

**LONGITUDINAL DIFFERENCES IN WHITE MATTER INTEGRITY  
BETWEEN APOE  $\epsilon$ 4 CARRIERS VERSUS NONCARRIERS**

By

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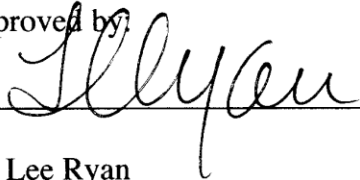
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**Abstract**

Alzheimer's disease (AD) is a neurodegenerative disorder that causes atrophy in gray and white matter (WM) as well as cognitive declines. Even though recent literature suggests that the apolipoprotein E (APOE)  $\epsilon 4$  allele increases cognitive deficiencies in older adults, especially through increasing risk for the late-onset form of AD, relatively little is known about longitudinal differences in brain white matter integrity changes between APOE  $\epsilon 4$  carriers and noncarriers. Data was obtained from 53 individuals (ages 55-91) which included 34 APOE  $\epsilon 4$  noncarriers and 19 APOE  $\epsilon 4$  carriers. From diffusion weighted images (DWI) fractional anisotropy (FA) maps were calculated at two time points 2.7 years apart, on average, in order to measure differences in the change in white matter integrity over time between the APOE  $\epsilon 4$  groups. Regions of interest (ROI) were selected based on significant regions of difference at the first scan. No significant decreases in WM integrity were observed over time for the ROIs selected. However, in the left superior temporal white matter there was a significant increase in FA over time for the  $\epsilon 4$  non-carriers but not the carriers. A similar, trending pattern was found in the left middle frontal white matter. Interestingly, these were two ROIs in which  $\epsilon 4$  carriers had greater volumes at initial scan, suggesting that the regions of advantage with regard to white matter efficiency that  $\epsilon 4$  carriers had compared to noncarriers disappear over time. Further analysis into associations between changes in white matter and changes in cognition over time should be undertaken.

## **Introduction**

It is estimated that by the year 2030, approximately 65.7 million people will be living with some form of dementia, with this number doubling every 20 years (Bendlin et al., 2009). Amongst these individuals, many will suffer from Alzheimer's disease (AD), a neurodegenerative disorder that progressively causes declines in memory and later declines in other cognitive functions. From a physiological standpoint, AD is mainly characterized by the formation of extracellular neuritic plaques containing the amyloid-Beta peptide ( $A\beta$ ) and intraneuronal accumulation of neurofibrillary tangles associated with hyperphosphorylated tau protein. The  $A\beta$  accumulation that plays a large role in neurotoxicity has been marked as the initial pathological event in the progression of AD (Jiang, et al, 2014). From a cognitive standpoint, the progressive neurodegeneration that occurs leads to volume loss and lesions in areas of the brain important for encoding, retrieving, and consolidating memories (de la Torre, 2015). For example, even in early onset forms of a dementia, those affected by AD show a decline of gray matter density.

In AD patients widespread decreases in gray matter volume relative to controls is seen in temporal regions including the amygdala and hippocampus bilaterally, the left inferior temporal gyrus, left parahippocampal gyrus, and frontal regions, including the orbitofrontal cortex, frontal poles bilaterally, the left inferior temporal gyrus, and left parahippocampal gyrus, among other regions (Irish et. al, 2014). This degradation of brain areas may not only negatively impair memory but also language, concept formation, problem solving, and could potentially cause changes in personality (Weintraub et al, 2012). There is currently no cure for AD. However, several initiatives have been made to attempt to treat the symptoms of AD in their earliest stages. For this reason, it is important to identify AD risk factors and measure the impact they have on

cognitive processes before diagnosis. One such risk factor is the Apolipoprotein E (APOE)  $\epsilon 4$  allele, which is associated with increased risk for late-onset AD with decreased cognitive functions even before AD diagnosis (Ryan, et al., 2011).

Carriers of the  $\epsilon 4$  variant of the APOE allele typically have an increased risk of developing the disease, especially those who are homozygous carriers, although not everybody with the allele is guaranteed to develop AD. The APOE gene is best known to play a role in cholesterol transport, metabolism, and breakdown of the amyloid-beta peptide (NIH, 2011). However, the specific  $\epsilon 4$  form of the allele can inhibit this breakdown, possibly associated with the increase in the risk for developing AD (NIH, 2011). Three allelic variants of the APOE gene exist and have differential effects on risk for developing AD: the APOE  $\epsilon 2$ , which is the most rare, shows a moderately protective effect against AD (Bendlin et al, 2009); the APOE  $\epsilon 3$  allele, which is the most frequently occurring allele does not directly increase nor decrease AD risk; and the APOE  $\epsilon 4$  allele which increases AD risk, although it is not the sole factor in genetic risk for the disease (Corder, et. al, 1994).

The APOE  $\epsilon 4$  allele also seems to play a role in structural and metabolic differences in brain regions that parallel those affected by AD even prior to onset of AD, affecting both gray and white matter volumes (Van Duijn, et. al, 1991). APOE  $\epsilon 4$  carriers have, on average, smaller volumes in the medial temporal lobe and decreased cortical thickness in the entorhinal cortex. In another study comparing gray matter volume in overall healthy individuals who had subjective memory impairment, APOE  $\epsilon 4$  carriers compared to noncarriers showed GM atrophy in the inferior temporal gyrus, inferior parietal lobule, anterior cingulum, middle frontal gyrus, and precentral gyrus, in addition to some atrophy in hippocampal volume (Lee et al., 2014). In regard to health of white matter (WM) or white matter integrity,  $\epsilon 4$  carriers show poorer white

matter integrity in the brain's temporal regions as well as frontal, occipital, and parietal white matter regions (Ryan, et al., 2011). Degradation in white matter integrity in  $\epsilon 4$  carriers is similar to findings of decreased white matter integrity in patients with mild cognitive impairment and AD (Maier-Hein, et al, 2014).

Limited research has been conducted on examining changes in white matter integrity over time within individuals. These studies are crucial as they provide a more in-depth understanding of cross-sectional observations in APOE carriers versus noncarriers. Both APOE  $\epsilon 4$  carriers and noncarriers will show noticeable degeneration in white matter integrity over their lifespan, due to normal aging processes (Lockhart et. al., 2014). However, it is not clear if  $\epsilon 4$  carriers will show significantly greater changes in white matter integrity as they age, since they are at increased risk of developing AD. White matter integrity is measured through a neuroimaging method known as diffusion weighted imaging (DWI; Adluru, et. al, 2014). DWI is a form of magnetic resonance imaging (MRI) that is helpful in studying changes in the integrity of brain tissue and provides information on the degree and directionality of water diffusion in the tissue (Ryan, et al, 2011). Because water displacement occurs faster along axons rather than across them, DWI simplifies the ability to visualize and quantify WM pathways (Westlye et al., 2012). The most frequently used DWI measures are fractional anisotropy (FA) and mean diffusivity (MD). MD represents the average rate of water diffusion regardless of directionality, while FA reflects how direction specific the diffusion of water is (Nyberg et. al, 2014; Ryan & Walther, 2014). FA measurements range from 0 to 1, with 0 being an eigenvalue that represents free water diffusion within an axon, resulting in less efficient communication of signals throughout the brain. On the other hand, an FA value of 1 represents water diffusion in an organized and confined linear manner, resulting in much more efficient and directed signal communication from cell to cell. Less effective

communication in the brain may be attributed to swelling or demyelination. Therefore, it is important to consider how white matter degradation results in less effective communication of signals within the brain of those at increased risk for developing AD.

***Aim***

The present study sought to expand the work of Ryan and colleagues (2011) by investigating longitudinal differences in changes in white matter integrity over time in APOE  $\epsilon 4$  carriers versus non-carriers. Changes in white matter integrity were measured across two time points (approximately 2.7 years apart) for each person and then compared for average changes across  $\epsilon 4$  groups using a mixed-design ANOVA with time point (Time 1 (T1) and Time 2 (T2)) as a within subjects factor and  $\epsilon 4$  status ( $\epsilon 4$ - and  $\epsilon 4$ +) as a between subjects variable.

***Hypothesis***

We predicted a decrease in white matter integrity across the brain over time for both  $\epsilon 4$  carriers and non-carriers. However, we specifically predicted a significantly greater decrease in  $\epsilon 4$  carriers versus non-carriers (*Figure 1*).

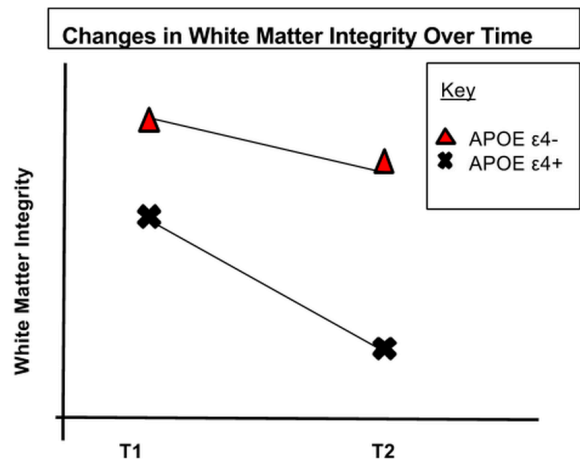


Figure 1. Predictions for this study.

## Method

### *Participants*

To test the hypothesis, data was taken from 53 community dwelling late middle age and older (58 – 91 years) adults ( $\epsilon 4-$ ,  $n=34$ ;  $\epsilon 4+$ ,  $n=19$ ) from Tucson, Arizona and the surrounding areas. Participants were recruited from the Cognition and Neuroimaging Laboratory and Aging and Cognition Unit at the University of Arizona. APOE  $\epsilon 4$  carriers and non-carriers are matched on education and sex ( $p$ 's  $> .05$ ); however, the groups significantly differed on age ( $p < .05$ ; see Table 1). Therefore, we controlled for the effect of age when analyzing the data. Participants' education levels ranged from 12 to 20 years and ages ranged from 58 to 91 years for APOE noncarriers ( $\epsilon 4-$ ) and 55 to 78 years for carriers ( $\epsilon 4+$ ). The University of Arizona Institutional Review Board reviewed and approved this project. Informed consent was obtained from all subjects.

Table 1. Demographics based on *APOE*  $\epsilon 4$  status

	$\epsilon 4-$ (34)	$\epsilon 4+$ (19)
Age at T1 (Mean (SEM)) <sup>†</sup>	71.48 (1.23)	67.69 (1.53)
Age at T2 (Mean (SEM))*	74.24 (1.22)	70.27 (1.52)
Years of Education (Mean (SEM))	15.97 (.44)	15.79 (.69)
Time difference (years; T2-T1; Mean (SEM))	2.76 (.11)	2.58 (.08)
Sex (M/F)	9/25	3/16
* $p < .05$ ; <sup>†</sup> $p < .10$		

### *Genotyping procedures*

Saliva samples taken from each subject (using Oragene DNA Collection Kits; DNA Genotek, Ottawa, Ontario, Canada) were genotyped for both *APOE* rs429358 and *APOE* rs7412 at the Translational Genomics Research Institute in Phoenix, Arizona. Genotyping used TaqMan allelic discrimination (Applied Biosystems, Foster City, CA) and ABI Prism 7000 sequence detection (Applied Biosystems, Foster City, CA).

### *Neuroimaging*

All MRI data was extant and was collected at the University of Arizona Medical Center using a GE 3.0 T Signa VH/I whole body echo-speed scanner equipped with an 8-channel phased array head coil (HD Signa Excite, General Electric, Milwaukee, WI). The diffusion tensor images were acquired with an echo planar imaging sequence corrected for spatial distortion (GE ASSET) in 25 directions with two averages. Images were eddy current corrected in FSL. Diffusion tensor values were obtained for each voxel in DTI Studio Version 2.4. These included three eigenvalues and eigenvectors. This study investigated a particular measure of white matter integrity, fractional anisotropy (FA). FA and B0 maps were computed based on tensor values for each participant. Custom templates were created in SPM8 and included all participants B0 images which were aligned to the anterior and posterior commissure. FA maps were coregistered to the B0 images and were normalized to the custom templates. A high-resolution white matter map was used to mask the values and ensure that FA was only being examined in WM regions. Then FA values were measured in identified white matter regions across the whole brain. Differences between APOE groups were calculated in SPM8 using two independent samples t-tests (scan time 1 and 2), controlling for age and ICV. Data was collected at two time points (T1 and T2) approximately 2.7 years apart. Difference in years between T1 and T2 did not significantly differ between  $\epsilon 4$  groups (see Table 1). T1 and T2 ranged from 1.60 to 4.10 years for APOE  $\epsilon 4^-$  and from 2.00 to 3.80 years for APOE  $\epsilon 4^+$ . Note that separate custom templates for T1 and T2 were used to normalize FA values. In each t-test, clusters of significant difference were defined as  $p < .05$  with a minimum of 10 contiguous voxels. Both  $\epsilon 4^- > \epsilon 4^+$  and  $\epsilon 4^+ > \epsilon 4^-$  contrasts were run at each time point.

Seven regions of interest (ROIs) were selected to be analyzed in SPSS for changes in FA from T1 to T2. They included the left parietal lobe, left middle frontal gyrus, right cingulum of the corpus callosum, left parahippocampal gyrus, right superior temporal lobe, left superior temporal lobe, and the right inferior frontal gyrus. Spherical ROIs were created in MarsBar (<http://marsbar.sourceforge.net/>) each with a 5mm radius. From the ROIs, FA values were extracted from each participant. These values were then entered into SPSS for further analysis. In SPSS repeated measures general linear models were run to test the 2x2 ( $\epsilon 4$  status x time point) mixed ANOVA.

## Results

Whole brain analysis was completed for T1 and T2 separately. Overall,  $\epsilon 4$  noncarriers tended to have greater FAs than carriers when controlling for age and intracranial volume at both time points. See Figure 2. For,  $\epsilon 4$  noncarriers had greater FA than noncarriers in more regions within the prefrontal, parietal, occipital cortices and the cerebellum compared to carriers at both time points. Meanwhile,  $\epsilon 4$  carriers tended to have greater FA than noncarriers in areas on and surrounding the corpus callosum as well as in the cingulum bundle. These effects were more numerous in T2 than T1. Seven ROIs were selected based on regions of significant difference between APOE noncarriers and carriers. They included the following:

- $\epsilon 4^- > \epsilon 4^+$  at T1: left parietal lobe, left parahippocampal gyrus, right superior temporal lobe, right inferior frontal gyrus
- $\epsilon 4^+ > \epsilon 4^-$  at T1: right cingulum, left superior temporal lobe, and left middle frontal gyrus

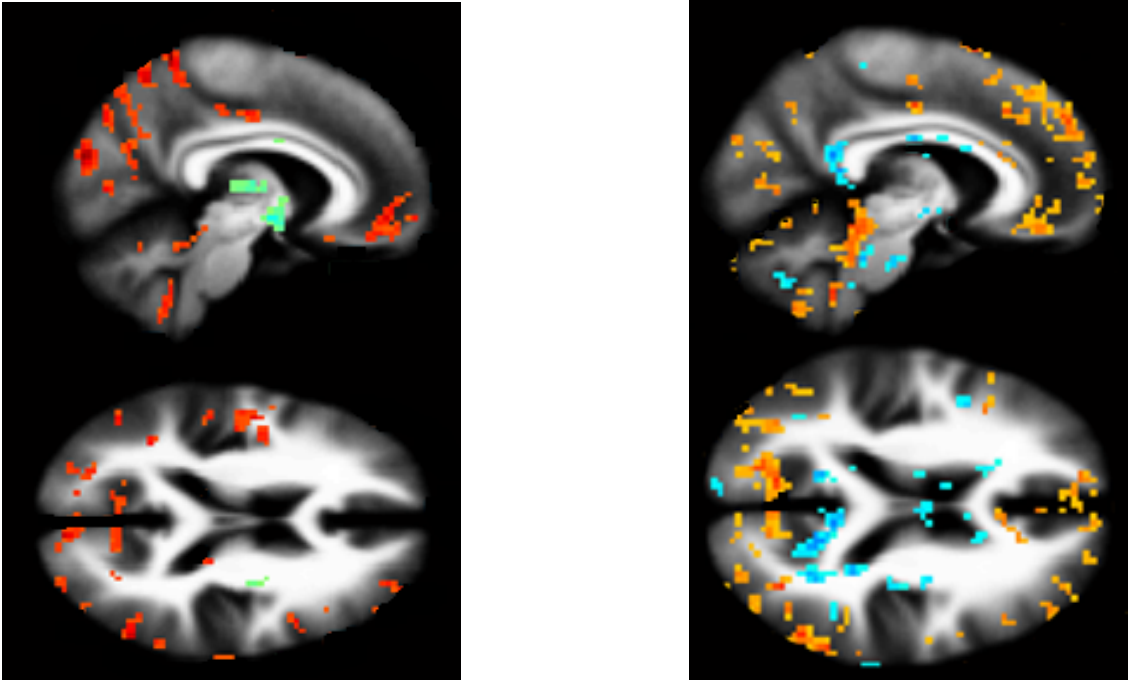


Figure 2. Regions of significant difference in FA at T1 (left) and T2 (right). Warm colors =  $\epsilon 4^- > \epsilon 4$ . Cool colors =  $\epsilon 4^+ > \epsilon 4^-$ .

### *Longitudinal effects*

Based on 2x2 ( $\epsilon 4$  status x time point) mixed ANOVAs, in ROIs that had greater FA in  $\epsilon 4^-$  noncarriers compared to  $\epsilon 4^+$  at T1, there were no significant differences in change of FA from T1 to T2 between  $\epsilon 4$  groups (left parietal lobe:  $F(1, 51) = 2.70, p = 0.11$ ; left parahippocampal gyrus:  $F(1, 51) = 2.69, p = 0.10$ ; right superior temporal lobe  $F(1, 51) = 0.72, p = 0.40$ ; and the right inferior frontal gyrus  $F(1, 51) = 0.24, p = 0.63$ ).

In contrast, ROIs that had higher FA for  $\epsilon 4^+$  compared to  $\epsilon 4^-$  at T1 showed longitudinal differences in FA change between APOE groups, except the cingulum ( $F(1, 51) = 1.04, p = 0.31$ ). The left superior temporal lobe ( $F(1, 51) = 4.94, p = 0.05$ ) displayed an unusual significant increase in FA values over time for  $\epsilon 4^-$ , while there was no significant decrease in FA values for  $\epsilon 4^+$ . Likewise, the left middle frontal gyrus ( $F(1, 51) = 3.38, p = 0.07$ ) showed a marginal

effect, such that there was no change in  $\epsilon 4+$  FA, but there was an increase in FA for  $\epsilon 4-$  over time. See Figures 3 and 4.

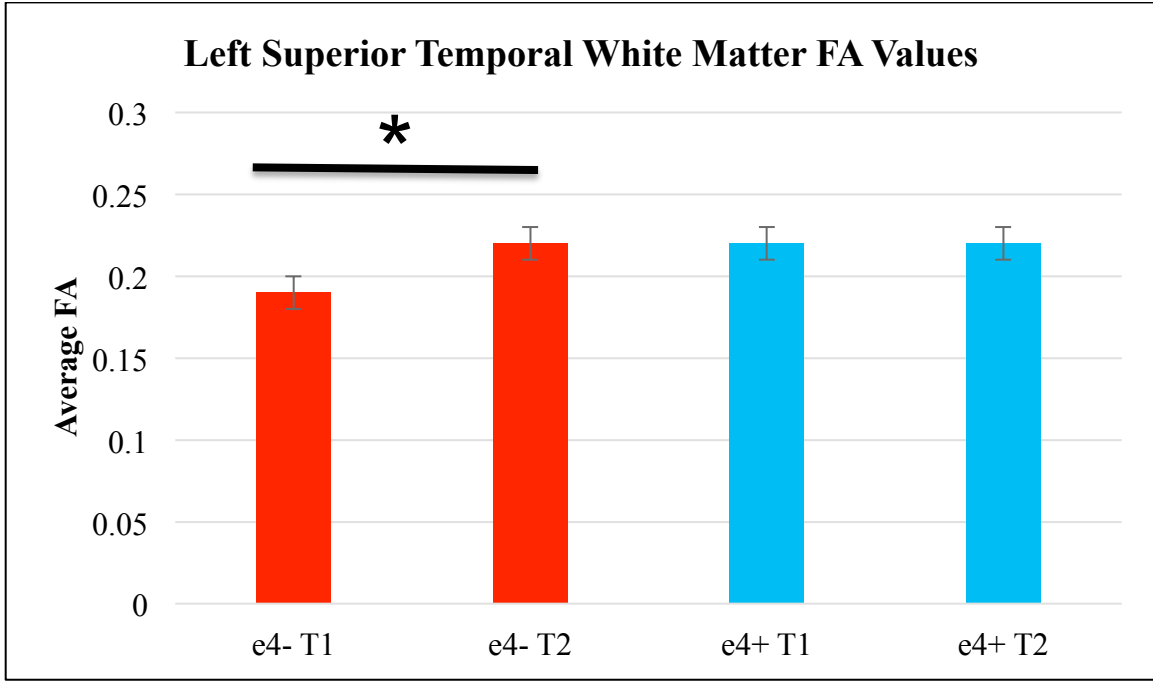


Figure 3. APOE  $\epsilon 4$  noncarriers had significant increases in FA in the left superior temporal white matter over time while  $\epsilon 4$  carriers did not change over time ( $F(1, 51) = 4.94, p = 0.05$ ).

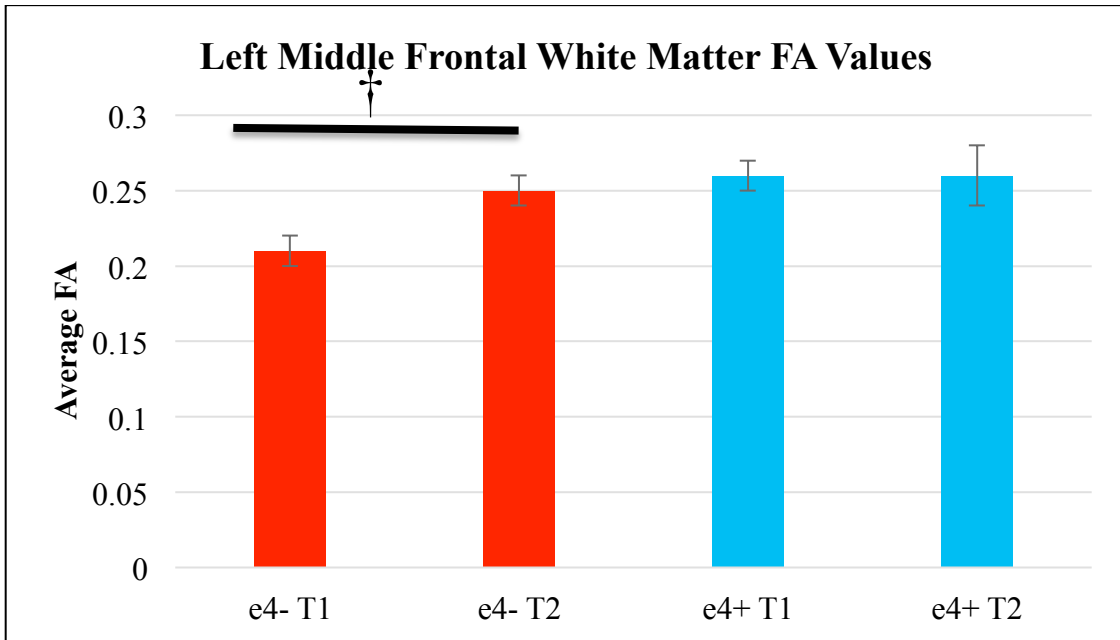


Figure 4. FA marginally increased in  $\epsilon 4$  noncarriers over time while remaining relatively stable in  $\epsilon 4$  carriers in the left middle frontal white matter ( $F(1, 51) = 3.38, n = 0.07$ ).

## **Discussion**

It is well documented that APOE  $\epsilon 4$  status increases risk for AD. For example, Huang et al. (2004) explored whether the risk of dementia and AD was due to a positive FH and the APOE gene over a longitudinal study lasting 6 years. Of the 907 participants in the study (aged 75 years or older), 31.1% reported a family history of dementia and 203 of the 265 people who later developed dementia, later developed AD. It was observed that there was a combined effect of FH and APOE  $\epsilon 4+$  on the risk of AD, demonstrating a higher risk in  $\epsilon 4+$  participants who had two or more first-degree relatives with dementia. Overall, having both FH and the APOE  $\epsilon 4$  allele indicated notable effects on the risk of AD. Participants who had two or more first-degree relatives or siblings with dementia significantly increased the risk of dementia in carriers of the APOE  $\epsilon 4$  allele. In contrast, APOE  $\epsilon 4-$  with positive FH of dementia was not associated with increased AD risk.

We selected seven regions of interest (ROIs) to investigate for changes in FA over time. Four regions were those in which  $\epsilon 4$  noncarriers had significantly greater FA compared to  $\epsilon 4$  carriers. This included the left parietal lobe, left parahippocampal gyrus, right superior temporal gyurs, and the right inferior frontal gyrus. The three remaining regions were taken from areas in which  $\epsilon 4$  carriers had greater FAs compared to noncarriers, specifically the left middle frontal gyurs, the right cingulum of the corpus callosum, and the left superior temporal lobe.

Contrary to our hypothesis, across groups, white matter integrity did not always decrease over time. In regions like the left parietal lobe, the left parahippocampal gyrus, the right superior temporal lobe, and the right inferior frontal gyrus there were no significant differences in change of FA over time between the  $\epsilon 4$  carriers and noncarriers. However, there were some significant differences in change of FA over time between  $\epsilon 4$  carriers and noncarriers where FA was greater

in carriers compared to noncarriers at T1. The right cingulum of the corpus callosum showed no significant difference in FA values over time. However, the left superior temporal lobe significantly increased in FA value over time for  $\epsilon 4$  noncarriers, while displaying no significant change in FA values for  $\epsilon 4$  carriers. This observation differed from my original hypothesis in that FA values across the brain over time would decrease in both  $\epsilon 4$  carriers and non-carriers, but significantly more in  $\epsilon 4+$ . Likewise, a marginal effect was seen in the left middle frontal gyrus, showing no change in  $\epsilon 4$  carriers but an increase in FA values over time for  $\epsilon 4$  noncarriers. Increases in FA over time were unexpected, and it is not clear whether this is a result of actual increased efficiency in  $\epsilon 4$  noncarriers or a methodological error.

A number of studies have shown opposing findings to what was produced in this study. The findings of Westlye et al. (2012) saw no direct associated risk of developing AD due to the allelic effects of APOE. Similar to the present study, Westlye et al. (2012) used diffusion weighted imaging in order to test differences in white matter integrity between  $\epsilon 4$  carriers and non-carriers. They observed decreases in FA values as well as increases in radial diffusion between carriers of the  $\epsilon 2/\epsilon 3$  alleles compared with  $\epsilon 3/\epsilon 3$  carriers, contrary to previous literature stating that individuals with an  $\epsilon 2$  variant of APOE show moderately protective effects against AD. In addition, it was found that  $\epsilon 2$  and  $\epsilon 4$  carriers showed similar differences in functional connectivity compared to  $\epsilon 3$  homozygotes, which may indicate that allelic effects of APOE in healthy adults do not necessarily translate into similar differences in diffusion. However, it was later stated that few  $\epsilon 2/\epsilon 3$  carriers and a wide age range of participants were limitations to the study.

Furthermore, in a study conducted by Okonkwo, et al. (2012) which longitudinally explored GM atrophy and cognitive declines based on two AD risk factors, APOE  $\epsilon 4$  and family history

(FH) of AD, neither FH nor APOE  $\epsilon 4$  independently contributed to declines in regional GM or cognition. Instead, FH was associated with increased GM atrophy in the right posterior hippocampus of APOE  $\epsilon 4^-$ , whereas, this effect was not present for APOE  $\epsilon 4^+$  (Okonkwo, et al, 2012). This suggests that future studies, including those investigating white matter integrity, should take into account multiple risk factors for AD. Compared to the present study, however, these individuals were younger (average age: 54 years) which may also be driving the interactions between AD risk factors, and it may be that in later life APOE and FH of AD do have independent effects on brain structure.

In a study of a broader age-range of 273 participants (i.e., those aged 55-95 years), Nyberg et al. (2014) examined the APOE effects on white matter using DWI. Tract-based analyses of diffusion did not reveal significant APOE effects nor any significant interactions between the genotype and the participants' age. Overall, FA values showed no significant evidence that APOE  $\epsilon 4$  carriers had changes in WM microstructure. While voxelwise analysis showed  $\epsilon 4$  related WM decreases in the anterior and posterior midline regions, this did not "meet whole-brain corrections for multiple comparisons," suggesting less robust effects of APOE on WM than have been suggested in past studies.

Nevertheless, many studies have supported the notion that  $\epsilon 4$  carriers have poorer WM integrity compared to noncarriers. Persson et al. (2006) observed lower FA values in  $\epsilon 4$  carriers compared to noncarriers in both the medial temporal lobe and the posterior corpus callosum using diffusion-tensor imaging. In contrast, the present study found lower FA in the outer areas of the corpus callosum for  $\epsilon 4$  noncarriers compared to carriers. Interestingly, Persson and colleagues found similar patterns in both young and old subjects (the age range was between 49

and 79 years), implying that APOE may influence brain structures beginning from a younger age than what is expected in patients of early onset dementia.

Furthermore, in a study conducted by Alduru, et al., (2014), FH and APOE  $\epsilon$ 4 status was assessed through a cross-sectional study on white matter microstructure in cognitively healthy late middle-aged adults (aged 47-67 years). Using a large sample size of 343 participants who underwent diffusion imaging, the authors examined regions like the medial and lateral parietal, medial and lateral temporal, and inferior frontal cortices along with the fiber tracts that connect them such as the uncinate, fornix, cingulum, and the corpus callosum. Similar to the earlier findings of Okonkwo et al. (2012; the same research group), there were no sole effects of APOE  $\epsilon$ 4 on white matter microstructure, but there were interactions between APOE  $\epsilon$ 4 and age on mean diffusivity (MD) in the superior longitudinal fasciculi, a region thought to be affected in AD and mild cognitive impairment, which carries fibers that connect parts of the cerebrum to each other, including the frontal, occipital, parietal, and temporal lobes. Changes in MD were also seen in the cingulum bundle, a region that contains fibers that connect association cortices in the frontal, parietal, and temporal lobes, thalamus, insular cortices, and the hippocampal formation. This MD interaction showed that older participants (over 65 years of age) who were  $\epsilon$ 4 carriers had higher MD. This region is thought to be affected in AD and mild cognitive impairment, which carries fibers that connect parts of the cerebrum to each other, including the frontal, occipital, parietal, and temporal lobes. Unexpectedly, a higher FA was seen in participants with a greater risk of developing AD based on FH. The authors attribute this to a possible decrease in the number of frontal white matter nerve fibers, a degenerative process that is inversely accompanied by increased myelination (Alduru, et al., 2014). Rhesus macaque monkeys in a study conducted by Bowley et al., (2010) showed a loss of axons but a preservation

of the myelin sheaths that surround the axons, which could possibly result in higher FA. This may be what we are observing in the present study in regions, like the right cingulum of the corpus callosum, the left superior temporal lobe, and the left middle frontal gyrus, where  $\epsilon 4$  carriers had higher FA than noncarriers at T1.

Taken together, further examination of the APOE gene will aid in the future understanding of the contributions of other AD-risk genes on white matter microstructure (Alduru, et al. 2014). While, the importance of APOE for long-term potentiation, synaptic development, dendrite formation, and axonal guidance, as well as interacting with several age-related pathogens including the  $\beta$ -amyloid accumulation and clearance has been suggested (Westlye et al. 2012), evidence for these effects on a larger macrostructure (e.g., white matter integrity) are mixed. The present study demonstrated the importance of neuroimaging methods such as diffusion tensor imaging, for detecting white matter alterations in carriers of the APOE  $\epsilon 4$  allele (Alduru et al. 2014). However, diffusion imaging has limitations, such as the inability to take into account crossing fibers within a voxel which may be misrepresented as having low white matter integrity.

In addition, this study indicated the importance of conducting a longitudinal study that compared two different time points, T1 and T2, to examine the change in white matter integrity throughout a certain length of time. Based on this study, it is not certain if white matter integrity is degenerating at a quicker rate in APOE  $\epsilon 4$  carriers compared to noncarriers as mentioned in the Ryan et al., (2011) study, therefore, longitudinal studies with greater time intervals will be useful in shedding light on this process. Furthermore, Alexopoulos et al. (2011) reported that even healthy young APOE  $\epsilon 4$  carriers have smaller hippocampal volumes than  $\epsilon 2$  carriers. Although this difference is not seen in cognitive functions, it could indicate that degenerative properties of the APOE  $\epsilon 4$  allele are later exposed in earlier adulthood. On the other hand, Khan

et al., (2014) acquired MRI images from approximately 1,000 healthy adolescents (with a mean age of 14 years), which was a much larger sample size compared to the Alexopoulos et al., (2011) study, and found no hippocampal volume differences between carriers and noncarriers of APOE  $\epsilon 4$  or  $\epsilon 2$  alleles. Finding no differences between  $\epsilon 2$  and  $\epsilon 4$  carriers regarding hippocampal volumes or cognitive functions could also confirm the fact that effects of the APOE  $\epsilon 4$  allele are not seen until later adulthood. Therefore, the aforementioned studies confirm the importance of conducting a longitudinal study to examine the effects of APOE  $\epsilon 4$  neurodegeneration over a longer span of time. Perhaps, however, by older adulthood carriers and noncarriers show equal declines in brain structure over time as is largely suggested from our findings.

This study was faced with some limitations. First, limitations of diffusion tensor imaging could have resulted in skewed FA values, slight changes in the MRI scanner over time may have impacted our results. However, we would expect the influence to be spread equally across groups. This may be part of the reason why we sometimes saw increases in FA overtime across both groups. Another potential limitation was our small sample size of only 53 people, as well as studying particular ROIs over time rather than whole brain analysis from T1 to T2. Demographic information, not controlled for, like gender, may have also had an effect on the results (e.g., perhaps gender interacts with  $\epsilon 4$  status to effect white matter integrity differentially for men versus women). Lack of longitudinal data on males makes this difficult to explore.

After closely examining the physiological effects of carrying the APOE  $\epsilon 4$  allele in terms of white matter integrity, an ideal future step would be to study the cognitive effects that the  $\epsilon 4$  allele has on AD-related functional symptoms. Therefore, future directions would involve taking the results from this study and longitudinally exploring how this would affect cognitive functions (e.g., performance on memory tasks) to find out if there is a correlation between region specific

degeneration and declines in cognitive dysfunction. Similar work has been conducted by Ryan et al. (2011), where two measures of diffusion, apparent diffusion coefficient (ADC) and FA, were used to examine executive function and memory function. The results found that frontal diffusion were more closely associated with a decline in executive function across both  $\epsilon 4$  carriers and noncarriers, because changes in frontal lobe diffusion typically come as a result of normal, healthy aging and not specifically as a result of being an  $\epsilon 4$  carrier. On the other hand, it was observed that APOE  $\epsilon 4$  related white matter degeneration affected the temporal lobes because degeneration in that specific region is more closely associated with the presence of the  $\epsilon 4$  allele (Ryan, et al., 2011). However, this study did not capture the associations between white matter integrity and rates of cognitive decline over time.

In summary, contrary to my hypothesis, across groups, white matter integrity did not always decrease over time. This may be related to undetected axonal changes that are being repaired through extra myelination, causing an increase in FA values (Bowley et al., 2010). Overall, more regions of the brain had higher white matter integrity for  $\epsilon 4$  noncarriers than carriers. Unexpectedly, in the regions we chose to investigate for changes in white matter integrity over time, the rates of FA decline/incline did not typically differ between groups. Surprisingly, when rate of change in FA did differ over time it was such that  $\epsilon 4$  noncarriers' white matter integrity increased from T1 to T2 while  $\epsilon 4$  carriers' FA remained relatively unchanged. Moreover, the regions showing this effect were those that were originally higher in FA for  $\epsilon 4$  carriers compared to noncarriers at T1. Limitations to the present study were a small sample size and limitations in diffusion imaging techniques. For example, DWI does not take into account crossing fibers, and may underestimate FA in voxels that contain crossing fibers. In future analyses, we hope to

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examine longitudinal effects of APOE  $\epsilon$ 4 on cognitive functions in order to confirm a correlation between region specific degeneration in the brain and a decline in cognitive dysfunction.

## References

- Adluru N, Destiche DJ, Yuan-Fu Lu S, et. al. (2014). White matter microstructure in late middle-age: Effects of apolipoprotein E4 and parental family history of Alzheimer's disease. *NeuroImage: Clinical* 4: 730–742.
- Alexopoulos, P., Richter-Schmidinger, T., Horn M., et al., (2011). Hippocampal Volume Differences Between Healthy Young Apolipoprotein E e2 and e4 Carriers., *Journal of Alzheimer's Disease* 26: 207-210.
- Bendlin BB, Carlssona CM, Gleason CE, et. al. (2009). Midlife predictors of Alzheimer's disease. *Maturitas* 65: 131–137.
- Bowley M, Cabral H, Rosene DL, Peters A, et al., (2010). Age changes in myelinated nerve fibers of the cingulate bundle and corpus callosum in the rhesus monkey. *Journal of Comparative Neurology*: 518(15): 3046-3064.
- Corder EH, Saunders AM, Risch NJ, Strittmatter WJ, Schmechel DE, Gaskell PC Jr, et al. (1994). Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. *Nat Genet*; 7(2): 180-4.
- de la Torre, JC, (2015). Do we try mending Humpty Dumpty or prevent his fall? An Alzheimer's Disease Dilemma, *Journal of Alzheimer's Disease*, 1-6.
- Huang W, Qiu C, von Strauss, Eva, et al., (2004). APOE Genotype, Family History of Dementia and Alzheimer's Disease Risk, *Archive Neurology*, 61: 1930-1934.
- Irish M, Hornberger M, Wahsh SE., (2014). Grey and White Matter Correlates of Recent and Remote Autobiographical Memory Retrieval – Insights from the Dementias, *PLOS One* 9(11): 1-14.
- Jiang, T., Chang RC-C., Rosenmann, H., Yu, J-T., (2014). Advances in Alzheimer's Disease: From Bench to Bedside. *Hindawi*: vol. 2015: 1-3.
- Khan W, Giampietro V, Ginestet C, et al., (2014). No Differences in Hippocampal Volume between Carriers and Non-Carriers of the ApoE e4 and e2 Alleles in Young Healthy Adolescents, *Journal of Alzheimer's Disease* 40: 37-43.
- Lee Y-M, Ha J-M, Lee B-D, et al., (2014). Impact of Apolipoprotein E4 Polymorphism on the Gray Matter Volume and the White Matter Integrity in Subjective Memory Impairment without White Matter Hyperintensities: Voxel-Based Morphometry and Tract-Based Spatial Statistics Study under 3-Tesla MRI, *Jornal of Neuroimaging* 00: 1-6.

- Lockhart SN, Decarli C, (2014). Structural imaging measures of brain aging. *Neuropsychology Rev* 24: 271 – 289.
- Maier-Hein KH, Westin CF, Shenton ME, et al., (2014). Widespread white matter degeneration preceding the onset of dementia. *Alzheimer's & Dementia*. 1-9.
- National Institutes of Health (NIH). (2011). National Institute on Aging: Alzheimer's Disease Education and Referral Center. *Alzheimer's Disease Genetics Fact Sheet*; (11)1-8.
- Nyberg L, Salami A, (2014). The APOE e4 allele in relation to brain white-matter microstructure in adulthood and aging, *Scandinavian Journal of Psychology*, 55: 263-267.
- Okonkwo OC, Xu G, Dowling NM, Bendlin BB et al., (2012). Family history of Alzheimer disease predicts hippocampal atrophy in healthy middle-aged adults, *Neurology* 78(22): 1769-76.
- Persson J, Lind J, Larsson M, et al., (2006). Altered brain white matter integrity in healthy carriers of the APOE e4 allele: A risk for AD?, *Neurology*, 66: 1029-1033.
- Ryan, L., Walther, K., Bendlin, B.B., Lue L-F., Walker, D.G., & Glisky, E.L. (2011). Age-related differences in white matter integrity measured by diffusion tensor imaging and cognitive function are related to APOE status, *NeuroImage*, 54(2), 1565-77.
- Ryan, L., Walther, K., (2014). White Matter Integrity in Older Females is Altered by Increased Body Fat, *Obesity* 00(00): 1-8.
- Van Duijn CM, Clayton D, Chandra V, Fratiglioni L, Graves AB, Heyman A, et al., (1991). Familial aggregation of Alzheimer's disease and related disorders: a collaborative re-analysis of case-control studies. *Int J Epidemiol*; 20 Suppl 2: 13-20.
- Weintraub, S., Wicklund AH., Salmon, D., (2012). The Neuropsychological Profile of Alzheimer's Disease. *Cold Spring Harb Perspect Med* 2: 1-18.
- Westlye, LT., Reivang, I., Rootwelt, H., Espeseth, T., (2012). Effects of the APOE on brain matter microstructure in healthy adults, *Neurology* 79: 1961-1969.