yu et al., 2014). At present, the *ε4* allelic variant of *APOE* – *APOE ε4* – is the best established genetic risk factor for the development of late-onset Alzheimer's Disease (AD) (Corder et al., 1993, Strittmatter et al., 1993). The involvement of genetic risk factors such as *APOE ε4* in sporadic late-onset AD has been profoundly demonstrated (Saunders et al., 1993, Sherrington et al., 1995, Bertram et al., 2007, Reitz et al., 2011, Lockhart and DeCarli, 2014).

Structural neuroimaging patterns related to *APOE ε4* in elderly individuals described grey matter atrophy in the medial temporal structures (Chen et al., 2007, Donix et al., 2010b, Hua et al., 2010, Risacher et al., 2010, Lu et al., 2011, Roussotte et al., 2014) such as the subiculum (Burggren et al., 2008, Suthana et al., 2010) and CA1 subfield (Kerchner et al., 2014) of the hippocampus (Donix et al., 2010a, Chiang et al., 2011, O'Dwyer et al., 2012, Taylor et al., 2014), although contrasting results were published as well (Jack et al., 1998, Du et al., 2006, Schuff et al., 2009, Taylor et al., 2014). Moreover, higher cortical beta-amyloid deposition (Reiman et al., 2009, Morris et al., 2010, Fleisher et al., 2013), glucose hypometabolism in brain regions typically impaired in AD (Rimajova et al., 2008, Protas et al., 2013, Fouquet et al., 2014) and changes in brain function during an encoding memory task (Filippini et al., 2011) were previously described in elderly cognitive intact individuals carrying the *APOE ε4* allele. No interaction effects were found of APOE *ε4* status on the relationship between brain beta-amyloid levels and grey matter network disruption (Tijms et al., 2016). So far, the exact pathophysiological mechanism through which *APOE ε4* contributes to the aetiology and progression of the disease remains unclear.

In vitro and in vivo studies demonstrated that *APOE ε4* allele is associated with axonal degeneration (Tesseur et al., 2000) and structural modifications in intracellular microtubules (Nathan et al., 1995), thereby raising the possibility of mechanistically impacting white matter (WM) microstructure (Heise et al., 2011, Westlye et al., 2012, Heise et al., 2014). More than half of the individuals diagnosed with AD display WM microstructural alterations (Chalmers et al., 2005) that can be investigated *in vivo* by diffusion tensor imaging (DTI).

DTI detects the amplitude and directional coherence of water molecule diffusion and, since water molecule diffusion is usually constrained along the main fiber direction by axonal membranes and myelin sheaths, this feature can be used to measure WM structural integrity (Pierpaoli and Basser, 1996, Behrens et al., 2007). In particular, Fractional Anisotropy (FA) measures are generally high in healthy, structurally intact, coherently organized WM tissues (Acosta-Cabronero and Nestor, 2014). However,

there was also evidence of reduced FA in healthy cognitively intact adults in region of crossing fibers between the corticospinal tract and the superior longitudinal fasciculus (Douaud et al., 2011) as previously reported also in diseased tissue (Amlien and Fjell, 2014).
Whereas, high Mean Diffusivity (MD), Radial Diffusivity (radD) and Axial Diffusivity (axD) measures may potentially be used to detect tissue breakdown, myelin loss and axonal injury respectively (Beaulieu, 2002, Song et al., 2002, Song et al., 2005, Kumar et al., 2011, Kumar et al., 2013).

Previous studies investigating DTI indexes in AD patients showed a consistent pattern of decreased FA and increased MD, radD and axD, suggesting the presence of WM tracts disconnection in this population (Amlien and Fjell, 2014, Zhang et al., 2014). Although brain WM integrity, in older adults carrying *APOE ε4,* have been previously investigated in several monocentric studies (Gold et al., 2012, Felsky and Voineskos, 2013, Lyall et al., 2014), the reproducibility of these results in multicenter studies has not been sufficiently examined. In the present study, we investigated how the *APOE ε4* variant alters the brain WM microstructure in healthy older individuals recruited in the European multicenter DTI Study on Dementia (EDSD).

EXPERIMENTAL PROCEDURES

Participants

Sociodemographic, clinical and neuroimaging data were selected from the retrospective multicenter European Diffusion Tensor Imaging Study on Dementia (EDSD) database (Teipel et al., 2011, Fischer et al., 2012, Teipel et al., 2012, Dyrba et al., 2013, Dyrba, 2014, Kilimann et al., 2014, Kljajevic et al., 2014, Teipel et al., 2014, Tsao et al., 2014, Brueggen et al., 2015, Dyrba et al., 2015, Brueggen et al., 2016). The EDSD is a framework created to study the multicenter variability and diagnostic accuracy of DTI derived markers in patients with prodromal Alzheimer's disease (AD) and AD dementia. It was founded in 2010 and is coordinated by the German Center for Neurodegenerative Diseases (DZNE) in Rostock (Germany). Initially, MRI data, including DTI sequences of healthy control subjects (HC) and AD patients were retrospectively collected from 10 European centers leading in the field of AD research. The EDSD database has collected data from eleven European centers: Amsterdam (The Netherlands), Brescia (Italy), Cambridge (United Kingdom), Dublin (Ireland), Frankfurt (Germany), Freiburg (Germany), Milan (Italy), Mainz (Germany), Mannheim (Germany), Munich (Germany), and Rostock (Germany). As of March 2016, the EDSD sample consists of 139 Alzheimer's patients, 160 Mild Cognitive Impairment patients and 194 Healthy controls. An inclusion criterion for each center in order to upload the data of HC required that they were free of cognitive ~~complaints~~ impairment. Healthy subjects were recruited via advertisement, e.g. in newspapers. During anamnesis and neuropsychological assessment it was ruled out that they had cognitive complaints or medical diseases, including neurological and psychiatric diseases (such as depression or substance abuse). In the present study, we selected 85 healthy control individuals that underwent *APOE* genotyping conducted according to the standard methods derived from Amsterdam (renamed Center 1), Dublin (Center 2), Munich (Center 3), and Rostock (Center 4). Quality control of DTI scans was done visually to exclude scans with conspicuous artefacts such as ghosting, blurring due to motion, or strong susceptibility artefacts, and scans on which the brain was not entirely delimited within the field of view. Because of poor/incomplete head coverage preventing the creation of the mean FA image and its skeleton, 20 DTI scans were excluded from ~~the~~ analysis. The sample was subsequently enriched by integrating 9 supplementary DTI scans of individuals carrying *APOE ε4* coming from Rostock center. The analysis was carried out on 74 healthy cognitively normal older individuals categorized into 31 *APOE ε4* carriers (*APOE ε4*+) and 43 *APOE ε4* non-carriers (*APOE ε4*-). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, and the applicable revisions at the time of the investigation. The study was approved by the local ethics committee in each participating center. Written informed consent was provided from all participants or their representatives.

Image Acquisition

Images were acquired using four different magnetic resonance imaging (MRI) scanners. The detailed image acquisition protocol has been described in Table 1 while Figure 1 report a representative FA map for each site. Because of a possible effect of the scanner type on the data, centers were treated as covariates in the statistical analysis.

TBSS Image Processing and atlas-based ROI analyses

The DTI toolbox of FSL (4.1) (available at http://www.fmrib.ox.ac.uk/fsl/) was used for the DTI data pre-processing. Voxelwise statistical analysis of the FA data was carried out using TBSS (Tract-Based Spatial Statistics (Smith et al., 2004)), part of FSL (Smith, 2002). First, FA images were created by fitting a tensor model to the raw diffusion data using FDT, and then brain-extracted using BET (Smith, 2002). All subjects' FA data were then aligned into a common space using the nonlinear registration tool FNIRT (*www.fmrib.ox.ac.uk/analysis/techrep)*, which uses a b-spline representation of the registration warp field (Rueckert et al., 1999). Next, the mean FA image was created and thinned to create a mean FA skeleton, thresholded at FA > 0.2, which represents the centres of all tracts common to the group. Each subject's aligned FA data were then projected onto the skeleton. In addition, the nonlinear warps and the skeleton projection achieved with FA images were applied to the MD, radD and axD maps to bring them into standard space (Jovicich et al., 2014). Finally, data fed into voxelwise cross-subject statistics.

Moreover, an atlas-based ROI analysis for FA, MD and radD maps was performed in the WM tracts resulted significance from voxelwise cross-subject statistics. In particular, we focused on the corpus callosum (genu, body and splenium), the internal and external capsule, the inferior fronto-occipital and inferior longitudinal fasciculi, the cingulum, the right corona radiate, the right posterior thalamic radiation and the right superior longitudinal fasciculus. These WM ROIs are pre-defined in the JHU-ICBM-FA-1mm atlas. We back-projected the ROIs with a non-linear co-registration to each subject's FA map in the MNI152 space obtained from TBSS. Each ROI label was overlapped with the FA TBSS skeleton space to remove any CSF and gray matter voxels. These new ROIs were then used in each subject's FA, MD and radD maps (MNI152 space) to extract the FA, MD and radD metrics in each above mentioned tract (Jovicich et al., 2014). Subject motion, was extracted from each DTI acquisition using the tool FLIRT from FSL (Jenkinson et al., 2002).

Statistical Analysis

Demographic, clinical, cognitive data, global and tracts related FA, MD, radD, and axD mean values

Group differences between APOE ε4+ and APOE ε4- were assessed using the Chi-Square test for categorical variables and using the Mann-Whitney U-test for continuous variables. For global and tracts related FA, MD, radD and axD mean values as well as motion, group differences were adjusted for site, age and gender using linear regression. At first, the group by age interaction was added in the linear models and it was tested for global and specific WM tracts. The interaction was removed when no significant effect was detected. Effects were tested using likelihood ratio test. P values were corrected for multiple testing using Benjamini-Hochberg correction. Cohen's f^2 was used to measure the effect size of ApoE4 status for global metrics and specific WM tracts. Moreover, stratification by centers was performed to assess group differences in DTI global metrics. For this analysis Mann-Whitney U-test was used and the effect size was calculate as proposed by Cohen (Cohen 1988) according to the following formula: z/sqrt(N). P values were corrected for multiple testing using Benjamini-Hochberg correction. Statistical analysis was performed using R 3.3.2.

TBSS: General Linear Model. First, design matrix and contrast for the General Linear Model were generated including center, gender and age as covariate (Number of EVs: 7, Number of Points: 74, Number of Contrasts: 2). Then, voxelwise statistics of DTI images were performed using the randomise FSL's tool for nonparametric statistical thresholding (Nichols and Holmes, 2002) using the Threshold-Free Cluster Enhancement option. The number of permutations was set at 5000 (Nichols and Holmes, 2002). MD, FA, axD, and radD values within the skeleton were compared between *APOE ε4+* and *APOE ε4-* groups controlling for the family-wise error (FWE) rate (two tailed, $p < 0.05$). We then compared MD, FA, axD, and radD values between *APOE ε4+* and *APOE ε4-* groups within each single center (two tailed $p < 0.05$).

RESULTS

Clinical, demographic, and neuropsychological findings

In the non-carrier group the allelic frequencies for APOE were: ε2/ε3=7 ε3/ε3=36, while in the carrier group were: ε2/ε4=5; ε3/ε4=23; ε4/ε4=3. Subject groups did not differ in terms of age at MRI date, sex, education, and global cognition measured using the MMSE, both considering the whole sample (Table 2) and the stratification by single center (Table 3).

Comparisons of DTI Indexes: FA, MD, radD, axD

DTI metrics were not influenced by subject motion, as described by rotation parameters reported in Table 2. Comparison between APOE ε4+ and APOE ε4- in terms of global FA, MD, radD, and axD mean values revealed significant results exclusively for FA and radD. A significant FA reduction and increase radD in APOE ε4+ compared to APOE ε4- was observed (FA: $p < 0.001$, Cohen $f = 0.31$; radD: $p < 0.001$, Cohen $f = 0.31$, Table 2). The comparison within centers showed a significant reduction of FA in the APOE ε4+ compared with the APOE ε4- cases in Center 4 ($p = 0.024$ $r = 0.55$, Table 3), while an increase of axD was found in Center 2 ($p = 0.028$ $r = 0.70$) and an increase of radD was detected exclusively in Center 4 ($p = 0.007$ Cohen $r = 0.70$, Table 3). The distribution of DTI metrics across centers is described in the Figure 2. No significant group*age interaction was found for FA, MD, axD, radD (Table A1).

TBSS: Decreased Fractional Anisotropy (FA), in APOE ε4+ vs. APOE ε4-

Compared with APOE ε4-, individuals with APOE ε4+ showed significant widespread reduction of FA across the entire skeleton (Figure 3). In particular, major differences between the two groups were located in all components of corpus callosum: genu ($p < 0.001$, Cohen $f = 0.44$), body ($p = 0.001$, Cohen $f = 0.17$) and splennium ($p < 0.001$, Cohen $f = 0.37$, Table A2), bilaterally in the internal capsule (right: $p < 0.001$, Cohen $f = 0.68$; left: $p < 0.001$, Cohen $f = 0.47$, Table A2), in the right and left external capsule (right: $p < 0.001$, Cohen $f = 0.27$; left: $p = 0.003$, Cohen $f = 0.15$, Table A2), bilaterally in the inferior fronto-occipital and inferior longitudinal fasciculi (right: $p = 0.004$, Cohen $f = 0.13$; left: $p = 0.009$, Cohen $f = 0.11$, Table A2), and in the cingulum (right: $p < 0.001$, Cohen $f = 0.33$; left: $p = 0.015$, Cohen $f = 0.10$, Table A2) particularly its anterior part (Figure 3). Table A2 reports p-values for group differences of specific FA white tracts. No significant group*age interaction was found for all white matter tracts considered (Table A1).

Subsequently, the analysis was repeated separately for each center. After controlling for the family-wise error, results remained significant only for Center 4 ($p < 0.05$). However, the uncorrected p-maps showed a similarly widespread reduction of FA within each center (Figure 4). No effects in FA in the opposite direction, i.e. increased FA in APOE ε4+ vs APOE ε4-, were found; even when uncorrected p-values were scrutinized.

TBSS: Increased Mean Diffusivity, in APOE ε4+ vs. APOE ε4-

Compared with APOE ε4-, individuals with APOE ε4+ showed significant increase of MD in the right hemisphere (Figure 5), particularly in the genu of corpus callosum ($p = 0.002$, Cohen $f = 0.16$, Table A2), in the right internal capsule ($p = 0.004$, Cohen $f = 0.13$, Table A2) in the right corona radiate ($p = 0.016$, Cohen $f = 0.09$, Table A2) and in the right superior longitudinal fasciculus ($p = 0.020$, Cohen $f = 0.08$, Table A2). No significant group*age interaction was found for all white matter tracts considered (Table A1). Subsequently, the analysis was repeated separately for each center. After controlling for the family-wise error, results remained significant only for Center 4 ($p < 0.05$) (Figure 6). However, the uncorrected p-maps showed similar MD maps within each center (Figure 6). No effects in MD in the opposite direction, i.e. increased FA in APOE ε4+ vs APOE ε4-, were found; even when uncorrected p-values were scrutinized.

TBSS: Increased Radial Diffusivity (radD), in APOE ε4+ vs. APOE ε4-.

An increase in radD was found in APOE ε4+ compared to APOE ε4- (Figure 7), in particular in the genu and splenium of corpus callosum ($p < 0.001$, Cohen $f = 0.41$ and $p < 0.001$, Cohen $f = 0.33$ respectively, Table A2), bilaterally in the internal capsule (right: $p < 0.001$, Cohen $f = 0.65$; left: $p < 0.001$, Cohen $f = 0.57$, Table A2), in the right and left inferior fronto-occipital and inferior longitudinal fasciculi (right: $p < 0.001$, Cohen $f = 0.24$; left: $p < 0.001$, Cohen $f = 0.22$, Table A2), in the anterior and posterior part of the cingulum bilaterally (right: $p < 0.017$, Cohen $f = 0.89$; left: $p < 0.004$, Cohen $f = 0.13$, Table A2) and in the external capsule bilaterally (right: $p < 0.001$, Cohen $f = 0.89$; left: $p < 0.003$, Cohen $f = 0.13$, Table A2) (Figure 7). No significant group*age interaction was found for all white matter tracts considered (Table A1). Statistical comparisons within centers revealed a significantly increased radD in the APOE ε4+ group in the Center 4 (Figure 8). Centers 1, 2, and 3, uncorrected p-maps revealed a trend of increased radD in the APOE ε4+ compared to APOE ε4- (Figure 8). No increased in radD was observed in the APOE ε4- group relative to the APOE ε4+, even when uncorrected p-values were scrutinized.

DISCUSSION

In the present study, we explored the impact of the *APOE ε4* genotype on WM microstructure in cognitively intact older adults recruited in the EDSD multicenter study. To our knowledge this is the first multicenter study investigating a broad range of WM microstructure indices on a population of cognitively healthy elderly individuals. Indeed, the majority of studies published so far on the effect of *APOE ε4+* on the WM microstructure in cognitively intact older individuals have previously explored exclusively FA and MD indices, while the present study has also considered radD, and axD indices. Our results showed FA reduction and concomitant higher MD and radD in brain areas affected by AD. No significant differences in axD were found between *APOE ε4+* and *APOE ε4-*. In addition *APOE ε4-* cases showed no decrease of FA or increase of MD and radD in any tract compared to *APOE ε4+* cases.

Several evidences have showed an increased risk to develop AD in elderly individuals carrying APOE *ε4*. In particular, evidence have reported in APOE *ε4* heterozygotes individuals compared to APOE *ε3* homozygotes a risk of developing AD of 4 times higher above 60 years old (Reinvang et al., 2013). Furthermore, previous studies described how cognitive intact and mild cognitive impairment individuals carrying APOE ε4 compared to APOE *ε4* non-carriers had an increase of brain beta-amyloid and tau load (Reiman et al., 2009, Small et al., 2009, Morris et al., 2010), the two major pathophysiological hallmarks of AD. This evidence highlights the importance of investigating indices of white matter microstructure related to population of older age adults at high risk to develop AD.

Despite our data were collected at mutlicentric level in a non-homogenized clinical settings using different DTI acquisition protocols, our findings are generally consistent with previous DTI studies, showing alterations of cerebral WM in elderly *APOE ε4+* compared with *APOE ε4-* individuals (Persson et al., 2006, Smith et al., 2010, Heise et al., 2011, Ryan et al., 2011). We found a lower WM integrity in individuals carrying *APOE ε4* in WM tracts characteristically associated with early AD pathology, such as the corpus callosum, the cingulum, and the inferior longitudinal and fronto-occipital fasciculi (Medina et al., 2006, Rose et al., 2006, Xie et al., 2006, Firbank et al., 2007, Huang et al., 2007, Sydykova et al., 2007, Teipel et al., 2007, Filippini et al., 2009, Tsao et al., 2014, Lee et al., 2016). In particular, in agreement with previous evidence, we found lower FA and higher radD values in *ε4* carriers compared to non-carriers in the genu and splenium of corpus callosum (Persson et al., 2006, Smith et al., 2010, Ryan et al., 2011, Adluru et al., 2014, Tsao et al., 2014, Lee et al., 2016). This finding also agrees with prior MRI studies observing macroscopic WM lesions in both normal aging (Bartzokis, 2004, Filippini et al., 2009) and AD patients (Janowsky et al., 1996, Teipel et al., 2003) in the corpus callosum. Morphological differences in the corpus callosum may point to regional and cell-type specific neuronal neurodegeneration (Hampel et al., 1998); indeed, WM fibres of the splenium originate from the temporoparietal regions (Conturo et al., 1999), which are characteristically affected in the early stages of AD (Thompson et al., 2001, Ewers et al., 2011a).

In agreement with previous studies, using the same method, we found a significant widespread reduction of FA in the posterior portion of cingulum, in the inferior fronto-occipital and longitudinal fasciculi in elderly

non-demented *APOE ε4*+ participants (Heise et al., 2011, Zhang et al., 2015).

In line with previous literature results, cognitive intact individuals carrying APOE ε4 showed a significant increase of MD in the genu of corpus callosum (Heise et al., 2011), in the corona radiata, internal capsule and superior longitudinal fasciculus (Heise et al., 2011, Westlye et al., 2012, Adluru et al., 2014). A study conducted on a smaller sample of elderly individuals carrying *APOE ε4* from the EDSD database (Kljajevic et al., 2014) reported a modest effect of *APOE ε4* on MD in the lentiform nucleus in healthy controls comparing *APOE ε4*+ with *APOE ε4*-. The number of overlapping subjects between the present work and the one of Kljajevic and colleagues was 35 out of 74 subjects considered in the present Manuscript. These inconsistent DTI findings may be partially due to the small sample size and the use of different methods (such as FSL, SPM).

In addition, a significantly increased radD in elderly *APOE ε4*+ individuals was detected. Few studies investigating this marker in *APOE ε4* carriers have been conducted so far. Our results are in line with the findings described in the manuscript by Westlye and colleagues (2012), showing an increased radial diffusivity in *APOE ε4*+ compared to *APOE ε4*-. Interestingly, preliminary results showed an increase of radial diffusivity in elderly individuals at risk of AD, defined as the presence of APOE ε4 and family history of dementia, thus raising the possibility that reduced WM integrity may contribute to AD onset (Ewers et al., 2011b, Gold et al., 2012). Decreased FA and increased radD were found in the inferior longitudinal fasciculus indicates a substantial involvement of WM fibres connecting the occipital and medial temporal lobe regions (Catani et al., 2003), including the amygdala/hippocampal head regions anteriorly and the ventral visual association areas posteriorly. It is well known that these tracts – involved in face recognition (Fox et al., 2008), visual perception (ffytche and Catani, 2005) and visual memory processing (Ross, 2008) – are affected in AD (Liu et al., 2011). Furthermore, a recent study showed as healthy elderly *APOE ε4*+ carriers exhibited topographical alterations in both WM and functional networks, in particular with a reduced efficiency in the parahippoacampal gyrus mediated by the effect of APOE variants on memory performances (Chen et al., 2015). In addition, a further study found shorter neuronal fibres bundles lengths in the left uncinate fasciculus of *APOE ε4*+ carriers related to severe deficits in semantic memory (Yasmin et al., 2008).

The comparison between APOE ε4+ and APOE ε4- within centers revealed a trend in each subgroups of decreased FA and increased MD, axD and radD in the APOE ε4+ groups. This evidence supports the claim that the overall effect is not due to center effects and that the investigation of indexes of WM microstructure at multicenter level, using different MRI protocols of acquisition, is feasible and lead to results previously replicated at monocentric level. However, previous studies showed that DTI scalar measurements are dependent on several factors, among them: the use of different b value, the number of diffusion directions and the voxel size. In particular, a study detecting any dependency of the FA values on the applied b-value, found significantly different mean FA values between the DTI acquisitions using a b-value of 700 s/mm2 and those using a b-value of 1000 s/mm2 in the genu of right internal capsule and the anterior limb of left internal capsule (Bisdas et al., 2008). In our study most of the centre presented similar

b-value. Center 4 displayed substantial higher FA values compared to the FA values of other Centers. Previous studies have showed that FA values are mainly affected by changes in the number of gradients and voxel resolution (Barrio-Arranz et al., 2015). In particular, high voxel size resulted in decreasing FA average. Center 4 shows a lower voxel size compared to other centers indicating a likely impact in increasing FA values. Moreover, a high number of gradient directions were shown to decrease FA (Jones, 2004). Our findings are in line with these previous results displaying decrease FA in the center with less gradient directions.

Despite the above results, findings on the effect of *APOE ε4* in cognitively normal older individuals are still inconclusive, including studies describing no significant impact of the *APOE ε4* allele on WM microstructure damages in middle-aged (Bendlin et al., 2010) and older (Nyberg and Salami, 2014) cognitive intact individuals. Furthermore, our results did not report any interaction between age and APOE as described in previous studies (Heise et al., 2011, Westlye et al., 2012), however contrasting results were reported (Adluru et al., 2014).

Some limitations of the present study need to be addressed. Firstly, due to the small sample size we cannot generalize our results. Secondly, due to the potentially limited anatomical specificity of the TBSS method (Bach et al., 2014), further analyses should be performed with additional techniques. For instance, the diffusion tensor imaging tractography dissection method (Catani et al., 2002) followed by a manual correction preformed by expert anatomists would allow to carry out more accurate measurements of white matter structures close to the cortical grey matter or subcortical nuclei such as the fornix and the uncinate (Acosta-Cabronero and Nestor, 2014). In addition, this manuscript does not investigate more potentially sensitive indices of white matter microstructure such as those from the NODDI model (Zhang et al., 2012) and from the advanced spherical deconvolution, such as the HMOA index (Dell'Acqua et al., 2013). Then, although center was included as a covariate of no interest in the group comparisons, our results might be affected by the different protocols of DTI acquisition used in each center. However, previous physical and clinical phantom studies based also on the EDSD database have revealed limited variability of DTI data when some minimal standards of acquisition are met (Landman et al., 2007, Teipel et al., 2011). Finally, we would like to underline that the multicenter nature of the EDSD study might not be the ideal condition to investigate the effect of APOE ε 4 genotype on the white matter microstructure, however at the same time the multicenter structure of the EDSD study allows the use of DTI among scanners and clinical settings. This is an essential aspect for the translation of imaging markers from the research bench to the clinical context.

In conclusion, this is the first multicenter study investigating a large spectrum of DTI indexes of WM microstructure. Despite the multicenter and not harmonized nature of EDSD DTI data, our findings support previous evidence regarding the impact of the *APOE ε4* genotype on WM integrity in cognitive intact older individuals. In particular, an early alteration of WM in the corpus callosum, in the cingulum, and in the inferior fronto-occipital and longitudinal fasciculi was found. These findings suggest that a reduction of WM tracts integrity may represent early pathological changes related to underlying AD pathology in healthy

elderly individuals carrying APOE *ε4* (Hampel et al., 2010). In view of the heterogeneity of results previously described in the literature, our study adds information on possible causes of inter-individual WM heterogeneity that may be genetically determined by the presence of APOE genotype. Future studies should be conducted in even larger cohorts of subjects carrying APOE *ε4* alleles both at preclinical and prodromal level of AD progression in order to further clarify the effect of APOE *ε4* on WM microstructure damages throughout the entire AD continuum.

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Table 1. Acquisition parameters of DTI scans

Center	Scanner type	Tesla	DTI						
			TR	TE	Fa	Number of diffusion directions	b-values	Voxel size	Gap (mm)
CENTER 1	Siemens Sonata	1.5	8500	86	90	60	0, 1000	2x2x2	0
CENTER 2	Siemens Trio	3.0	11,450	52	90	15	0, 800	2x2x2	0
CENTER 3	Siemens Trio	3.0	9300	102	90	12	0, 1000	2x2x2	0
CENTER 4	Siemens Verio	3.0	8200	93	90	20	0, 1000	1x1x2.4	0.4

TR = Repetition time (ms); TE = Echo time (ms); TI = Inversion time (ms); Fa = Flip angle (degrees).

Table 2. Sociodemographic, global cognition and white matter microstructure parameters of 74 older adults cognitively normal individuals of the EDSD database.

	APOE ε4+ (N=31)	**APOE ε4-** (N=43)	**p-value**
Site			
Center 1	4 (12.90%)	7 (16.28%)	0.940
Center 2	8 (25.81%)	9 (20.93%)	
Center 3	7 (22.58%)	11 (25.58%)	
Center 4	12 (38.71%)	16 (37.21%)	
Age (years)	67.85 ± 8.42	69.75 ± 5.73	0.600
Gender			
Female	16 (51.61%)	23 (53.49%)	0.940
Male	15 (48.39%)	20 (46.51%)	
Education	13.81 ± 3.17	13.67 ± 4.35	0.600
MMSE	28.93 ± 0.80	28.74 ± 1.20	0.922
Gobal FA	0.46 ± 0.04	0.48 ± 0.04	**<0.001**
Global MD ($mm^2/s\ 10^{-3}$)	0.76 ± 0.05	0.75 ± 0.06	0.102
Global radD ($mm^2/s\ 10^{-3}$)	0.53 ± 0.08	0.49 ± 0.10	**<0.001**
Global axD ($mm^2/s\ 10^{-3}$)	1.18 ± 0.05	1.17 ± 0.05	0.537
Rotation (mm)	0.15 ± 0.20	0.22 ± 0.26	0.537

Note. Counts, percentages, means and standard deviations are shown for the two groups, as well as p-values, to indicate statistically significant group differences.
p-values are corrected for multiple testing using Benjamini-Hochberg method and adjusted for age, gender and center for DTI results.

Table 3. Description of socio-demographic features, global cognitive performances and indexes of white matter integrity stratified by number of cases per center.

	Center 1			Center 2			Center 3			Center 4		
	APOE ε4+ (N = 4)	**APOE ε4- (N = 7)**	**p-value**	**APOE ε4+ (N = 8)**	**APOE ε4- (N = 9)**	**p-value**	**APOE ε4+ (N = 7)**	**APOE ε4- (N = 11)**	**p-value**	**APOE ε4+ (N = 12)**	**APOE ε4- (N = 16)**	**p-value**
Age (years)	76.81 ± 4.29	74.30 ± 5.49	0.938	68.75 ± 8.40	67.56 ± 5.03	0.985	63.57 ± 6.48	68.27 ± 7.32	0.840	66.75 ± 8.81	70.00 ± 4.07	0.938
Gender (F/M)												
Female	3 (75.00%)	5 (71.43%)	0.985	4 (50.00%)	6 (66.67%)	0.938	4 (57.14%)	4 (36.36%)	0.938	5 (41.67%)	8 (50.00%)	0.938
Male	1 (25.00%)	2 (28.57%)		4 (50.00%)	3 (33.33%)		3 (42.86%)	7 (63.64%)		7 (58.33%)	8 (50.00%)	
Education (years)	16.00 ± 0.00	17.00 ± 3.21	0.938	13.25 ± 3.41	13.78 ± 7.16	0.938	14.00 ± 4.24	13.09 ± 3.73	0.938	13.33 ± 2.81	12.56 ± 2.37	0.840
MMSE	29.00 ± 0.00	28.86 ± 1.46	0.938	29.75 ± 0.46	28.89 ± 1.62	0.840	28.86 ± 0.38	29.18 ± 0.75	0.935	28.30 ± 0.82	28.31 ± 1.01	1.000
Gobal FA	0.41 ± 0.03	0.43 ± 0.01	0.339	0.43 ± 0.02	0.44 ± 0.02	0.935	0.50 ± 0.02	0.51 ± 0.02	0.938	0.48 ± 0.03	0.52 ± 0.02	**0.028**
Global MD ($mm^2/s\ 10^{-3}$)	0.86 ± 0.02	0.85 ± 0.03	0.938	0.77 ± 0.04	0.76 ± 0.02	0.938	0.73 ± 0.03	0.73 ± 0.03	0.938	0.73 ± 0.02	0.71 ± 0.03	0.345
Global radD ($mm^2/s\ 10^{-3}$)	0.66 ± 0.03	0.64 ± 0.03	0.840	0.58 ± 0.04	0.57 ± 0.02	0.938	0.52 ± 0.04	0.51 ± 0.03	1.000	0.47 ± 0.07	0.38 ± 0.03	**0.007**
Global axD ($mm^2/s\ 10^{-3}$)	1.25 ± 0.08	1.22 ± 0.06	0.938	1.18 ± 0.03	1.14 ± 0.02	**0.028**	1.17 ± 0.03	1.18 ± 0.05	0.983	1.16 ± 0.05	1.16 ± 0.03	0.938

Note. Counts, percentages, means and standard deviations are shown for the two groups, as well as p-values, to indicate statistically significant group differences.
p-values are corrected for multiple testing using Benjamini-Hochberg method.

Figure Captions

Figure 1 Sample single-subject FA maps across different scanners for qualitative comparison. FA maps were selected from participant's non carrying APOE ε4. For DTI acquisition parameters see Table 1.

Figure 2 Forest plots illustrating the distributions of DTI metrics within each group (ε4+/ ε4-), both across and within each center. Diamonds indicate mean, Lower/ Upper Confidence Interval (CI) at 95%.

Figure 3 TBSS results: areas of decreased fractional anisotropy (FA) (red-yellow), in APOE ε4+ *vs.* APOE ε4-. Images are adjusted for scanner acquisition protocols, age and gender and for multiple comparisons at $p<0.05$. Numbers refer to Z axis. Radiological convention: Right (R) = Left (L) Hemisphere, skeleton shown in green; FWE= family-wise error.

Figure 4 Fractional Anisotropy maps adjusted for age and gender and stratified by center (each column), showing the contrast: APOE ε4- > APOE ε4+. Only significant results are depicted (results FWE corrected for Center 4 and uncorrected for Center 1, 2, 3). Numbers refer to X axis in the upper row, Y axis in the middle row and Z axis in the lower row. Radiological convention: Right (R) = Left (L) Hemisphere, skeleton shown in green.

Figure 5 TBSS results: areas of increased Mean Diffusivity (MD) (red-yellow), in APOE ε4+ *vs.* APOE ε4-. Images are corrected for age, gender and scanner acquisition protocols and for multiple comparisons at $p<0.05$. Numbers refer to Z axis. Radiological convention: Right (R) = Left (L) Hemisphere, skeleton shown in green; FWE= family-wise error.

Figure 6 Mean Diffusivity maps, stratified by center (each column), showing the contrast APOE ε4- < APOE ε4+. Only significant results are depicted (results FWE corrected for Center 4 and uncorrected for Center 1, 2, 3). Numbers refer to X axis in the upper row, Y axis in the middle row and Z axis in the lower row. Radiological convention: Right (R) = Left (L) Hemisphere, skeleton shown in green.

Figure 7 TBSS results: areas of increased Radial Diffusivity (radD) (red-yellow), in APOE ε4+ *vs.* APOE ε4-. Images are corrected for age, gender and scanner acquisition protocols and for multiple comparisons at $p<0.05$. Numbers refer to Z axis. Radiological convention: Right (R) = Left (L) Hemisphere, skeleton shown in green; FWE= family-wise error.

Figure 8 Radial Diffusivity maps, stratified by center (each column), showing the contrast APOE ε4- < APOE ε4+. Only significant results are depicted. (results FWE corrected for Center 4 and uncorrected for

Center 1, 2, 3). Numbers refer to X axis in the upper row, Y axis in the middle row and Z axis in the lower row. Radiological convention: Right (R) = Left (L) Hemisphere, skeleton shown in green.