THE ROLE OF BIOMARKERS, WHITE MATTER MICROSTRUCTURE, AND EDUCATION ON MEMORY PERFOMANCE IN OLDER ADULTS WITH VARYING COGNITIVE STATUSES

by

KHARINE R. JEAN-BUISSERETH

(Under the Direction of L. Stephen Miller)

ABSTRACT

APOE4 and phosphorylated tau (p-tau) are two biomarkers associated with Alzheimer's disease (AD) and poorer memory performance in older adults. Examination of the mechanisms by which these biomarkers impact memory is of particular importance in individuals with varying cognitive function. Given APOE4 and p-tau's likely impact on axonal integrity, there is evidence that suggests these biomarkers may play a role in affecting white matter microstructure (WM). The current study was interested in understanding the role of white matter microstructure in mediating the relations between APOE4 and memory. There is some evidence to suggest that APOE4 and p-tau interact such that individuals with both high genetic and high biological risk exhibit worse performance on memory tasks. Our study proposes that one manner in which APOE4 and p-tau interact to impact memory is through the degradation of white matter microstructure in WM tracts important for memory (e.g., hippocampal cingulum, fornix). Increased education has been found to buffer individuals at greater genetic and biological risk from cognitive impairment relative to those with low educational level; investigating the role of education in potentially buffering the effects of significant biomarker

burden is also of great importance for the current study. Understanding these relations provides further understanding of the mechanisms by which genetic and biological risk factors impact memory and helps to explain some of the heterogeneity that exists in the presentation of Alzheimer's disease.

INDEX WORDS: APOE4, P-Tau, White Matter Microstructure, Education, Alzheimer's Disease, Mild Cognitive Impairment

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KHARINE R. JEAN-BUISSERETH

BS, University of Georgia, 2015

MS, University of Georgia, 2019

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2022

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KHARINE R. JEAN-BUISSERETH

Major Professor:	L. Stephen Miller
Committee:	Kerstin Gerst Emerson
	Gregory Strauss

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
August 2022

DEDICATION

This dissertation document is dedicated to my mother, Carline Jean, and my father, Rene Jean, who have helped me to see further by allowing me to stand on their shoulders. I also want to dedicate this to my husband, who has remained my safe place throughout this process. This document is also dedicated to my siblings, aunts, uncles, cousins, nieces, nephews, godchildren, friends, and church community. My community has supported me in so many ways throughout my life and particularly during my graduate school years, and for that I am thankful.

ACKNOWLEDGEMENTS

I would like to acknowledge my amazing advisor, L. Stephen Miller, who has played a major part of my development within the field of neuropsychology. I would also like to acknowledge my lab mates throughout the years who have supported me both professionally and personally. I would like to acknowledge my committee members for their helpful critiques and guidance. Special acknowledgement to Violeta Rodriguez for her statistical support.

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CHAPTER 1

INTRODUCTION

Older adults aged 65 years and over are a growing population, and this age group is expected to make up approximately 20% of the population in the U.S by the year 2030 (Vespa, Armstrong, & Medina, 2018). With the increased life expectancy and the increase in older adult population, it is important to consider the increased risk of negative health outcomes that are associated with older age (Rockwood et al., 2008). For example, the risk of neurodegenerative disorders, such as Alzheimer's disease (AD), increases in older adulthood (Lyketsos, Chen, & Anthony, 1999). It is important to note that most older adults do not experience significant cognitive decline outside of normal age-related cognitive changes. However, identification of older adults who may be at higher risk of neurodegenerative disorders is important. Understanding how genetic, biological, and modifiable environmental risk factors interact to affect the development of Alzheimer's disease and influence its progression is of considerable interest for treatment and prevention.

APOE

A genetic biomarker of special interest has been apolipoprotein e (APOE), a blood protein involved in transporting cholesterol, triglycerides, and other fats throughout the body and plays an important role in how these fats are metabolized (Gregg et al., 1986). There are three main types of apolipoprotein E alleles: e2, e3, and e4, which have a worldwide prevalence rate of approximately 8.4%, 77.9%, and 13.7%, respectively (Liu, Kanekiyo, Xu, & Bu, 2013). Evidence suggests the e2 allele may be neuroprotective against AD and progression from mild

cognitive impairment (MCI) to AD by increasing amyloid-beta clearance in the brain (Conejero-Goldberg, et al., 2014; Reiman et al., 2020). APOE2 homozygotes had an 87% lower Alzheimer's dementia odds ratio than APOE3 homozygotes and a 99.6% lower AD odds ratio than APOE4 homozygotes in a recent 5,000-person neuropathological study (Reiman et al., 2020). The e2 allele has also been associated with reduced cardiovascular risk disease (Lopez, Krastins, & Ning, 2014). Although mixed, there is also evidence suggesting the e2 allele is associated with negative cerebrovascular outcomes, such as increased white matter hyperintensity load and risk of brain infarcts (Anand et al., 2009). The e3 allele is the most common allele and has generally not been related to diseases in the human population and is thought to be neither protective nor a risk factor for the development of disease (Lopez, Krastins, & Ning, 2014; Davignon, Gregg, & Sing, 1988).

The apolipoprotein e4 allele, however, has generally been found to have a negative influence on older adults' health, as it has been associated with increases in amyloid plaques (i.e., protein clumps) within the brain (Davignon et al., 1988; Caselli, Walker, Sue, Sabbagh, & Beach, 2010). In a sample of middle-aged adults, a significant linear trend was found such that as the number of e4 alleles increased, the cortical beta amyloid burden increased proportionally (Mecca et al., 2018). The increase in these toxic plaques (i.e., beta amyloid plaques) in the brain can lead to neuronal death (Mahley & Rall, 2000). When compared to the e3 allele, it is thought the e4 allele is less effective at transporting brain cholesterol which may have significant negative consequences on central nervous system cholesterol homeostasis and neuronal health (e.g., synaptic activity, neuroplasticity; Liu et al., 2013). Positive e4 status (i.e., carriers of the e4 allele) has been related to decreased synaptic activity, vascular function, neurogenesis, and increased risk for dementia and Alzheimer's disease (Liu et al., 2013; Slooter, et al., 1997,

Bétard, et al., 1994). A meta-analysis examining the prevalence estimates of the e4 allele in individuals with Alzheimer's disease found a worldwide overall estimate prevalence rate of 48.7% and homozygote prevalence rate of 9.6% (Ward et al., 2012). These estimates were significantly heterogeneous and varied upon region, definition of AD, age, and where the sample was collected (e.g., community sample vs clinic sample). E4 status has been related to AD risk in a dose-dependent manner, with homozygotes (carrying 2 alleles) exhibiting much higher increased risk and earlier onset of AD than e4 heterozygotes (carrying only 1 allele; Liu et al., 2013). More specifically, heterozygotes have an approximately three-fold increased risk of developing AD and e4 homozygotes have approximately an eight to fifteen-fold increased risk of developing AD (Goltermann et al., 2019).

APOE4 and Cognitive Function in Healthy and Pathological Aging

E4 carriers have been found to have worse cognitive function than those without the allele (Perna, Mons, Rujescu, Kliegel, & Brenner, 2016; Wisdom, Callahan, & Hawkins, 2011). E4 allele presence alongside the presence of other environmental factors (e.g., cardiovascular disease, lower education, lower literacy level) increases the risk of cognitive decline in older adults (Perna et al., 2016; Kaup et al., 2015). A meta-analysis by Wisdom and colleagues (2011) found that cognitively healthy older adults with the e4 allele exhibited worse cognitive performance on tasks related to episodic memory, executive functioning, perceptual speed, and overall global cognitive ability (Wisdom et al., 2011). In a sample of older adults with amnestic MCI, Alzheimer's disease, and healthy controls, APOE4 carriers had lower scores on tasks of global cognitive functioning and functional activities (Kerchner et al., 2014). A review by O'Donoghue and colleagues (2018) suggest that while the effect of APOE4 on cognition in healthy adults is controversial, generally the presence of the e4 allele on cognition is negative

and is likely due to multiple neurobiological mechanisms which need to be further investigated (O'Donoghue, Murphy, Zamboni, Nobre, & Mackay, 2018).

Episodic memory is one of the first and most prominent signs of Alzheimer's dementia and thus APOE4's impact on memory, specifically, has been examined (Kerchner et al., 2012). In individuals with subjective cognitive concerns, e4 carriers had worse baseline memory and accelerated memory decline (Samieri et al., 2014). Kerchner and colleagues (2014) found that e4 carriers performed worse on a composite measure of episodic memory, although their performance on other cognitive domains were similar to noncarriers' performance. This effect was dose-dependent, with increased number of the e4 allele being related to worse episodic memory performance. These findings were similar for healthy controls and those with amnestic MCI and Alzheimer's disease. In a sample of healthy older adults with increased risk for AD due to high beta amyloid burden, APOE4 carriers showed a moderately faster decline on memory tasks after a 54-month time period (Lim et al., 2015). This suggests that APOE4 carriership exacerbates high beta-amyloid burden's effect on memory. Across the Alzheimer's and Lewy Body Dementia spectrum, Saeed and colleagues (2018) found that e4 carriers were significantly impaired on long-delay free recall of words (Saeed et al., 2018). They also found that APOE4 was associated with smaller hippocampal volumes in a dose dependent manner and global learning was associated with hippocampal volumes only among e4 positive individuals (Saeed et al., 2018). This finding suggests APOE4 status has effects on brain structure which may impact cognitive performance.

Phosphorylated Tau

The neuropathology of Alzheimer's disease is significant for abnormal hyperphosphorylation of the tau protein. Normally, tau protein is important for microtubule

formation, playing a role in the stabilization of neuronal microtubules in the development of cell processes and intracellular support (Buerger et al., 2006; Mandelkow & Mandelkow, 1998). Hyperphosphorylation of the tau protein is engaged in the detachment of tau from microtubules. It is suggested that the mechanisms by which phosphorylated tau negatively impacts brain structure in Alzheimer's disease is potentially through the breakdown of microtubules in neurons. Given hyperphosphorylated tau's lack of microtubule-promoting activity, it interacts with normal tau and reduces normal tau's ability to assemble tubulin into microtubules which can lead to the aggregation of p-tau and the formation of neurofibrillary tangles and synaptic damage (Alonso, Zaidi, Grundke-Iqbal & Iqbal, 1994; Buerger et al., 2006; Mandelkow & Mandelkow, 1998; Rajmohan & Reddy, 2017). Total tau and phosphorylated tau in CSF provide valuable information for the diagnosis of Alzheimer's disease and differential diagnosis from other neurodegenerative disorders (e.g., frontotemporal dementia, Lewy body dementia; Hampel et al., 2010). In AD, CSF p-tau predicts the rate of cognitive decline at different stages of the disease (Hampel et al., 2010). CSF p-tau reliably predicted progression to AD in a European sample of MCI individuals with an accuracy rate of 80% (Ewers et al., 2007).

Regarding APOE4 and p-tau, APOE4 is thought to be related to increased cellular tau phosphorylation and enhances the release of phosphorylated tau (Wadhwani, Affaneh, Van Gulden, & Kessler, 2019). More information about the interaction between APOE4 and CSF tau is needed. Koch and colleagues (2017) found that when compared to e4 non-carriers, CSF tau negatively impacted cortical plasticity, led to faster AD progression, and led to faster cognitive decline in e4 carriers (Koch et al., 2017). Animal models suggest APOE4 may cause tau-dependent impairment of hilar GABAergic interneurons which impact the learning and memory

in mice and thus help explain memory and learning deficits in AD (Andrews-Zwilling et al., 2010).

White Matter Microstructure

Given APOE is the major lipids distributor in the central nervous system and tau is important in microtubule integrity, both APOE and tau play a vital role in the promotion (e.g., formation of synapses, microtubule formation) and/or protection (e.g., repair of acute CNS trauma) of neurons, as APOE is secreted by astrocytes and provides delivery of important lipids for neuronal health and tau promotes axonal and synaptic plasticity in the developing brain but becomes pathological in the adult brain (Liu et al., 2013; Mahley, Weisgraber, & Huang, 2006). Given the role of APOE and tau in axonal health, examination of white matter fibers (i.e., bundles of axons) and their relation to these biomarkers is of great interest. A method used to examine the integrity of white matter tracts is diffusion tensor imaging (DTI). DTI measures the movement of water molecules amongst the axons of the brain to determine the integrity of the white matter tracts. Diffusion-weighted images are collected across time and within various orientations to determine the directionality and magnitude of water movement within different regions of the brain. These measurements are then paired with a mathematical model (e.g., diffusion tensor model) to describe the diffusion within each voxel and are fit to a tensor, an ellipsoid that is characterized by its three eigenvectors (v1, v2, v3) and their associated eigenvalues ($\lambda 1$, $\lambda 2$, and $\lambda 3$; Figure 1; Madden & Parks, 2016). Eigenvectors provide information about the orientation of the tensor whereas eigenvalues describe the strength of diffusion along the associated axes and can be combined to calculate different metrics of anisotropy and diffusion (Madden & Parks, 2016; Melhem et al., 2002; Rajagopalan Jiang, Stojanovic-Radic, Yue, & Pioro, 2017). $\lambda 1$ represents the rate of diffusion along or parallel to the orientation of the

fibers while λ2 and λ3 provide information about the rate of diffusion perpendicular to the axonal fibers (Mukherjee, Berman, Chung, Hess, & Henry, 2008).

Within healthy white matter tracts, water molecules primarily move in an anisotropic manner, meaning that water flows in one direction. Abnormally high levels of isotropic movement (i.e., water molecules moving diffusely in all directions) is considered a possible indication of degradation of the white matter tract (Chanraud, Zahr, Sullivan, & Pfefferbaum, 2010). DTI uses four main metrics comprised of λ1, λ2, and λ3 to characterize anisotropy and diffusion (Figure 1). Fractional anisotropy (FA), is a standard summary measure of white matter integrity, particularly anisotropy, and is calculated using the equation $\sqrt{(\lambda_1^2-\lambda_2)^2+(\lambda_2-\lambda_3)^2+(\lambda_1-\lambda_3)^2} / [2(\lambda_1^2+\lambda_2^2+\lambda_3^2)]$. FA measures the diffusivity of water along the primary axis (v1) running parallel to the tract in relation to the other two axes (v2 and v3) running perpendicular to the tract (Alexander, Lee, Lazar, & Field, 2007; Madden & Parks, 2016). FA values range from 0 to 1, where higher FA values represent greater anisotropy within a region (Chanraud et al., 2010). FA has been shown to be sensitive to white matter microstructural changes (Alexander et al., 2007; Madden et al., 2016). For example, in normal appearing white matter (NAWM) located near white matter hyperintensities (WMH), there was a significant correlation between WMH volume and NAWM FA. After 4 years, FA decreased within NAWM and was related to worse functional outcome (Moscufo et al., 2018). This suggests that DTI FA is sensitive to microstructural changes that are occurring despite white matter appearing normal on conventional MR images.

Mean diffusivity (MD) is another standard summary measure calculated using the equation $(\lambda_1+\lambda_2+\lambda_3)/3$ and represents the average magnitude of diffusion across all three axes within a voxel (Mukherjee et al., 2008). Higher MD is generally representative of greater

diffusivity and isotropy and has been shown to increase overtime in brain regions most impacted by aging in healthy older adults (Mukherjee et al., 2008; Rieckmann et al., 2016).

While MD is considered a sensitive measure of broad pathology, research suggest MD may not have great specificity in detecting white matter reductions in anisotropy which can be caused by several factors such as reduced parallel diffusivity and/or increased perpendicular diffusivity (Alexander et al., 2007, Bartzokis et al., 2012). Radial diffusivity (RD) and axial diffusivity (AxD) are other DTI metrics thought to provide greater directionally specific information which can be useful in determining myelin or axonal integrity (Madden & Parks, 2016). RD reflects diffusivity along the secondary axes (λ_2 and λ_3) and is thought to indicate aspects of myelin integrity, with radial diffusivity increasing with demyelination and decreasing with myelination (Suzuki, Matsuzawa, Kwee & Nakada, 2003; Rieckmann et al., 2016). Axial diffusivity (AxD) reflects diffusivity along the longitudinal or primary axis and is thought to represent axonal integrity. AxD is measured on a normalized scale and ranges from 0 to 1. Currently there is no consensus in the directional relation between axial diffusivity and normal aging, with some studies finding higher AxD values (e.g., Kumar et al., 2013; Operto et al., 2018) and others finding lower or mixed findings for AxD values (Bennet, Madden, Vaidya, Howard, D., & Howard, J., 2010) representing age-related changes in the brain.

Neuroimaging studies have consistently found that white matter degeneration is a neuropathological feature of Alzheimer's disease and progresses over the course of the disease (Acosta-Cabronero, Williams, Pengas, & Nestor, 2010; Steketee et al., 2016). Individuals with AD were found to have lower FA in the cingulum (cingulate gyrus and hippocampus), inferior and superior longitudinal fasciculus, and other WM tracts as compared to controls (Steketee et al., 2016). Chen and colleagues (2020) found that individuals with amnestic MCI and AD

showed higher diffusivity (e.g., MD, RD) in fiber tracts within the left hemisphere compared to healthy controls (Chen et al., 2020). Consistent with these findings, AD was associated with disrupted white matter microstructure (low FA, high MD, AxD, and RD) compared to healthy older adults in widespread regions of interest, including the hippocampus (Mayo et al., 2019). A meta-analysis examining white matter microstructural changes from MCI to AD found that significantly decreased FA in patients with AD were identified in the fornix, hippocampus, cingulate gyrus, cingulate bundle, and other key regions involved in AD pathology (Qin, Guo, McClure, & Mu, 2020). In a longitudinal study examining structural changes in AD, after one year, individuals with AD saw decreased FA and increased MD in the hippocampal cingulum which was not seen in healthy age-matched controls (Mayo et al., 2017). These findings show that AD is associated with microstructural changes in multiple WM tracts (Mayo et al., 2017; Qin et al., 2020)

APOE4 and White Matter Microstructure

The APOE4 allele may be related to microstructural changes in the integrity of the brain's white matter through neuronal toxicity and brain atrophy (Honea, Vidoni, Harsha, & Burns, 2009). APOE4 has been thought to also influence white matter integrity through reduced neural repair, oxidative stress, and by negatively affecting myelin-producing oligodendroglia (Ryan et al., 2011). Compared to non-carriers, reductions in FA in the parahippocampal white matter have been reported in e4 carriers (Nierenberg et al., 2005; Tsao et al., 2014). Decreased FA in the splenium of the corpus callosum, anterior frontal lobe white matter, inferior temporal lobe white matter, hippocampus, and the cingulum have also been observed (Persson et al., 2006). Heise and colleagues (2011) found older adults with the e4 allele exhibited greater MD relative to non-carriers in widespread areas (e.g., corona radiata, cingulum, corpus callosum,

external capsule, internal capsule, and superior longitudinal fasciculus) using whole-brain analysis with tract-based spatial statistics (TBSS; Heise, Filippini, Ebmeier, & Mackay, 2011). Furthermore, the e4 allele has been found to have an additive negative effect on normal age-related changes in white matter integrity (Operto et al., 2018).

For those with the e4 allele, reductions in the integrity of white matter in these areas are consistent with those reported in individuals with MCI, AD, and other dementias (Kantarci et al., 2001; Kantarci et al., 2005; Huang et al., 2007). AD patients have been found to have lower FA values and larger MD values in the parahippocampal and limbic area white matter bilaterally (Kljavevic et al., 2014). This may suggest that normal aging carriers of the e4 allele may be experiencing preclinical pathological changes associated with neurodegenerative disorders. A systematic review by Harrison and colleagues (2020) found that APOE4 carriers had lower FA and increased MD and RD, all suggesting worse white matter microstructure, in multiple white matter tracts (Harrison et al., 2020). Kerchner and colleagues (2014) found that the relation between APOE4 and episodic memory was mediated by CA1-SRLM thinning (Kerchner et al., 2014).

P-Tau and White Matter Microstructure

Phosphorylated tau has been associated with increased rate of hippocampal atrophy progression in individuals with AD, even when controlling for AD severity and duration (Hampel et al., 2005). Therefore, p-tau is a considerable factor in predicting structural disease progression in AD. P-tau has been found to be a significant predictor of changes in white matter connection strength (Kim et al., 2019). Stenset and colleagues (2011) reported decreased FA and increased RD (i.e., worse microstructure) in the cingulum (a region of the brain important for memory) of individuals with subjective cognitive concerns and mild cognitive impairment,

suggesting loss of myelin may contribute to early white matter changes in individuals at risk of developing AD (Stenset et al, 2011). P-tau was found to be significantly related to hippocampus and fornix WM disruption in those with amnestic MCI and mild AD (Magalhaes et al., 2020). Alm and colleagues (2019) found that studies examining the relation between total tau and DTI measures found that there were fewer significant associations reported with FA, but relationships between tau and measures of diffusivity were much more common (Alm & Bakker, 2019). This provides evidence that the mechanism by which p-tau impacts white matter microstructure is likely through impacting radial and axonal integrity of the axon. While there are fewer studies that have examined the relation between p-tau and DTI metrics, greater levels of p-tau has typically been related to lower FA and higher MD in individuals with MCI and cognitively normal individuals within the cingulum, frontal lobe, thalamus, and corpus callosum (Alm & Bakker, 2019).

Overall, it appears that APOE4 and p-tau negatively impact white matter microstructure in healthy and pathological aging. E4 carriership and increased p-tau are related to lowered FA and greater diffusivity within several regions (e.g., hippocampus, fornix, cingulum) that play a key role in memory. Understanding whether white matter microstructure is a manner by which APOE4 and p-tau impact memory in older adults is therefore an area that needs to be further explored.

Role of Cognitive Reserve in Alzheimer's Disease and Cognitive Performance

Cognitive reserve (CR) is the ability to maintain performance despite brain damage or aggregation of neuropathology (Stern, 2013). CR has been found to help explain, in part, the differential development and progression of Alzheimer's disease (Stern, 2013). Cognitive reserve is typically measured with the use of proxy measures (e.g., education, occupational attainment,

intellectual functioning; Stern, 2013). Education has been a proxy measure of cognitive reserve that has been found to be one of the strongest predictors of cognition in older adults (Crowe et al., 2013; Roe, Xiong, Miler, & Morris, 2007). Education has also been found to be related to brain structure. Tang and colleagues (2017) found that education was positively related to subregions of the hippocampus (Tang, Varma, Miller, & Carlson, 2017). For AD patients with a high genetic risk for AD (i.e., APOE4 homozygotes or e4 homozygotes with a family history of AD), lower education was associated with reduced cortical thickness of the hippocampus, subiculum, entorhinal cortex, parahippocampal cortex, and other medial temporal regions (Baumgaertel et al., 2016). In this same study, education was not significantly related to cortical thickness in the medial temporal lobe for individuals with no genetic risk for AD. This suggests that education may be particularly important for those with greater risk of disease burden. In a cross-sectional study examining a sample of participants in predementia stages (i.e., subjective cognitive decline and MCI) and with probable AD dementia, education had a positive effect on cognitive performance in memory, attention, executive functioning, visuospatial ability, and overall global cognitive function, even when controlling for the degree of cerebral atrophy (Groot et al., 2018). This suggests education serves to mitigate cognitive symptoms in AD. For individuals with Alzheimer's disease, education has been found to buffer the effects of tau on cognitive function with highly educated AD patients tolerating greater tau pathology than their lower educated counterparts (Hoenig et al., 2017). Education may also protect against tauopathy in individuals with abnormal cognitive function, as Rolstad and colleagues' (2010) study found that greater education was related to lower concentrations of t-tau in individuals with MCI (Rolstad et al., 2010). However, the moderating role of education on biomarkers of AD still needs to be explored as not all studies have found education to be a significant moderator of AD

symptomology. Bauer and colleagues recently found that the relation between a biomarker composite (i.e., Tau/Aβ_{42} ratio) and a memory composite was not moderated by education (Bauer, Brown, & Gold, 2020).

Current Study

The current study's purpose was to evaluate brain mechanisms underlying the relation between APOE4 and memory, and the relations between APOE4, p-tau, white matter microstructure, and memory performance in individuals diagnosed with subjective cognitive concerns, MCI, or mild Alzheimer's disease. In addition, we sought to evaluate how education may serve to moderate the relations between these genetic and biological factors and how they impact memory. The first aim of this study was to determine whether white matter microstructure within regions involved in memory mediate the relation between APOE4 and memory performance. Our second aim was to determine whether an interaction between APOE4 status and p-tau existed and impacted the mediating role of white matter microstructure. We hypothesized that APOE4 carriers would perform worse on tasks of memory, and that white matter microstructure in regions associated with memory (i.e., fornix and the hippocampal cingulum) would mediate this relation. Furthermore, we proposed that the mediating role of white matter microstructure on the relation between APOE4 and memory would be moderated by p-tau. We hypothesized that individuals with high levels of p-tau who are e4 carriers would have worse white matter microstructure and subsequently perform worse on tasks of memory performance. A third aim of the current study was to determine the role of education as a potential moderator of the moderated mediation model. We hypothesized that for those who are highly educated, the effect of the APOE4 and p-tau interaction on white matter microstructure is reduced. Conceptual models of the proposed aims are visualized in Figures 1.2, 1.3, and 1.4.

CHAPTER 2

METHODS

Participants

Participants came from the Alzheimer's Disease Neuroimaging Initiative (ADNI; adni.loni.usc.edu). The ADNI project's purpose is to determine how imaging data, biological markers, clinical, and neuropsychological assessments can be used in combination to contribute in the prediction of onset, identification, and progression of Alzheimer's disease. This is a public dataset and can be accessed at www.adni-info.org. Researchers can gain access and ask questions related to the topics of interest for the ADNI project. The primary investigator for the ADNI project is Michael Weiner, MD, VA Medical Center and University of California, San Francisco. Participants for the current study will include individuals at least 65 years old who were categorized as having significant memory concerns (SMC), MCI, or AD, and were willing to undergo imaging, biomarker, and neuropsychological assessment. Individuals were identified as MCI or AD based on a set of inclusion criteria described in detail at http://adni.loni.usc.edu/methods/documents/. Briefly, SMC participants self-reported having significant memory concerns; this was quantified using the Cognitive Change Index (CCI), a measure of self-reported changes in memory and cognitive function over the years. SMC participants had to have a Clinical Dementia Rating (CDR) score of zero, score within the normal range for cognitive functioning, and their informant did not express concern with progressive memory difficulties. MCI subjects had an MMSE score between 24-30, a memory complaint, objective memory loss measured by education-adjusted scores on neuropsychological

testing, absence of significant cognitive deficits in other domains, largely preserved activities of daily living, and an absence of dementia. Mild/early AD subjects had an MMSE score between 20-26, Clinical Dementia Rating score of .5 or 1, and met NINCDS/ADRDA criteria for probable AD.

The ADNI project data has been collected over four cohorts (i.e., ADNI1, ADNI2, ADNIGO, and ADNI3). For this study, participants were derived from ADNI2 and ADNIGO. The exact number of participants to be included in this study was based upon the availability of combined data across all variables of interest. Given variability in genetic, biological, and neuroimaging data acquired from the different cohorts, participants were included from ADNI2 and ADNIGO who had available data for the proposed analyses. This means for each aim, participants were included based on whether they had the available data to assess that aim.

APOE4

During a screening visit, whole blood was collected from participants. The two SNPs (rs429358, rs7412) that define the epsilon 2,3, and 4 alleles were genotyped using DNA extracted by Cogenics from a 3 mL aliquot of ethylenediaminetetraacetic acid (EDTA) blood. Briefly, blood samples were placed in centrifuge, spun, and separated into three layers (i.e., red blood cells, plasma, and white blood cells/buffy coat). DNA was extracted from the white blood cells to genotype APOE nucleotides. TaqMan quantitative polymerase chain reaction assays were used for genotyping APOE nucleotides. Details are described in further detail by Shaw and colleagues (2009).

CSF phosphorylated tau

CSF p-tau was acquired through lumbar puncture at baseline and at other timepoints. Baseline CSF p-tau was used for this study. Shaw and colleagues (2009) describe in detail the

process by which CSF p-tau was extracted. Briefly, CSF tau levels were measured using Innogenetics (INNO-BIA AlzBio3) immunoassay kit-based reagents in the multiplex xMAPLuminex platform (Luminex). Details describing ADNI project methods for the measurement, analysis, assessment of quality control, and reporting of CSF p-tau can be found here: www.adni-info.org (accessed September 2020).

Assessment of Memory

Participants in the ADNI study were given a neuropsychological battery that includes measures of attention, working memory, executive function, language, and memory. For the purposes of the current aims, we examined performance on measures of memory. From the battery of neuropsychological tests administered, a composite memory score (ADNI-Mem) was created by the ADNI project (Crane et al., 2012). This composite measure was developed by combining the following assessments: Logical Memory (immediate recall, delayed recall), Rey Auditory Verbal Learning Test (RAVLT; trials 1-5, interference trial, immediate and delayed recall, recognition), AD Assessment Scale-Cognitive (ADAS-Cog; word list trials 1-3, recall, and recognition), and Mini Mental Status Exam (MMSE; 3 words recall). The ADNI-Mem composite has been psychometrically evaluated to be valid, as it was found to be slightly better at detecting change than total RAVLT recall scores and as good or better than all other scores at predicting conversion from MCI to AD (Crane et al., 2012).

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RAVLT is a list learning test that measures learning and memory (Rey, 1964). A list of 15 unrelated words are read. After the list is read, immediate recall is elicited. The list is then read 4 more times, with immediate recall elicited after each time the list is read for a total of 5 learning trials. Number of correctly recalled words is recorded for each trial. After a 20-minute delay, free recall of the list is elicited. Then, a yes/no recognition test is administered which includes 15 words from the original list along with 15 randomly intermingled distractor words.

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Mini-Mental State Exam

MMSE is a screening instrument which is used to measure cognitive impairment (Folstein, Folstein, & McHugh, 1975). Memory related tasks on this measure includes a recall task where participants must recall three words that were previously repeated.

Educational Attainment

Self-reported years of education provided by each participant within the ADNI database was used as our measure of educational attainment.

MRI Acquisition

Imaging data was collected from ADNI GO and ADNI 2. ADNI 1 was excluded given the MRI acquisition was done on a 1.5 Tesla MRI scanner and due to other incompatible techniques done by the ADNI 1 acquisition protocol. Participant data was collected from several sites on a 3-Tesla MRI scanner. The following parameters were used to collect the 3D Magnetization Prepared-Rapid Gradient Echo (MPRAGE) T1-weighted sequence: repetition time (TR) =2300 ms; echo time (TE) = 2.98 ms; inversion time (TI) =900 ms; 170 sagittal slices; within plane FOV = 256 × 240 mm2; voxel size = 1.1 × 1.1 × 1.2 mm3; flip angle = 9 °; bandwidth = 240 Hz/pix. Echo-planar imaging sequencing was used to collect T2 FLAIR scans with the following parameters: TR = 9000 ms, TE = 90 ms, and TI = 2500 ms, number of slices = 42, slice thickness = 5 mm. DTI sequences were collected using the following parameters: 256 × 256 matrix; voxel size: 2.7 × 2.7 × *2.7 mm^3; TR = 9000 ms; scan time = 9 min. There were 46 separate images acquired for each DTI scan. Five T_2-weighted images with no diffusion sensitization (b0 images) and 41 diffusion-weighted images (b = 1000 s/mm^2). Every MPRAGE image is linked with related files which have undergone pre-processing correction steps which were provided by the Mayo Clinic and are replicated and

included below (also available online: http://adni.loni.usc.edu/methods/mri-tool/mri-analysis/#mri-pre-processing-container).

1. Gradwarp: gradwarp is a system-specific correction of image geometry distortion due to gradient non-linearity. The degree to which images are distorted due to gradient non-linearity varies with each specific gradient model.

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3. N3: N3 is a histogram peak sharpening algorithm that is applied to all images. It is applied after grad warp and after B1 correction for systems on which these two correction steps are performed. N3 reduces intensity non-uniformity due to the wave or the dielectric effect at 3T. 1.5T scans also undergo N3 processing to reduce residual intensity non-uniformity.

Diffusion Tensor Imaging

Diffusion weighted images (DWIs) underwent preprocessing steps that are outlined in the ADNI protocol. This protocol is consistent with previously published work by Nir and colleagues (2013) using ADNI 3T MR data (Nir et al., 2013). These procedures will be discussed here briefly. To correct for head motion and eddy current, raw DWI volumes were aligned to the average b0 image using the eddy current and motion correction tool from the Functional MRI of the Brain (FMRIB) Software Library (FSL; www.fmrib.ox.ac.uk/fsl). ROBEX, which is an automated brain extraction program used for manually "skull-stripping" MRI data, and FreeSurfer were used to remove extra-cerebral tissue from T1-weighted anatomical scans (Iglesias et al., 2011; Fischl et al., 2004). MNI *nu_correct* tool

(www.bic.mni.mcgill.ca/software/) was used to correct for intensity inhomogeneity of anatomical scans. Brain Extraction Tool (BET) from FSL was used to extract non-brain tissue from DWIs (Smith, 2002). FSL *flirt* was used to align data from different participants into the same 3D coordinate space with each T1-weighted anatomical image linearly aligned to a standard template (Jenkinson, Bannister, & Smith, 2002). FSL *flirt* was also used to conduct Echo-planar imaging (EPI) correction for EPI-induced susceptibility artifacts. The subsequent 3D deformation fields were then applied to the remaining 41 DWI volumes before estimating diffusion parameters. A single diffusion tensor was modeled for every voxel of the brain. Scalar anisotropy and diffusivity maps were obtained from the resulting diffusion tensor eigenvalues (λ_1, λ_2 and λ_3). The FA image from the Johns Hopkins University (JHU) DTI atlas was linearly, then elastically registered to each subject's distortion corrected FA image. Deformation was applied to the stereotaxic JHU Eve white matter (WM) atlas labels. The average FA, MD, RD, and AxD for every subject were then extracted from 43 regions of interest and made available for download. Mean FA and RD values of the white matter tracts within the ROIs (i.e., fornix, hippocampal cingulum) were individually used as the measures of white matter microstructure. For our primary analyses, right and left hemisphere values were averaged together to create a single mean value for each ROI used in the statistical analysis. Mean diffusivity and axial diffusivity were examined in an exploratory fashion.

Statistical Procedures

Demographic information about the study sample is provided in Table 2.1 along with t-test and chi-square results examining any differences between the e4 groups. Bivariate and point-biserial correlations were run to examine the simple correlations between all the variables of

interest (Table 2.2). Given age is a strong predictor of structural brain integrity in older adults, it was included as a covariate in our analyses.

Addressing Missing Data. According to Lo & Jagust (2012), missing data within the ADNI dataset are not missing completely at random and likely due to conditional features (Lo & Jagust, 2012). They found that for those with MCI and AD, individuals who had a family history of AD were more likely to have CSF variables measured at baseline. Also, APOE4 positive AD participants and those with AD positive family histories tended to remain in the ADNI study. Within our dataset, 16 participants were missing CSF p-tau data. Given the amount of missing data in our study was not too small to where the consequences would not be felt, nor too high as to ruin the investigation (i.e., our missing data is > 5% and less than 40% of our total sample), it was decided that an intervention strategy to retrieve the information, such as an imputation method, was appropriate (Collins et al., 2001; Fernández-Garcia, Vallejo-Seco, Livácic-Rojas, & Tuero-Herrero, 2018; Little & Rubin, 2002). Multiple imputation (MI) method was used to address the 16 missing CSF p-tau values in our study. MI performs several imputations (i.e., plausible values) for each missing data point. The result is multiple datasets having slightly different values for the imputed data. Each data set is analyzed and then all the results are combined into one using formulas developed by Rubin (1987). For this study, 10 imputations were chosen, given our missing data points account for approximately 10% of our total data (Fernández-Garcia et al., 2018). Furthermore, participant data was stratified by biomarker-specific missingness predictors (i.e., grouped by diagnostic status and APOE4 status) to impute CSF p-tau data that was as closely consistent with what can be expected from participants with similar genetic and cognitive backgrounds.

Aim 1 Statistical Analysis. To address the first aim (Figure 1.2), the Statistical Package for Social Sciences (IBM SPSS Version 27.0) was used to analyze the data using a simple mediation model. A multiple linear regression was used to determine the direct relation between APOE4 status on ADNI-Mem score. The indirect effect of APOE4 status on memory through white matter integrity in a priori regions of interest were tested for its significance using standard procedures identified by Hayes (2012a&b), which provides explicit instructions on using Hayes' PROCESS Macro in SPSS to determine the significance of the indirect effect using the bootstrap approach to obtain confidence intervals. A mediation model for each DTI metric was used in the analyses. Mean FA and RD values of the white matter tracts within the ROIs (i.e., fornix, hippocampal cingulum) were individually used as the measures of white matter microstructure. Mean diffusivity and axial diffusivity were examined in an exploratory fashion.

Aim 2 Statistical Analysis. To address the second aim (Figure 1.3), the Statistical Package for Social Sciences (IBM SPSS Version 27.0) was used to analyze the data using a moderated mediation model. A multiple linear regression was used to determine the direct relation between APOE4 status on ADNI-Mem score. The indirect effect of APOE4 status on memory through white matter integrity in a priori regions of interest were tested for its significance using standard procedures identified by Hayes (2012a&b), which provides explicit instructions on using the PROCESS Macro in SPSS to determine the significance of the indirect effect using the bootstrap approach to obtain confidence intervals. From there, the moderation of the mediation determines whether the indirect effect differs in size or strength as a function of CSF p-tau levels. This was done using Hayes' Model 7. A mediation model for each DTI metric was used in the analyses. Mean FA and RD values of the white matter tracts within the two ROIs

were individually used as the measures of white matter microstructure. Mean diffusivity and axial diffusivity were examined in an exploratory fashion.

Aim 3 Statistical Analysis. To assess the exploratory third aim (Figure 1.4), SPSS was used to conduct a moderated-moderated-mediation analysis using Hayes' PROCESS Macro in SPSS. Procedures for analyzing this model (Model 11) is provided in Hayes' 2018 article (Hayes, 2018). This model determined whether the moderating effect of p-tau levels on the indirect effect (Model 7) differs in size or strength based upon education level. Mean FA and RD values of the white matter tracts within the ROIs (i.e., fornix, hippocampal cingulum) were individually used as the measures of white matter microstructure. There was a mediation model for each DTI metric. Mean diffusivity and axial diffusivity were examined in an exploratory fashion.

Power Analysis

This study included data that had already been collected. A sensitivity analysis was conducted in G*Power to determine the size of the effect our simple mediation analysis was powered to detect. Based on a power of .80, sample size of 74 (our smallest group size), and 3 predictors, the simple mediation model was powered to detect a medium effect (f^2=.156). To conduct a power analysis for the moderated mediation, Table 4 of Preacher and colleagues (2007) was used to determine the minimal sample size needed to achieve power of at least .80; minimal sample size suggested was 100 and no greater than 200 to detect a medium effect (Preacher, Rucker, & Hayes, 2007). Regarding multiple comparisons, one common technique to address this is the Bonferroni technique, which seeks to reduce false positives. This is a fairly conservative technique that has been suggested to be used when a large number of comparisons are made and not preplanned (Armstrong, 2014). The Benjamin-Hochberg approach to address

multiple comparisons and decrease the false discovery rate (FDR) is another technique that is less stringent, but also is typically used when many hypotheses are tested simultaneously (Benjamin & Hochberg, 1995; Chen, Feng, & Yi, 2017). It has been suggested that if a study has preplanned comparisons, the number of comparisons within a family of tests is small, and the risk of false negatives is greater than the risk of false positives, then not controlling for multiple comparisons is an option if the authors' claims are cautious. Given only 2 models were used to assess the main DTI metrics (i.e., FA and RD), corrections for multiple comparisons were not done.

CHAPTER 3

Fractional Anisotropy of the Fornix Mediates the Relation Between APOE4 and Memory Performance in Older Adults with Varying Cognitive Statuses[11]

[1] Jean, K.R., & Miller, L.S. To be submitted to *Archives of Clinical Neuropsychology*

ABSTRACT

The Apolipoprotein (APOE) e4 allele has been related to poorer cognitive outcomes, particularly poorer memory performance, in individuals with impaired cognitive function. However, the potential mechanisms by which APOE4 impacts memory need to be further examined. This study sought to better understand the potential mediating role white matter microstructure may play within the relation between APOE4 and memory in subjectively and objectively cognitively impaired older adults. Participants included 161 older adults (M = 74 years, 40.4% female, 92% White, 74 e4 non-carriers, 87 e4 carriers) with subjective and objective cognitive impairment from the Alzheimer's Disease Neuroimaging Initiative (ADNI) dataset. A composite memory score created by the ADNI project was used as the outcome variable. Mean fractional anisotropy (FA) and radial diffusivity (RD) values of white matter tracts within regions of interest (i.e., fornix (FX), hippocampal cingulum (CGH)) were individually used as the measures of white matter microstructure. Hayes' Process Macro Model 4 was used to run a mediation analysis. Indirect effects were tested using bootstrapping procedures. APOE4 was significantly related to FA of the FX but was not significantly related to FA or RD of the CGH or RD of the FX. APOE4 and FA of the fornix were significantly related to memory. FA of the fornix was a significant mediator between APOE4 and memory function (95% CI: -.1477, -.0039). FA and RD of the CGH and RD of the fornix were not significant mediators. Findings provide evidence that a potential mechanism by which APOE4 impacts memory performance in individuals with subjective and objective cognitive impairment is through white matter microstructural changes within the fornix, leading to poorer memory function.

INTRODUCTION

Though most older adults do not experience significant cognitive decline, increased age is a risk factor for Alzheimer's disease (AD) and other age-related neurodegenerative disorders (Hou et al., 2019). Several genetic, biological, and environmental risk factors for AD have been identified in the literature and there is great interest in understanding the mechanisms by which these risk factors for AD impact cognitive functions in older adults. The apolipoprotein e (APOE) gene, particularly the e4 allele, has been identified as a biomarker for AD. APOE is a blood protein responsible for transporting cholesterol, triglycerides, and other fats throughout the body and is also involved in the metabolism of fats (Gregg et al., 1986; Liu, Kanekiyo, Xu, & Bu, 2013). The e4 allele has been generally related to negative cognitive outcomes in older adults, with the literature suggesting a dose-dependent effect. For example, e4 heterozygotes (one e4 allele) have a three-fold increased risk of developing AD while individuals with both e4 alleles (homozygotes) have an eight to fifteen-fold increased risk (Goltermann et al., 2019). It is thought the e4 allele is less efficient at transporting lipids within the CNS, thus impacting the cholesterol homeostasis and general neuronal health (i.e., synaptic activity, neuroplasticity) of axons within the brain (Liu et al., 2013). Being a carrier of the e4 allele has been related to reduced synaptic activity, vascular function, neurogenesis, and increased risk for age-related dementias (Liu et al., 2013; Slooter, et al., 1997, Liu et al., 2013; Bétard, et al., 1994). One of the possible mechanisms by which APOE4 may increase AD risk is through poorer clearance of beta amyloid which results in greater beta amyloid plaques within the brain, a signature neuropathology of AD (Caselli et al., 2010; Huang, Weisgraber, Mucke, & Mahley, 2004). Other

potential mechanisms include neuroinflammation, neurogenesis, and neuronal toxicity (Liu et al., 2013).

APOE4 and Cognition in Healthy and Pathological Aging

In healthy older adults, there is some controversary as to whether the e4 allele is related to negative cognitive outcomes, with several studies finding e4 carriers showing cognitive deficits compared to non-carriers and other studies finding no relation between e4 carriership and cognition (for a full review of these studies, see O'Donoghue, Murphy, Zamboni, Nobre, & Makay, 2018). However, generally the presence of the e4 allele on cognition is negative and is likely due to multiple neurobiological mechanisms which need to be further investigated (O'Donoghue et al., 2018). In a meta-analysis examining the relation between e4 carriership and cognitive function in healthy older adults, Wisdom and colleagues (2011) found that e4 carriers performed poorer on tasks of episodic memory, executive function, perceptual speed, and overall global cognitive ability (Wisdom et al., 2011). In older adults with identified memory problems (i.e., amnestic mild cognitive impairment), e4 carriers had poorer scores on a screener for global cognition, a measure of dementia severity, and on a measure of functional activities (Kerchner et al., 2014).

Since episodic memory is one of the first and most prominent signs of Alzheimer's dementia (Kerchner et al., 2012), understanding APOE4's impact on memory is particularly important. Kerchner and colleagues (2014) examined e4 carriers' performance on measures of episodic memory and found that e4 carriers performed worse on tasks of episodic memory, even though their performance in other cognitive domains were similar to noncarriers. Furthermore, this finding was dose-dependent, with homozygotes having worse episodic memory performance. Notably, this finding was consistent across healthy controls, MCI, and those with

AD. In a sample of healthy older adults with increased risk for AD due to high beta amyloid burden, APOE4 carriers showed a moderately faster decline on memory tasks after a 54-month time period (Lim et al., 2015). This suggests that APOE4 carriership may exacerbate high beta-amyloid burden's effect on memory. Across the Alzheimer's and Lewy Body Dementia spectrum, Saeed and colleagues (2018) found that e4 carriers were significantly impaired on long-delay free recall of words (Saeed et al., 2018). They also found that APOE4 was associated with smaller hippocampal volumes in a dose dependent manner and global learning was associated with hippocampal volumes only among e4 positive individuals (Saeed et al., 2018). This finding suggests APOE4 status has effects on brain structure which may impact cognitive performance.

APOE4 and White Matter Microstructure

Given APOE is the major lipids distributor in the central nervous system (CNS) and is important in microtubule integrity, APOE plays a vital role in the promotion (e.g., formation of synapses, microtubule formation) and protection (e.g., repair of acute CNS trauma) of neurons, as APOE is secreted by astrocytes and provides delivery of important lipids for neuronal health (Liu et al., 2013; Mahley, Weisgraber, & Huang, 2006). Thus, examination of white matter fibers and their relation to APOE4 is of great interest. It is thought the e4 allele may be related to microstructural changes in the integrity of the brain's white matter through neuronal toxicity and brain atrophy (Honea, Vidoni, Harsha, & Burns, 2009). APOE4 has been thought to influence white matter microstructure through reduced neural repair, oxidative stress, and by negatively affecting myelin-producing oligodendroglia (Ryan et al., 2011). Compared to non-carriers, reductions in fractional anisotropy (FA, a measure of axonal microstructure) in the parahippocampal white matter have been reported in e4 carriers (Nierenberg et al., 2005; Tsao et

al., 2014). Decreased FA in the splenium of the corpus callosum, anterior frontal lobe white matter, inferior temporal lobe white matter, hippocampus, and the cingulum have also been observed (Persson et al., 2006). Heise and colleagues (2011) found that older adults with the e4 allele exhibited greater mean diffusivity (MD) relative to non-carriers in widespread areas (e.g., corona radiata, cingulum, corpus callosum, external capsule, internal capsule, and superior longitudinal fasciculus) using whole-brain analysis with tract-based spatial statistics (TBSS; Heise, et al., 2011). The e4 allele's effect on normal age-related changes in white matter integrity has been found to have an additive negative effect, suggesting that APOE4 adds an additional burden to age-related changes, especially for e4 homozygotes (Operto et al., 2018). For those with the e4 allele, reductions in the structural integrity of white matter in these areas are consistent with those reported in individuals with MCI, AD, and other dementias (Kantarci et al., 2001; Kantarci et al., 2005; Huang et al., 2007). This suggests carriers of the e4 allele who are cognitively intact may be experiencing preclinical pathological changes associated with neurodegenerative disorders. Harrison and colleagues' (2020) systematic review found that e4 carriers had lower FA and increased MD and RD in multiple white matter tracts, all suggesting worse white matter microstructure (Harrison et al., 2020). Kerchner and colleagues (2014) found that the relation between APOE4 and episodic memory was mediated by CA1-SRLM thinning (Kerchner et al., 2014). This provides some evidence that the way in which APOE4 may be impacting memory performance is potentially through the degradation of the structural integrity of white matter tracts known to be involved in neurodegenerative processes.

Current Study

The purpose of the current study was to evaluate select brain mechanisms underlying the relation between APOE4 and memory. We sought to determine whether white matter

microstructure within regions involved in memory would mediate the relation between APOE4 and memory performance in individuals with subjective memory concerns, MCI, and AD. We hypothesized that APOE4 carriers would perform poorer on tasks of memory. We also hypothesized that white matter microstructure in regions associated with memory functioning (i.e., fornix and hippocampal cingulum) would mediate this relation.

METHODS

Participants

This study used data collected from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database, a publically available dataset (adni.loni.usc.edu). The goal of the ADNI project is to determine how integration of imaging data, biological markers, clinical, and neuropsychological assessments can contribute to the prediction, identification, and progression of AD. The primary investigator for the ADNI project is Michael Weiner, MD, VA Medical Center and University of California, San Francisco. Participants for this current study included individuals at least 65 years old with a diagnosis of "significant memory concerns", MCI or AD, and were willing to undergo imaging, biomarker, and neuropsychological assessment. Individuals were identified as expressing subjective memory concerns (SMC), MCI or AD based off a set of inclusion criteria described in detail at http://adni.loni.usc.edu/methods/documents/. Briefly, SMC participants self-reported having significant memory concerns; this was quantified using the Cognitive Change Index (CCI), a measure of self-reported changes in memory and cognitive function over the years. SMC participants had to have a Clinical Dementia Rating (CDR) score of zero, score within the normal range for cognitive functioning, and their informant did not express concern with progressive memory difficulties. MCI subjects had an MMSE score between 24-30, a memory complaint, objective memory loss measured by

education-adjusted scores on neuropsychological testing, absence of significant cognitive deficits in other domains, largely preserved activities of daily living, and an absence of dementia. Mild/early AD subjects had an MMSE score between 20-26, CDR score of .5 or 1, and met NINCDS/ADRDA criteria for probable AD. The ADNI project data has been collected over four cohorts (i.e., ADNI1, ADNI2, ADNIGO, and ADNI3). Participants in the current study were derived from the ADNI2 and ADNIGO databases. Given variability in genetic, biological, and neuroimaging data acquired from the different cohorts, participants were included who had available data for the majority of the variables proposed for the analyses.

The current study included a total of 161 older adults. Participants were on average 74 years of age, 40.4% female, college educated, majority White (92%), with an average MMSE score of 26/30. There were 74 non-carriers of APOE4 and 87 carriers of APOE4.

APOE4

During a screening visit, whole blood was collected from participants. The two SNPs (rs429358, rs7412) that define the epsilon 2,3, and 4 alleles were genotyped using DNA extracted by Cogenics from a 3 mL aliquot of ethylenediaminetetraacetic acid (EDTA) blood. Briefly, blood samples were placed in a centrifuge, spun, and separated into three layers (i.e., red blood cells, plasma, and white blood cells/buffy coat). DNA was extracted from the white blood cells to genotype APOE nucleotides. TaqMan quantitative polymerase chain reaction (PCR) assays were used for genotyping APOE nucleotides. Details are described in further detail by Shaw and colleagues (2009).

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purposes of the current aims, we examined performance on measures of memory. From the battery of neuropsychological tests administered, a composite memory score (ADNI-Mem) was created by the ADNI project (Crane et al., 2012). This composite measure was developed by combining the following assessments: Logical Memory (immediate recall, delayed recall), Rey Auditory Verbal Learning Test (RAVLT; trials 1-5, interference trial, immediate and delayed recall, recognition), AD Assessment Scale-Cognitive (ADAS-Cog; word list trials 1-3, recall, and recognition), and Mini Mental Status Exam (MMSE; 3 words recall). The ADNI-Mem composite has been psychometrically evaluated to be valid, as it was found to be slightly better at detecting change than total RAVLT recall scores and as good or better than all other scores at predicting conversion from MCI to AD (Crane et al., 2012).

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(TR) =2300 ms; echo time (TE) = 2.98 ms; inversion time (TI) =900 ms; 170 sagittal slices; within plane FOV = 256 × 240 mm2; voxel size = 1.1 × 1.1 × 1.2 mm3; flip angle = 9 °; bandwidth = 240 Hz/pix. Echo-planar imaging sequencing was used to collect T2 FLAIR scans with the following parameters: TR = 9000 ms, TE = 90 ms, and TI = 2500 ms, number of slices = 42, slice thickness = 5 mm. DTI sequences were collected using the following parameters: 256 × 256 matrix; voxel size: 2.7 × 2.7 × *2.7 mm^3; TR = 9000 ms; scan time = 9 min. There were 46 separate images acquired for each DTI scan. Five T_2-weighted images with no diffusion sensitization (b0 images) and 41 diffusion-weighted images (b = 1000 s/mm^2). Every MPRAGE image is linked with related files which have undergone pre-processing correction steps which were provided by the Mayo Clinic and are replicated and included below (also available online: http://adni.loni.usc.edu/methods/mri-tool/mri-analysis/#mri-pre-processing-container).

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Diffusion weighted images (DWIs) had already undergone preprocessing steps that are outlined in the ADNI protocol. This protocol is consistent with previously published work by Nir and colleagues (2013) using ADNI 3T MR data (Nir et al., 2013). These procedures are discussed here briefly. To correct for head motion and eddy current, raw DWI volumes were aligned to the average b0 image using the eddy current and motion correction tool from the Functional MRI of the Brain (FMRIB) Software Library (FSL; www.fmrib.ox.ac.uk/fsl). ROBEX, which is an automated brain extraction program used for manually "skull-stripping" MRI data, and FreeSurfer were used to remove extra-cerebral tissue from T1-weighted anatomical scans (Iglesias et al., 2011; Fischl et al., 2004). MNI *nu_correct* tool (www.bic.mni.mcgill.ca/software/) was used to correct for intensity inhomogeneity of anatomical scans. Brain Extraction Tool (BET) from FSL was used to extract non-brain tissue from DWIs (Smith, 2002). FSL *flirt* was used to align data from different participants into the same 3D coordinate space with each T1-weighted anatomical image linearly aligned to a standard template (Jenkinson, Bannister, & Smith, 2002). FSL *flirt* was also used to conduct Echo-planar imaging (EPI) correction for EPI-induced susceptibility artifacts. The subsequent 3D deformation fields were then applied to the remaining 41 DWI volumes before estimating diffusion parameters. A single diffusion tensor was modeled for every voxel of the brain. Scalar anisotropy and diffusivity maps were obtained from the resulting diffusion tensor eigenvalues (λ_1, λ_2 and λ_3). The FA image from the Johns Hopkins University (JHU) DTI atlas was linearly, then elastically registered to each subject's distortion corrected FA image. Deformation was applied to the stereotaxic JHU Eve white matter (WM) atlas labels. The average FA, MD, RD, and AxD for every subject were then extracted from 43 regions of interest and made available for

download. Mean FA and RD values of the white matter tracts within the ROIs (i.e., fornix, hippocampal cingulum) were individually used as the measures of white matter microstructure in the current study. For our primary analyses, right and left hemisphere values were averaged together to create a single mean value for each ROI used in the statistical analysis. Mean diffusivity and axial diffusivity were examined in an exploratory fashion.

Statistical Procedures

Bivariate and point-biserial correlations were run to examine the simple correlations between all the variables of interest (Table 2). Given age is a strong predictor of structural brain integrity in older adults, it was included as a covariate in our analyses. The Statistical Package for Social Sciences (IBM SPSS Version 27.0) was used to analyze the data using a simple mediation model. A multiple linear regression was used to determine the direct relation between APOE4 status on ADNI-Mem score. The indirect effect of APOE4 status on memory through white matter integrity in a priori regions of interest were tested for its significance using standard procedures identified by Hayes (2012a&b), which provides explicit instructions on using the PROCESS Macro in SPSS to determine the significance of the indirect effect using the bootstrap approach to obtain confidence intervals. A mediation model for each DTI metric was used in the analyses. Mean FA and RD values of the white matter tracts within the ROIs (i.e., fornix, hippocampal cingulum) were individually used as the measures of white matter microstructure. Mean diffusivity and axial diffusivity were examined in an exploratory fashion.

RESULTS

Sample Characteristics and Bivariate Correlations.

See Table 3.1 for demographic characteristics. The total sample included 161 participants (i.e., 74 e4 negative and 87 e4 positive participants). APOE4 carriers were significantly younger,

t(159) = 2.076, p=.039 and had lower MMSE (t(159) = 2.608, p=.010) and memory composite scores (t(159) = 3.815, p <.001). There was no difference amongst the group in education level, t(159) = -.286, p=.775.

Bivariate correlations are presented in Table 3.2. Age was significantly correlated with FA of the CGH (r = -.361, p<.001), RD of the CGH (r=.438, p<.001), FA of the fornix (r=-.340, p<.001), RD of the fornix (r=.361, p<.001), and APOE4 status (r=-.162, p=.039). Education was significantly related to sex (r=-.192, p=.015) and sex was significantly related to FA of the CGH (r=-.174, p=.027). A chi-square test was run to determine the relations between APOE4 status and sex, which was found to be non-significant (X^2=1.767, p=.184). To better understand these correlations, bivariate correlations were done and separated by APOE4 status. This revealed that for e4 noncarriers, sex was not related to FA of the CGH (r=-.228, p=.051) or any other variable of interest. However, for e4 carriers, sex was related to age (r=-.214, p=.046) and FA of the fornix (r=.238, p=.027), but was not related to FA of the CGH (r=-.119, p=.272).

APOE4, White Matter Microstructure, and Memory Performance

Visualization of the two ROIs (fornix, hippocampal cingulum) identified on a patient's FA map is presented in Figures 3.1 and 3.2. Primary regression results for FA measures are presented in Figure 3.3. When examining memory performance as the primary outcome variable and controlling for age, the overall model was significant (R^2=.1823, p = <.001). More specifically, APOE4 was significantly related to memory performance (*b*=-0.5247, p=.001) and FA of the fornix was also related to memory performance (p=.0293). FA of the CGH was not significantly related to memory performance, p=.0824. Though the direct effect of APOE4 status on memory remained significant when accounting for mediators (c' = -.5247, p=.001, CI = [-.7894, -.2600]), FA of the fornix was a significant mediator, characterized by a significant

indirect effect (a_2b_2 = -.060, CI [-.1477, -.0039]. FA of the CGH was not a significant mediator, a_1b_1 = .0117, CI [-.0254, .0628].

Primary regression results for RD measures are presented in Figure 3.4. When examining memory performance as the primary outcome variable, RD of the CGH was a significant predictor of memory performance (*b*=-4914.6336, p<.001), whereas RD of the fornix was not a significant predictor (*b*=41.1449, p=.8187). There was a direct effect of APOE4 on memory (c'=-.4761, p=.0001), however, neither RD of the CGH or RD of the fornix were significant mediators.

Exploratory Analyses

Exploratory results examining the other two DTI metrics (MD and AxD) were conducted and presented in Figures 5 and 6, respectively. For MD analyses, APOE4 was not a significant predictor of MD of the CGH (b=.00, p=.098), but was a significant predictor of MD of the fornix, b=.0001, p=.0448. MD of the CGH was a predictor of memory performance (b=-5011.61, p=<.001), however, MD of the fornix was not a predictor of memory performance (b=58.153, p=.756). Neither MD of the CGH (CI: -.2831, .0157) nor MD of the fornix (-.0394, .0518) were significant mediators. In terms of AxD, AxD of the CGH was a significant mediator (a_1b_1 = -0.1531, CI: -.3175, -.0082). Both the total effect (c=-0.573, p<.001) and direct effect (c' =-0.4237, p=0.003) of APOE4 on memory were significant, suggesting partial mediation after accounting for AxD of the CGH as a mediator. APOE4 was significantly related to AxD CGH (b=.00, p=0.0396) and AxD of the CGH was significantly related to memory performance (b=-4670.764, p<.001). AxD of the fornix was not a significant mediator (a_2b_2 = 0.0038, CI: -.0318, .0381).

DISCUSSION

The current study assessed select mechanisms by which APOE4 allele carriership negatively impacts memory performance in individuals experiencing subjective and objective cognitive changes. Results from this study are consistent with literature that suggests positive e4 carriership is negatively related to memory performance in samples of predominately White older adults. Our primary mediation analysis indicated that degradation of white matter microstructure within the fornix, as measured by FA of the fornix, was a significant mediator between the relation of APOE4 and memory performance. Contrary to our hypotheses, the CGH was not a significant mediator. Given the hippocampal cingulum's established role in memory performance, this finding was unexpected. However, our exploratory analyses suggest that MD rather than FA may be a better DTI metric to examine the potential effect of APOE4 on white matter degradation in the CGH. Also contrary to our hypotheses, white matter microstructure degradation within the CGH or fornix as measured by radial diffusivity, was not a significant mediator. Though this finding might suggest that radial diffusivity is a less sensitive marker of microstructure degradation in these regions, we found that APOE4 was related to RD of the fornix. RD was used in our primary analyses because it is thought to reflect myelin integrity, with radial diffusivity increasing with demyelination (Rieckmann et al., 2016). It was proposed that given APOE4 is potentially involved in dysregulation of lipid distribution, which is important for myelin health, it was important to examine RD within our primary analyses. However, our results suggest that the mechanism by which APOE4 impacts memory performance is likely through specifically reducing anisotropy (which is best measured by DTI FA) within the fornix and thus the current common method of examining RD in exploratory analyses is still warranted. This is important when considering potential interventional strategies

to possibly reduce the negative outcomes related to being a carrier of the APOE4 allele. Our findings suggest that for e4 carriers, intervention strategies targeting microstructural integrity of white matter is important to possibly reduce e4's effect on memory functioning.

This study has several limitations and possible areas for improvement that may be helpful to enhance this research area. A primary limitation to the current study was the preponderance of predominantly White, highly educated individuals and thus our ability to generalize the findings to other populations is very limited. This is important to consider as research suggest that being an APOE4 carrier is more common in Black individuals and is less strongly associated with AD risk (Evans et al., 2003; Marden et al., 2014). Given this, understanding the role of APOE4 in memory performance in Black individuals and in individuals of other races and ethnicities is important as APOE4 carriership may function differently within these groups. Another limitation is the collection of DTI data within this sample. DTI data was only collected for a subset of participants within the ADNI dataset and it is possible that the individuals who had DTI are somehow different than participants who did not receive DTI. Finally, the current study only examined white matter microstructure within two regions of interest as potential mediators and thus our findings only found partial mediation effects. The current sample was not powered enough to examine a fuller model that incorporated other potential brain mechanisms (e.g., cerebral profusion, white matter hyperintensities) that are also potential mediators and could explain more variance within the model.

Overall, results from the current study reveals that observed differences in memory performance based upon e4 carriership is partially mediated by white matter microstructural degradation within white matter tracts important for memory consolidation. This provides insight into the mechanistic properties underlying the APOE4 allele in effecting white matter

microstructure health. As mentioned earlier, there are likely several other factors that are unaccounted for within our model that have implications for further research to understand how these brain mechanisms may also contribute to differences in memory performance based upon APOE4 carriership. For e4 carriers whose backgrounds are similar to individuals within this study, greater emphasis should be placed upon interventions targeted at increasing or maintaining the health of their white matter microstructure, which is largely impacted by cerebrovascular health and should be a major target for e4 carriers.

CHAPTER 4

P-Tau and Education as Moderators of the Relation between APOE4 and Memory Performance in Older Adults with Varying Cognitive Status[2]

[2] Jean, K.R., Miller, L.S. To be submitted to Archives of Clinical Neuropsychology

ABSTRACT

White matter microstructure (WMM) potentially mediates the relation between APOE4 and memory performance. This study's purpose was to understand whether p-tau effects this mediation model and whether education level differentially impacts the relations between these genetic and biological biomarkers' influence on memory. Participants included 161 older adults (M=74 years, 40.4% female, 92% White, 74 e4 non-carriers, 87 e4 carriers) with subjective and objective cognitive impairment from the Alzheimer's Disease Neuroimaging Initiative (ADNI). A composite memory score created by ADNI was used as the outcome variable. Mean fractional anisotropy (FA) and radial diffusivity (RD) values of white matter tracts within regions of interest (i.e., fornix (FX), hippocampal cingulum (CGH)) were individually used as the measures of WMM. A moderated mediation was run to examine whether p-tau was a moderator of the mediation between APOE4, white matter microstructure, and memory. A dual moderated mediation analysis examined education as a moderator of the moderated mediation. Indirect effects were tested using bootstrapping procedures. In the FA moderated mediation model, APOE4 was significantly related to FA of the fornix and memory performance. FA of the CGH and FX were also related to memory performance. With FA of the fornix as the mediator, the conditional indirect effect was not significant. There was a trend suggesting at low and average levels of p-tau, FA of the fornix was a significant mediator but was non-significant at high levels of p-tau. The RD moderated mediation model was non-significant. The FA and RD dual moderated mediation models were non-significant. However, the APOE4 x p-tau interaction with FA of the fornix as the mediator suggested a trend. At low levels of p-tau, increased education

was related to a significant moderated mediation. Results suggest that FA of the fornix is a significant mediator between the relation of APOE4 and memory, and this may be dependent upon p-tau levels. When p-tau burden load was high, the path by which APOE4 impacts memory performance was not through white matter microstructure degradation. Additionally, the potential buffering effects of education may be most robust at lower levels of p-tau burden.

INTRODUCTION

Understanding risk and protective factors that impact cognitive changes in normal and pathological aging and understanding the interactions amongst these factors are of great importance. One genetic factor examined is the apolipoprotein e (APOE) gene, a blood protein involved in the transportation and metabolism of lipids throughout the body (Gregg et al., 1986; Liu, Kanekiyo, Xu, & Bu, 2013). Being a carrier of the e4 allele has been found to be related to negative cognitive outcomes and is considered one of the strongest biomarkers of late-onset AD, particularly in European ancestry older adult samples (de Rojas et al., 2021). A meta-analysis by Wisdom and colleagues (2011) found that the APOE4 allele was related to poorer cognitive function, particularly in the domains of episodic memory, executive function, perceptual speed, and overall global cognitive ability in samples of healthy older adults (Wisdom et al., 2011).

The mechanisms by which APOE4 impacts cognitive functions need to be further examined. One mechanism examined has been APOE4's influence on amyloid plaque deposition within the brain leading to neurodegeneration, though this is likely not the only mechanism by which APOE4 impacts Alzheimer's disease, especially since there have been findings that amyloid plaque deposition accumulation poorly correlate with AD clinical expression and tissue loss (Josephs et al., 2008). APOE4 is likely related to multiple neurobiological mechanisms that need to be further investigated. Potential manners by which APOE4 has been related to neurodegeneration is through increased immune reactivity, beta amyloid plaques, reduced efficiency in the transportation of cholesterol within the brain, and increased tangles (Shi et al., 2017). Increased tangle formation and abnormal hyperphosphorylation of the tau protein is very

common in the neuropathology of Alzheimer's disease. Tau protein plays a role in the stabilization of neuronal microtubules that are important for the development of cell processes and intracellular support involved in microtubule formation (Buerger et al., 2006; Mandelkow & Mandelkow, 1998). Hyperphosphorylation of the tau protein occurs when tau is detached from the microtubules. Hyperphosphorylated/abnormally phosphorylated tau (p-tau) reduces microtubule-promoting activity by interacting with normal tau and reducing its ability to assemble tubulin into microtubules. Furthermore, the aggregation of p-tau leads to neurofibrillary tangles and synaptic damage. These two mechanisms are likely the most prominent ways by which p-tau negatively impacts brain structure and function (Alonso, Zaidi, Grundke-Iqbal & Iqbal, 1994; Buerger et al., 2006; Mandelkow & Mandelkow, 1998; Rajmohan & Reddy, 2017). Total tau and phosphorylated tau in the cerebrospinal fluid (CSF) provide valuable information for the diagnosis of Alzheimer's disease and differential diagnosis from other neurodegenerative disorders (e.g., frontotemporal dementia, Lewy body dementia; Hampel et al., 2010). P-tau within the CSF has been found to predict progression to AD in a European sample of MCI individuals with an accuracy rate of 80% (Ewers et al., 2007; Hampel et al., 2005).

It has been suggested that the presence of the APOE4 allele in addition to the presence of other biological (e.g., high p-tau burden) and environmental factors (e.g., lower education, cardiovascular disease, low literacy), can increase an older adult's risk for cognitive decline (Benson et al., 2022; Perna et al., 2016; Kaup et al., 2015). When examining the relations amongst APOE4 and p-tau, APOE4 is thought to be related to increased cellular tau phosphorylation and enhances the release of phosphorylated tau (Wadhwani, Affaneh, Van Gulden, & Kessler, 2019). Koch and colleagues (2017) found that when compared to e4 non-

carriers, CSF tau negatively impacted cortical plasticity, led to faster AD progression, and led to faster cognitive decline in e4 carriers (Koch et al., 2017). Animal models suggest APOE4 may cause tau-dependent impairment of hilar GABAergic interneurons which impact the learning and memory in mice and thus help explain memory and learning deficits in AD (Andrews-Zwilling et al., 2010). In a large cohort of participants with mild cognitive impairment (MCI), Benson and colleagues (2022) found that APOE4 carriers had a higher concentration of p-tau than non-carriers in a dose-dependent manner, suggesting that the APOE4 allele may be involved in p-tau aggregation. However, they found no APOE4 x CSF interaction effect on the progression of rate of decline (Benson et al., 2022). More information about the interaction between APOE4 and CSF tauopathy is needed.

Several life experiences have been examined as possible protective factors that can potentially mitigate the negative effects of tauopathy and APOE4 allele status on brain health. Education has been considered a proxy measure of cognitive reserve that has been found to be one of the strongest predictors of cognition in older adults (Crowe et al., 2013; Roe, Xiong, Miler, & Morris, 2007). Cognitive reserve (CR) theory posits that some individuals are able to maintain cognitive performance despite brain damage or aggregation of neuropathology (Stern, 2013). CR has been found to help explain, to some degree, the differential development and progression of Alzheimer's disease amongst individuals suffering from the disease (Stern, 2013). Education has also been found to be related to brain structure. For example, education has been positively related to subregions of the hippocampus (Tang, Varma, Miller, & Carlson, 2017). For APOE4 homozygotes or APOE4 homozygotes with a family history of AD, lower education was associated with reduced cortical thickness of the hippocampus, subiculum, entorhinal cortex, parahippocampal cortex, and other medial temporal regions (Baumgaertel et al., 2016). Within

this same study, education was not correlated with cortical thickness in the medial temporal lobe for those with no genetic risk for AD, suggesting CR might be most beneficial for individuals with greater risk of AD. Education has been found to buffer the effects of tau on cognitive functions in a sample of individuals with AD. AD patients with higher education tolerated greater tau pathology than those with lower education (Hoenig et al., 2017). In individuals with mild cognitive impairment, Rolstad and colleagues' (2010) found that greater education was related to lower concentrations of tau. However, there is still more to be learned about the possible moderating role of education on biomarkers of AD as not all studies have found education to be a significant moderator of AD symptomology. For example, Bauer and colleagues (2020) found the relation between a biomarker composite (Tau/ Ab_{42} ratio) and a memory composite was not moderated by education.

It is important to further study how biological and environmental factors work together to impact APOE4's influence on clinical outcomes in populations experiencing some level of cognitive dysfunction. The current study seeks to examine whether an interaction between APOE4 status and CSF p-tau existed and impacted the mediating role of white matter microstructure. We proposed there would be an interaction between APOE4 and p-tau, such that the mediating role of white matter microstructure on the relation between APOE4 and memory would differ between individuals at high and low p-tau levels. We hypothesized that individuals with high levels of p-tau who are e4 carriers would have poorer white matter microstructure and subsequently have worse memory performance. Furthermore, we explored the potential role of education in possibly reducing the APOE4 x p-tau interaction on white matter microstructure.

METHODS

Participants

Participants for the current study were included from the Alzheimer's Disease Neuroimaging Initiative (ADNI; adni.loni.usc.edu), a publicly available dataset aimed at determining how imaging data, biological markers, clinical, and neuropsychological assessments can be used in combination to contribute in the prediction of onset, identification, and progression of Alzheimer's disease. The primary investigator for the ADNI project is Michael Weiner, MD, VA Medical Center and University of California, San Francisco. The ADNI project has several cohorts, participants for this project were included from the ADNI2/GO datasets. Our sample included 161 older adults (65+) who were labeled as subjective cognitive complaints/subjective memory concern (SCC/SMC), mild cognitive impairment (MCI), and Alzheimer's disease (AD) based off of specific inclusion criteria. Briefly, SMC subjects self-reported significant memory concerns, quantified by using the Cognitive Change Index (CCI) and had a Clinical Dementia Rating (CDR) of zero. SMC participants scored within the normal range for cognition, and their informant did not equate the expressed concern with progressive memory impairment. MCI subjects had an MMSE score between 24-30, a memory complaint, objective memory deficit measured by education-adjusted scores on neuropsychological testing, absence of significant cognitive deficits in other domains, largely preserved activities of daily living, and an absence of dementia. Mild/early AD subjects had an MMSE score between 20-26, Clinical Dementia Rating score of .5 or 1, and met NINCDS/ADRDA criteria for probable AD.

APOE4

During a screening visit, whole blood was collected from participants. The two SNPs (rs429358, rs7412) that define the epsilon 2,3, and 4 alleles were genotyped using DNA

extracted by Cogenics from a 3 mL aliquot of ethylenediaminetetraacetic acid (EDTA) blood. Briefly, blood samples were placed in a centrifuge, spun, and separated into three layers (i.e., red blood cells, plasma, and white blood cells/buffy coat). DNA was extracted from the white blood cells to genotype APOE nucleotides. TaqMan quantitative polymerase chain reaction (PCR) assays were used for genotyping APOE nucleotides. Details are described in further detail by Shaw and colleagues (2009).

CSF phosphorylated tau

CSF p-tau was acquired through lumbar puncture at baseline and at other timepoints. Baseline CSF p-tau was used for this study. Shaw and colleagues (2009) describe in detail the process by which CSF p-tau was extracted. Briefly, CSF tau levels were measured using Innogenetics (INNO-BIA AlzBio3) immunoassay kit-based reagents in the multiplex xMAPLuminex platform (Luminex). Details describing ADNI project methods for the measurement, analysis, assessment of quality control, and reporting of CSF p-tau can be found here: www.adni-info.org (accessed September 2020).

Assessment of Memory

Participants in the ADNI study are given a neuropsychological battery that includes measures of attention, working memory, executive function, language, and memory. For the purposes of the current aims, we examined performance on measures of memory. From the battery of neuropsychological tests administered, a composite memory score (ADNI-Mem) was created by the ADNI project (Crane et al., 2012). This composite measure was developed by combining the following assessments: Logical Memory (immediate recall, delayed recall), Rey Auditory Verbal Learning Test (RAVLT; trials 1-5, interference trial, immediate and delayed recall, recognition), AD Assessment Scale-Cognitive (ADAS-Cog; word list trials 1-3, recall,

and recognition), and Mini Mental Status Exam (MMSE; 3 words recall). The ADNI-Mem composite has been psychometrically evaluated to be valid, as it was found to be slightly better at detecting change than total RAVLT recall scores and as good or better than all other scores at predicting conversion from MCI to AD (Crane et al., 2012).

Logical Memory

Logical memory is a measure from the Wechsler Memory Scale-Revised (WMS-R). This test asks individuals to freely recall information from one short story after the story has been read aloud and again after 30 minutes. The short story consists of 25 bits of information (Wechsler, D, 1987). The total bits of information recalled after the immediate trial and the delayed trial are recorded. The total maximum score for immediate and delayed recall is 25 for each trial.

Rey Auditory Verbal Learning Test

RAVLT is a list learning test that measures learning and memory (Rey, 1964). A list of 15 unrelated words are read. After the list is read, immediate recall is elicited. The list is then read 4 more times, with immediate recall elicited after each time the list is read for a total of 5 learning trials. Number of correctly recalled words is recorded for each trial. After a 20-minute delay, free recall of the list is elicited. Then, a yes/no recognition test is administered which includes 15 words from the original list along with 15 randomly intermingled distractor words.

Alzheimer's Disease Assessment Scale-Cognitive

ADAS-Cog is a structured assessment that evaluates multiple cognitive domains (e.g., memory, reasoning, language, orientation, ideational praxis, and constructional praxis; Rosen, Mohs, & Davis, 1984). For the ADNI-Mem composite, only the memory related tasks were included (ADAS-Cog list trials 1-3, recall, and recognition). The ADAS-Cog includes a word list learning task and a word recognition task. The word list learning task includes 10 unrelated

words that are printed on cards. The participant is asked to read the words aloud for them to remember. This is repeated a total of 3 times, after each learning trial they are asked to recall as many words as they can. After a delay of 5 minutes, they are asked to recall the list. In the word recognition task, 12 cards with words printed on them are read aloud by the participant and they are asked to remember them. Then the participant is presented with 24 words (12 target words, 12 distractor) and asked to identify which words were part of the original list. Positive hits and positive negatives are recorded.

Mini-Mental State Exam

MMSE is a screening instrument which is used to measure cognitive impairment (Folstein, Folstein, & McHugh, 1975). Memory related tasks on this measure includes a recall task where participants must recall three words that were previously repeated.

Educational Attainment

Self-reported years of education provided by each participant within the ADNI database was used.

MRI Acquisition

Imaging data was collected from ADNI GO and ADNI 2 data. ADNI 1 was excluded given the MRI acquisition was done on a 1.5 Tesla MRI scanner and due to other incompatible techniques done by the ADNI 1 acquisition protocol. ADNI3 was excluded because DTI data was not completed at the time. Participant data was collected from several sites on a 3-Tesla MRI scanner. The following parameters were used to collect the 3D Magnetization Prepared-Rapid Gradient Echo (MPRAGE) T1-weighted sequence: repetition time (TR) =2300 ms; echo time (TE) = 2.98 ms; inversion time (TI) =900 ms; 170 sagittal slices; within plane FOV = 256 × 240 mm2; voxel size = 1.1 × 1.1 × 1.2 mm3; flip angle = 9 °; bandwidth = 240 Hz/pix. Echo-

planar imaging sequence was used to collect T2 FLAIR scans with the following parameters: TR = 9000 ms, TE = 90 ms, and TI = 2500 ms, number of slices = 42, slice thickness = 5 mm. DTI sequences were collected using the following parameters: 256 × 256 matrix; voxel size: 2.7 × 2.7 × *2.7 mm3; TR = 9000 ms; scan time = 9 min. There were 46 separate images acquired for each DTI scan. Five T2-weighted images with no diffusion sensitization (b0 images) and 41 diffusion-weighted images (b = 1000 s/mm2). Every MPRAGE image was linked with related files which underwent pre-processing correction steps which were provided by the Mayo Clinic and were replicated and included below (also available online: http://adni.loni.usc.edu/methods/mri-tool/mri-analysis/#mri-pre-processing-container).

1. Gradwarp: gradwarp is a system-specific correction of image geometry distortion due to gradient non-linearity. The degree to which images are distorted due to gradient non-linearity varies with each specific gradient model.

2. B1 non-uniformity: this correction procedure employs the B1 calibration scans noted in the protocol above to correct the image intensity non-uniformity that results when RF transmission is performed with a more uniform body coil while reception is performed with a less uniform head coil.

3. N3: N3 is a histogram peak sharpening algorithm that is applied to all images. It is applied after grad warp and after B1 correction for systems on which these two correction steps are performed. N3 reduces intensity non-uniformity due to the wave or the dielectric effect at 3T. 1.5T scans also undergo N3 processing to reduce residual intensity non-uniformity.

Diffusion Tensor Imaging

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and colleagues (2013) using ADNI 3T MR data (Nir et al., 2013). These procedures are discussed here briefly. To correct for head motion and eddy current, raw DWI volumes were aligned to the average b0 image using the eddy current and motion correction tool from the Functional MRI of the Brain (FMRIB) Software Library (FSL; www.fmrib.ox.ac.uk/fsl). ROBEX, which is an automated brain extraction program utilized for manually "skull-stripping" MRI data, and FreeSurfer were used to remove extra-cerebral tissue from T1-weighted anatomical scans (Iglesias et al., 2011; Fischl et al., 2004). MNI nu_correct tool (www.bic.mni.mcgill.ca/software/) was used to correct for intensity inhomogeneity of anatomical scans. Brain Extraction Tool (BET) from FSL was used to extract non-brain tissue from DWIs (Smith, 2002). FSL flirt was used to align data from different participants into the same 3D coordinate space with each T1-weighted anatomical image linearly aligned to a standard template (Jenkinson, Bannister, & Smith, 2002). FSL flirt was also used to conduct Echo-planar imaging (EPI) correction for EPI-induced susceptibility artifacts. The subsequent 3D deformation fields were then applied to the remaining 41 DWI volumes before estimating diffusion parameters. A single diffusion tensor was modeled for every voxel of the brain. Scalar anisotropy and diffusivity maps were obtained from the resulting diffusion tensor eigenvalues ($\lambda 1$, $\lambda 2$ and $\lambda 3$). The FA image from the Johns Hopkins University (JHU) DTI atlas was linearly, then elastically registered to each subject's distortion corrected FA image. Deformation was applied to the stereotaxic JHU Eve white matter (WM) atlas labels. The average FA, MD, RD, and AxD for every subject were then extracted from 43 regions of interest and made available for download. Mean FA and RD values of the white matter tracts within the ROIs (i.e., fornix, hippocampal cingulum) were individually used as the measures of white matter microstructure. For our primary analyses, right and left hemisphere values were averaged together to create a

single mean value for each ROI used in the statistical analysis. Mean diffusivity and axial diffusivity were examined in an exploratory fashion.

RESULTS

Sample Characteristics and Bivariate Correlations.

See Table 4.1 for demographic characteristics. The total sample included 161 participants. 74 participants were e4 negative and 87 participants were e4 positive. Within this sample, APOE4 carriers were significantly younger, $t(159) = 2.076$, $p=.039$ and had lower MMSE scores ($t(159) = 2.608$, $p=.010$) and memory composite scores ($t(159) = 3.815$, $p < .001$). APOE4 carriers had greater ptau levels, $t(159) = -3.303$, $p=.001$. The two groups were not different in education level, $t(159) = -.286$, $p=.775$.

Bivariate and point bi-serial correlations are presented in Table 4.2. Age was significantly correlated with FA of the CGH ($r = -.361$, $p<.001$), RD of the CGH ($r=.438$, $p<.001$), FA of the fornix ($r=-.340$, $p<.001$), RD of the fornix ($r=.361$, $p<.001$), and APOE4 status ($r=-.162$, $p=.039$). P-tau levels were positively related to e4 status ($r=.253$, $p=.001$), sex ($r=.187$, $p=.017$), RD of the CGH ($r=.199$, $p<.001$), and negatively related to education level ($r=-.344$, $p<.001$). Education was significantly related to sex ($r=-.192$, $p=.015$) and sex was significantly related to FA of the CGH ($r=-.174$, $p=.027$). A chi-square test was run to determine the relations between APOE4 status and sex, which was found to be non-significant ($X^2=1.767$, $p=.184$). In addition, bivariate correlations were run to examine the two groups separately to better understand these correlations. This revealed that for e4 noncarriers, sex was not related to FA of the CGH ($r=-.228$, $p=.051$) or any other variable of interest. However, for e4 carriers, sex was related to age ($r=-.214$, $p=.046$) and FA of the fornix ($r=.238$, $p=.027$), but was not related to FA of the CGH ($r=-.119$, $p=.272$).

Primary Analyses

Visualization of the two ROIs (fornix, hippocampal cingulum) identified on a patient's FA map is presented in Figures 4.1 and 4.2. Primary regression results for FA measures are presented in Figure 4.3. When examining memory performance as the primary outcome variable and controlling for age, the overall model was significant (R^2=.1823, p=<.001). Examination of the moderated mediation index revealed that p-tau was not a significant moderator when examining the pathway with FA of the CGH as a mediator (index of moderated mediation = -.0014 (95% CI[-.0049, .0008]). When examining the path with FA of the fornix as the mediator, the conditional indirect effect was also non-significant, (index of moderated mediation = .0026 (95% CI[-.0009, .0070]). However, there appeared to be a trend that suggested the mediation effect differed at varying levels of p-tau. More specifically, FA of the fornix was a significant mediator at low (effect = -.1106, SE =.002, 95% CI[-.2421, -.0140]) and average levels of p-tau (effect = -.071, SE = .04, 95% CI[-.1658, -.0083]), but at high levels of p-tau, FA of the fornix was not a significant mediator, (effect = -.0315, SE = .042, 95% CI [-.1322, .0341].

When examining memory performance as the primary outcome variable and controlling for age in the RD models, the overall model was significant (R^2=.2274, p=<.001). Examination of the moderated mediation index revealed p-tau was not a significant moderator of the mediation model through RD of the CGH (index of moderated mediation = .000, SE=.0049, 95% CI [-.0104, .0091]) nor through RD of the fornix (index of moderated mediation = -.0001, SE =.0007, 95% CI[-.0016, .0016]).

Exploratory Analyses

Given our restricted sample size, the potential role of education as a moderator of the moderated mediation model (Hayes' model 11) was examined in an exploratory fashion. Overall,

when examining the FA of the fornix path, education was not a significant moderator of the moderated mediation, (index of dual moderated mediation = .0004, SE = .0008, 95% CI [-.0012, .0022]. However, a trend was present. Examination of the APOE4 x p-tau interaction at various levels of education revealed that at low levels of p-tau, increased education was related to a significant mediation model. See Table 4.3 for the indirect effect at varying levels of ptau and education. When examining FA of the CGH path, education was not a significant moderator of the moderated mediation (index of dual moderated mediation = .0011, 95% CI [-.0001, .0032].

DISCUSSION

The goal of the current study was to better understand potential biological (p-tau) and environmental (education) moderators that influence the mediating role of white matter microstructure in the relation between APOE4 allele carriership and memory functioning in individuals who are experiencing subjective or objective cognitive changes. When examining whether p-tau was a moderator of the mediation model, results suggest that p-tau was not a significant moderator. However, there was a trend that suggested at low and average levels of p-tau, FA of the fornix was a significant mediator, but at high levels of p-tau, FA of the fornix was not a significant mediator. This finding suggests that it may be possible that at high levels of tau burden, white matter microstructure changes within the fornix may not be a mechanism by which APOE4 impacts memory functioning. This finding suggests that when neuropathology is high, particularly hyperphosphorylation within the brain, APOE4's impact on memory functioning may not be through white matter microstructural changes in the fornix. This is somewhat consistent with findings that have suggested that at greater levels of neuropathology (e.g., plaques, tangles) and clinical presentation of cognitive difficulties, other mechanisms of neurodegeneration (e.g., plaques, tangles) override the effect of APOE4 (Benson et al., 2022).

Thus, it may be the case that once more direct processes, such as plaques and tangles within the brain, have accumulated enough in brain regions involved in memory, the mediating role of white matter microstructure in the fornix is weakened and overridden by neurodegeneration through hyperphosphorylation. On the other hand, our findings also suggest that for APOE4 carriers with lower levels of p-tau, white matter microstructure within the fornix is a potential target for intervention to reduce APOE4's negative impacts on memory performance. Further, this finding suggests that varying levels of p-tau influences the mechanisms by which APOE4 affects memory, and thus interventional strategies should be identified, examined, and recommended based upon their mechanism of action and the patient's current AD neuropathology stage. This has clinical implications which would suggests that when someone is an older adult and APOE4 carrier, it may be important for them to receive testing to identify their level of p-tau which will help inform recommendations for them. For example, an APOE4 carrier with low p-tau may be recommended to engage in behaviors that promote white matter microstructural health, whereas an APOE4 carrier with higher rates of p-tau may be encouraged to seek more pressing interventions (e.g., exercise, newer interventions for tauopathy including interventions to reduce insulin resistance to reduce hyperphosphorylation). For example, animal studies have found that drugs that stimulate insulin secretion prevent aberrant tau phosphorylation (Hansel et al., 2016; Farr et al., 2019). Further, a meta-analysis by Campbell and colleagues (2018) found that for individuals with diabetes, the use of metformin was related to decreased risk of developing AD and reduced cognitive impairment. However, insulin secretion drug use in humans to reduce AD is a relatively new interventional strategy and grants are in the early stages of examining the potential underlying neuroprotective potential of insulin in reducing p-tau in humans (Kellar & Craft, 2020).

When examining the role of education as potentially differentially buffering the effects of p-tau burden and APOE4 status on memory performance through white matter microstructure, our exploratory findings suggest that education was not a significant overall moderator of the moderated mediation. However, examination of the indirect effect at various levels of p-tau revealed that at low levels of p-tau, increased education was related to a stronger indirect effect (APOE4 → FA of the fornix → Memory Performance). However, at average and high levels of p-tau, there was no significant indirect effect. These findings provide some evidence similar to what was mentioned before. At lower levels of neuropathology, particularly phosphorylation, increased years of education was related to a stronger indirect effect, suggesting FA of the fornix was a significant mediator. However, as hyperphosphorylation increased, this indirect effect was no longer significant. Nonetheless, the exploratory finding that at the lowest tau levels, FA of the fornix was a significant mediator as education increased suggests that when AD neuropathology is at lower stages, the APOE4 allele may impact memory through degradation of the fornix, particularly in those with higher education. An explanation for this could be that as education increases for those at the lowest levels of ptau, the mechanism by which APOE4 impacts memory through the fornix remains, as increased education is possibly buffering other AD mechanisms from overriding this effect. Then, as ptau levels increase, the buffering effect of increased education is depleted and the indirect effect of white matter microstructure in the fornix is no longer the driving force.

This study is not without some limitations. One limitation is that the participants were primarily White and highly educated. These factors impact the generalizability of our findings, especially as there are data that suggests the relation between APOE4 and cognitive functions in other races and ethnicities varies. For example, research suggests APOE4 carriership is more

common in Black individuals and is less associated with risk for AD when compared to the APOE4 and AD relationship in White samples (Evans et al., 2003; Marden et al., 2014). Another potential limitation was the way in which education was measured. Within this study, education was a moderator variable that was measured by self-reported years of education. There are studies that have determined that, while years of education tends to be a strong predictor of cognitive outcomes, quality of education is a much more ideal measure. Measures of reading ability have been one commonly used proxy measure of quality of education/cognitive reserve that has been found to be more related to cognitive functions than years of education and has been found to be a better predictor of decline in memory, particularly within samples of ethnic minorities (Dotson, Kitner-Triolo, Evans, & Zonderman, 2009; Manly, Schupf, Tang, & Stern 2005). However, given our study sample was largely White, we feel years of education is an adequate reflection of the value of the educational experience for our sample. Another limitation was the collection of DTI data. As this study was a part of a much larger multisite study, DTI data was only collected at a subset of sites and for a subset of participants. There is a possibility that those who received DTI were somehow different from the larger participants. Future studies should consider replication with a larger, more representative sample in terms of race/ethnicity and education level.

CHAPTER 5

GENERAL DISCUSSION

As the population of individuals aged 65 and older continues to grow, it is imperative that we seek ways to better understand risk and protective factors that impact neurodegenerative disorders, particularly Alzheimer's disease, as AD risk increases with increased age. The results presented in the above analyses provide some information into understanding white matter microstructure as one potential underlying mechanism by which APOE4 impacts memory.

Within our first aim, our analyses revealed that in our sample, APOE4 was related to memory performance, and FA of the fornix was a significant mediator of this relation. Firstly, this finding provided confirming evidence that in a sample of highly educated, White, older adults, being a carrier of the e4 allele is related to poorer memory performance. Secondly, it suggests the e4 allele is involved in degradation of white matter microstructure within the fornix and this negatively impacts memory performance within this group. White matter microstructural degeneration has been found to be a neuropathological feature of Alzheimer's disease and its progression over time (Acosta-Cabronero et al., 2010; Steketee et al., 2016). Being an e4 carrier, particularly for White individuals, has been found to be related to poorer cognition, increased risk of AD, and increased beta-amyloid deposition. Our findings suggests that e4 carriership may have a more direct role in degradation of white matter microstructure in a region of the brain that is particularly important for memory, which then leads to white matter degradation and its clinical expression as evidenced by poorer memory functioning. Though our primary analyses did not find FA of the CGH to be a significant mediator, exploratory analyses found that MD of

the CGH was a significant mediator. This may suggest that MD is a better DTI metric to examine the potential effects within the hippocampal cingulum.

For our second aim, we wanted to determine if the mediation model was different based upon levels of p-tau. Our findings indicated that within this moderated mediation model, the model with the hippocampal cingulum as a mediator was non-significant. This makes sense given this mediation model was not significant in our first aims. With the fornix as a mediator, the moderated mediation was not significant, however, there was a trend suggesting that at low and average levels of p-tau, FA of the fornix was a significant mediator. At high levels of p-tau, however, FA of the fornix was not a significant mediator. Our findings indicate that at high levels of hyperphosphorylation, APOE4's impact on memory functioning may not be through white matter microstructural degradation. This is in line with other work that has found increased AD neuropathology via increases in plaques and tangles may override the effects of other biomarkers of AD, such as APOE4 carriership (Benson et al., 2022). Perhaps once more direct processes, such as tangles, have accumulated enough within the brain, the mediating role of white matter microstructure in the fornix is weakened and dominated by hyperphosphorylation.

Our third aim sought to understand the potential buffering role of education. More specifically, we examined whether increased education would differentially affect the APOE4 x p-tau interaction's impact on the indirect effect. Results indicated a trend that suggested at low levels of p-tau (suggestive of less AD neuropathology), increased education was related to a stronger indirect effect. However, at average and greater levels of p-tau, there was no indirect effect regardless of education levels. This suggests when AD neuropathology is low, FA of the fornix may be a significant mediator, especially as education increases. This may indicate that at lower levels of neuropathology but a low level of education, e4's impact on memory functioning

may be through another mechanism that overrides the effect of increased education. However, it has been shown that individuals with greater education are able to tolerate higher brain pathology (e.g., amyloid burden) prior to the clinical expression (Kemppainen et al., 2008; Stern, 2013). This may explain how for those with higher education in this sample of individuals already exhibiting subjective and/or objective cognitive difficulties, e4's impact on memory through FA of the fornix strengthens.

The goal of this project was to better understand some of the potential mechanisms by which APOE4 impacts memory functioning and identify factors that may alter this relationship. Taken together, this project provided support that the APOE4 allele, which is thought to be less effective in transporting fats important for axonal health, plays a role in degradation within the white matter microstructure within the fornix, which then leads to poorer memory performance. This finding has several implications. While individuals cannot change their genetic makeup, understanding potential mechanisms by which APOE4 elicits negative cognitive outcomes can be beneficial when considering prevention and intervention strategies. Regarding prevention, our findings suggest that for individuals who have a background similar to those in our study, increasing white matter microstructural health throughout the lifetime might be particularly important. This can be done through several avenues that help support brain health, one being exercise, which can serve as both a preventative and interventional method (Clark et al., 2019). For individuals who are APOE4 carriers, interventions that may help improve the health of the fornix may be of special importance. Burzynska and colleagues (2017) found that a dance intervention was related to increased FA in the fornix and paralleled changes in RD and MD in the fornix after 6 months, which they attributed to both macro- and micro-structural reorganization of the fornix (Burzynska et al., 2017). However, they did not find that this

structural change was related to cognitive change after 6 months, but their studies' timeframe may have been too short to show clinical changes in cognitive function.

When we examined moderators of our model within our sample of White, highly educated older adults, our findings pointed to the idea that there are likely multiple mechanisms by which APOE4 impacts memory functioning when neuropathology load is high. While FA of the fornix is one mediator, our findings suggest at high levels of tauopathy, FA of the fornix was not a significant mediator. This implies that at a certain point, the impact of tauopathy likely overrides the mediating role of white matter microstructural degradation within the fornix. This is further supported when we examined education within this context which hinted that at lower levels of tauopathy, increased education was related to a significant mediation model. However, at greater levels of tauopathy, education was not a significant moderator and white matter microstructure was no longer a significant mediator. These findings point to one of the hypotheses of the cognitive reserve theory that suggests the protective effects of cognitive reserve becomes depleted as the level of pathology increases (Stern, 2009; Soldan et al., 2013).

The findings from this study have provided insight into some mechanisms by which APOE4 may be indirectly impacting memory functioning in a sample of White, highly educated individuals experiencing subjective and/or objective cognitive difficulties. Given Black individuals are at an increased risk of developing Alzheimer's disease, have greater rates of vascular burden, and higher rates of e4 carriership (though e4 may not be as related to negative cognitive health as it is for White Americans), there is much work that needs to be done in understanding the role of APOE4 as an AD biomarker in Black individuals and understanding white matter microstructural health as a mechanism by which APOE4 may be related to cognitive function for this group. Though not much can be done about an individual's genetic

composition, understanding the mechanisms that lead to poorer cognitive health for individuals can be the one of the starting points to which preventative and interventional strategies can be based upon.

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Table 2.1 Demographic Table

Variables	**APOE4 negative (n=74)**	**APOE4 positive (n=87)**	**Total (n=161)**	**p value**
Age	75.06 (7.24)	72.84 (6.30)	73.86 (6.82)	.039
Education	15.78 (2.78)	15.91 (2.71)	15.85 (2.74)	.775
Race	White = 89.2% Black = 5.4% Asian = 2.7% Multi-racial = 2.7% **Ethnicity** Non-Hispanic = 90.5%	White = 94.3% Black = 2.3% Asian = 2.3% Multi-racial = 1.1% **Ethnicity** Non-Hispanic = 93.1%	White = 91.9% Black = 3.7% Asian = 2.5% Multi-racial = 1.9% **Ethnicity** Non-Hispanic = 91.9%	.639
**Sex AAB	Female = 45.9% Male = 54.1%	Female = 35.6% Male = 64.4%	Female = 40.4% Male = 59.6%	.184
**Diagnosis	SMC = 24.3% MCI = 56.8% AD = 18.9%	SMC = 10.3% MCI = 56.3% AD = 33.3%	SMC = 16.8% MCI = 56.5% AD = 26.7%	.021
ADNI Mem	.294 (.91)	-.221 (.80)	.016 (.89)	<.001

Note: **Chi-square test run

Table 2.2. Correlations

	1.	2.	3.	4.	5.	6.	7.	8.
1. Age	-							
2. Education	-.142	-						
3. Mean ptau	.039	-.344**	-					
4. FA CGH	-.361**	.041	-.114	-				
5. RD CGH	.438**	-.053	.199**	-.677**	-			
6. FA FX	-.34**	-.079	.100	.382	-.517**	-		
7. RD FX	.361**	.070	-.150	-.338**	.460**	-.897**	-	
8. e4 status	-.162*	.023	.253**	.103	.027	-.125	.102	-

Table 3.1. Demographic Characteristics

Variable	**APOE4 negative (n=74)**	**APOE4 positive (n=87)**	**Total (n=161)**	**p value**
Age	75.06 (7.24)	72.84 (6.30)	73.86 (6.82)	.039
Education	15.78 (2.78)	15.91 (2.71)	15.85 (2.74)	.775
Race	White = 89.2% Black = 5.4% Asian = 2.7% Multi-racial = 2.7% **Ethnicity** Non-Hispanic = 90.5%	White = 94.3% Black = 2.3% Asian = 2.3% Multi-racial = 1.1% **Ethnicity** Non-Hispanic = 93.1%	White = 91.9% Black = 3.7% Asian = 2.5% Multi-racial = 1.9% **Ethnicity** Non-Hispanic = 91.9%	.639
**Sex AAB	Female = 45.9% Male = 54.1%	Female = 35.6% Male = 64.4%	Female = 40.4% Male = 59.6%	.184
MMSE	27.32 (2.5)	26.23 (2.77)	26.73 (2.70)	.01
**Diagnosis	SMC = 24.3% MCI = 56.8% AD = 18.9%	SMC = 10.3% MCI = 56.3% AD = 33.3%	SMC = 16.8% MCI = 56.5% AD = 26.7%	.021
ADNI Mem	.294 (.91)	-.221 (.80)	.016 (.89)	<.001

*Note. Sex AAB = Sex assigned at birth.

Table 4.1. Demographic Table

Variables	**APOE4 negative (n=74)**	**APOE4 positive (n=87)**	**Total (n=161)**	**p value**
Age	75.06 (7.24)	72.84 (6.30)	73.86 (6.82)	.039
Education	15.78 (2.78)	15.91 (2.71)	15.85 (2.74)	.775
Race	White = 89.2% Black = 5.4% Asian = 2.7% Multi-racial = 2.7% **Ethnicity** Non-Hispanic = 90.5%	White = 94.3% Black = 2.3% Asian = 2.3% Multi-racial = 1.1% **Ethnicity** Non-Hispanic = 93.1%	White = 91.9% Black = 3.7% Asian = 2.5% Multi-racial = 1.9% **Ethnicity** Non-Hispanic = 91.9%	.639
**Sex AAB	Female = 45.9% Male = 54.1%	Female = 35.6% Male = 64.4%	Female = 40.4% Male = 59.6%	.184
MMSE	27.32 (2.5)	26.23 (2.77)	26.73 (2.70)	.01
Mean ptau	24.53 (14.14)	32.39 (15.78)	28.77(15.50)	.001

**Diagnosis	SMC = 24.3% MCI = 56.8% AD = 18.9%	SMC = 10.3% MCI = 56.3% AD = 33.3%	SMC = 16.8% MCI = 56.5% AD = 26.7%	.021
ADNI Mem	.294 (.91)	-.221 (.80)	.016 (.89)	<.001

Note: **Chi-square test run

Table 4.2. Bivariate and Point-biserial Correlations

	1.	2.	3.	4.	5.	6.	7.	8.	9.
1. Age	-								
2. Education	-.142	-							
3. Mean ptau	.039	-.344**	-						
4. FA CGH	-.361**	.041	-.114	-					
5. RD CGH	.438**	-.053	.199**	-.677**	-				
6. FA FX	-.34**	-.079	.100	.382	-.517**	-			
7. RD FX	.361**	.070	-.150	-.338**	.460**	-.897**	-		
8. e4 status	-.162*	.023	.253**	.103	.027	-.125	.102	-	
9. Sex	-.031	-.192*	.187*	-.174*	-.053	.116	-.143	-.105	-

Table 4.3. Conditional Moderated mediation at varying levels of the moderators

Ptau	Education	Effect	SE	95% LLCI	95%ULCI
13.2697	13.1141	-.0793	.0678	-.237	.0279
13.2697	15.8509	-.1083	.0597	-.2417	-.0115
13.2697	18.5877	-.1373	.0756	-.3124	-.0161
28.7734	13.1141	-.0453	.0422	-.1461	.0179
28.7734	15.8509	-.0578	.0413	-.1584	-.001
28.7734	18.5877	-.0703	.0581	-.2145	.0056
44.2772	13.1141	-.0112	.0488	-.1306	.0707
44.2772	15.8509	-.0073	.0552	-.1431	.0856
44.2772	18.5877	-.0034	.0941	-.2308	.1626

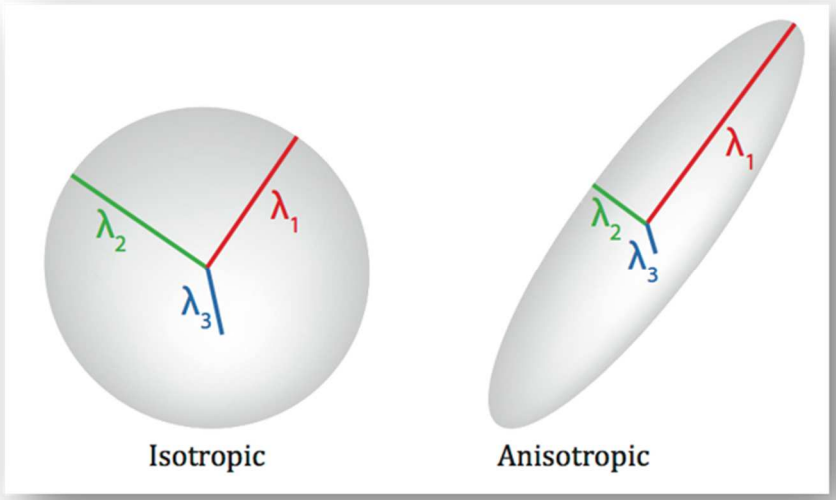

Figure 1.1. Visual schematic of a diffusion tensor and measures used to determine anisotropy and diffusion.

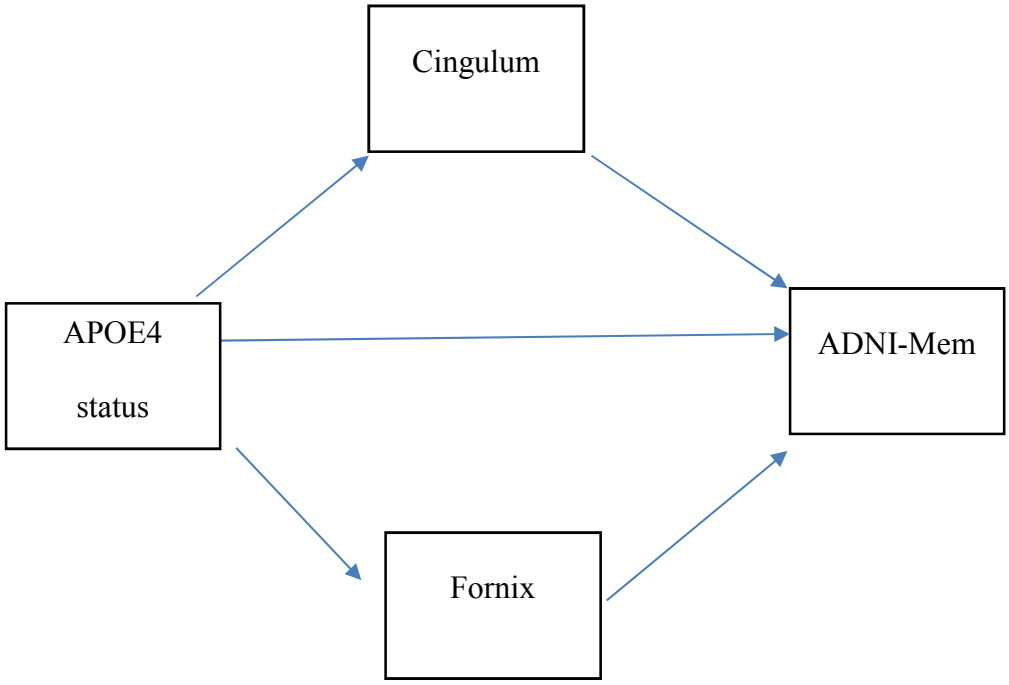

Figure 1.2. Proposed moderated mediation analysis depicting the relation between APOE4 status and memory performance mediated by a white matter tract and moderated by CSF p-tau levels.

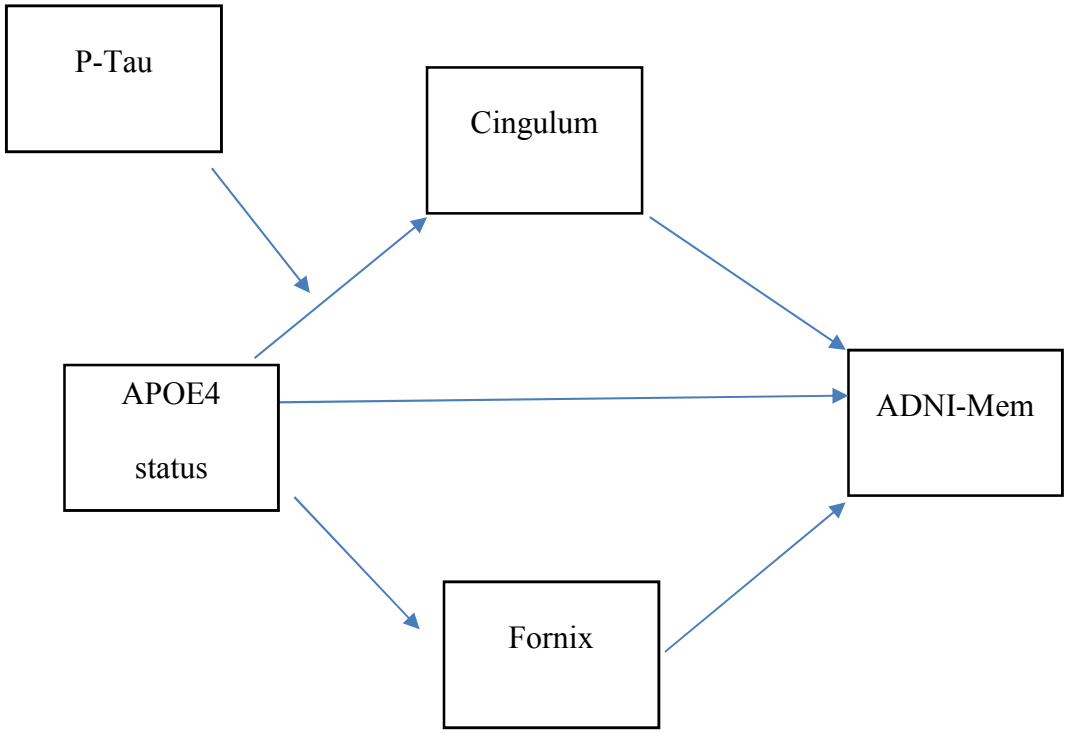

Figure 1.3. Proposed moderated mediation analysis depicting the relation between APOE4 status and memory performance mediated by a white matter tract and moderated by CSF p-tau levels.

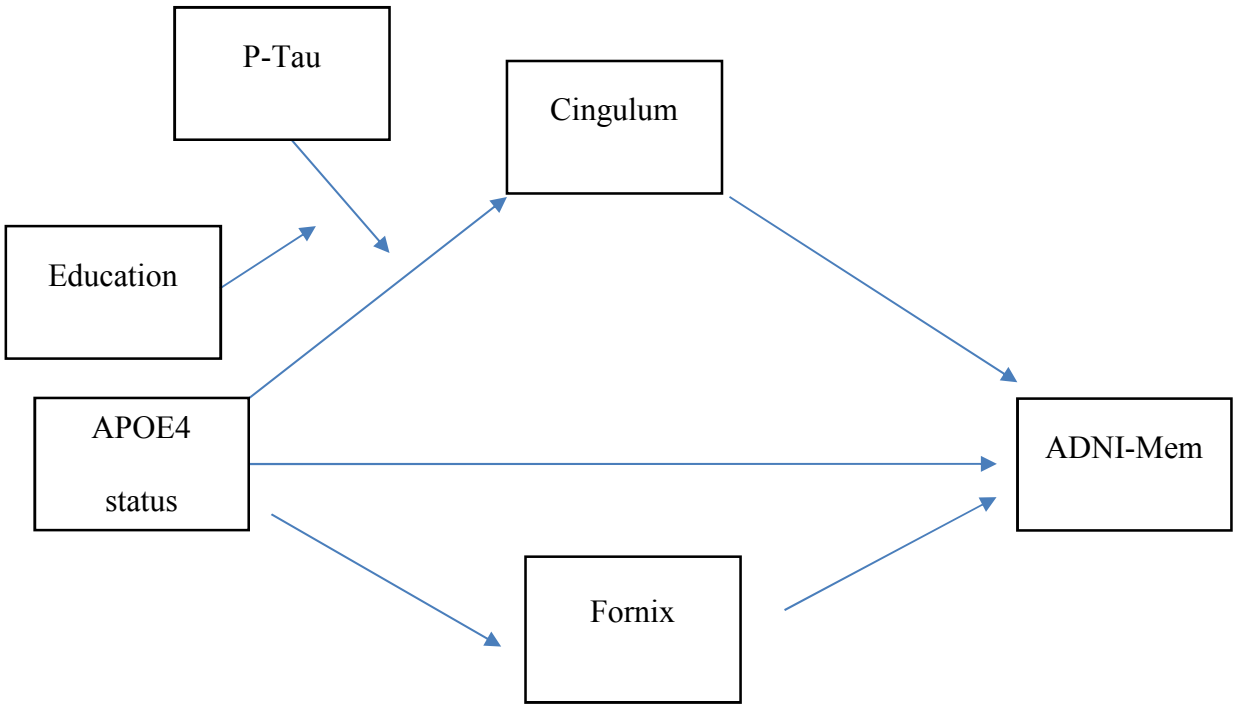

Figure 1.4. Proposed moderated-moderated mediation analysis depicting the moderated mediation analysis in Figures 2 and 3 moderated by education.

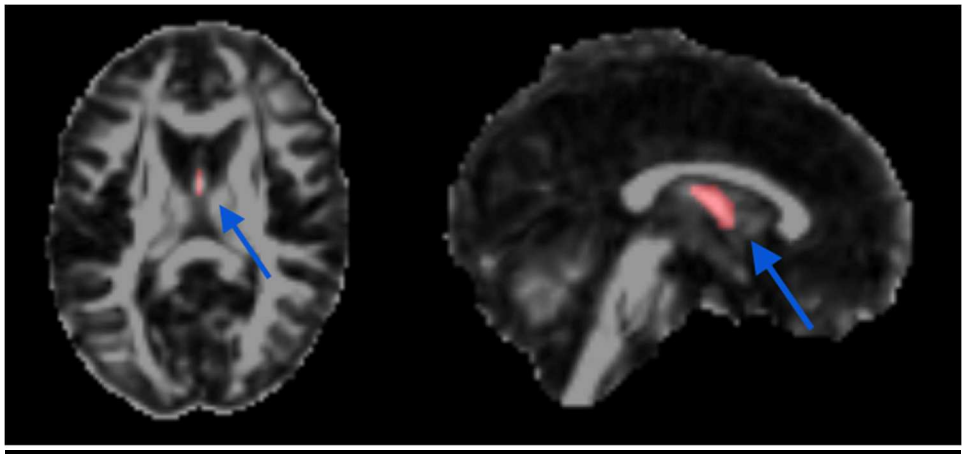

Figure 3.1. FA map of a participant with a superimposed ROI (fornix body & column) delineation. Images are shown in the axial and sagittal view.

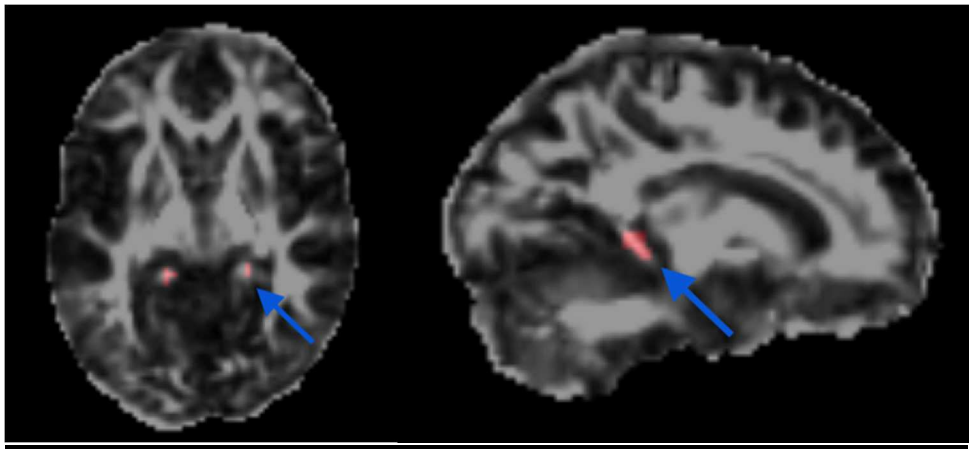

Figure 3.2. FA map of a participant with a superimposed ROI (hippocampal cingulum) delineation. Images are shown in the axial and sagittal view.

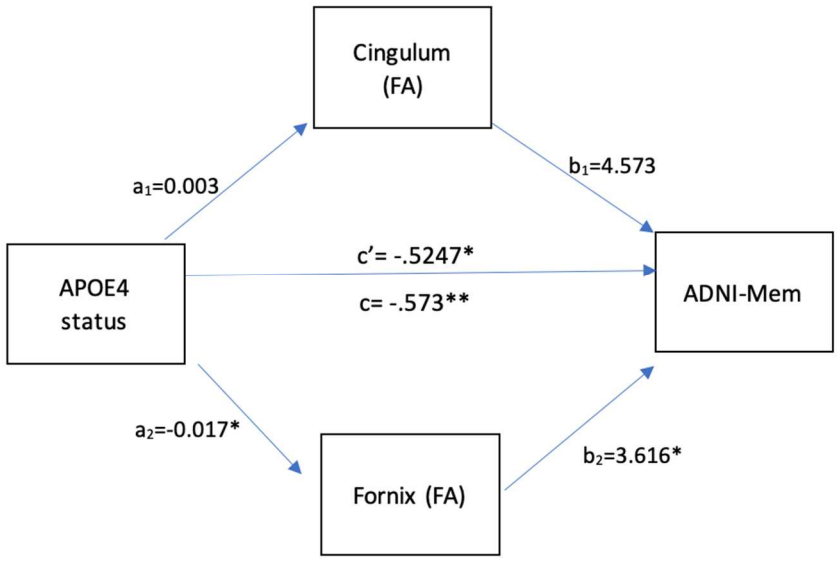

Figure 3.3. A statistical diagram of the mediation model for the relations amongst APOE4 status, FA regions of interests, and memory function controlling for age. Standardized regression coefficients are presented. *p<.05, **p=<.001

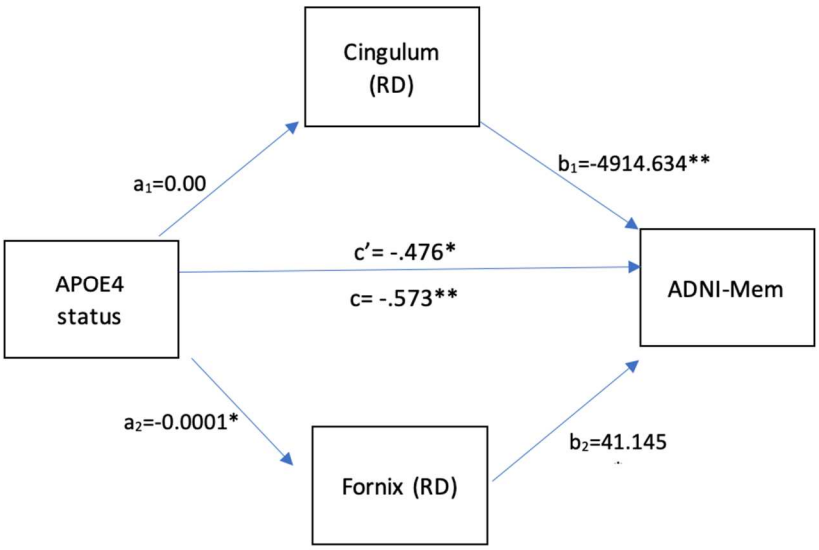

Figure 3.4. A statistical diagram of the mediation model for the relations amongst APOE4 status, RD regions of interests, and memory function controlling for age. Standardized regression coefficients are presented. *p<.05, **p<.001.

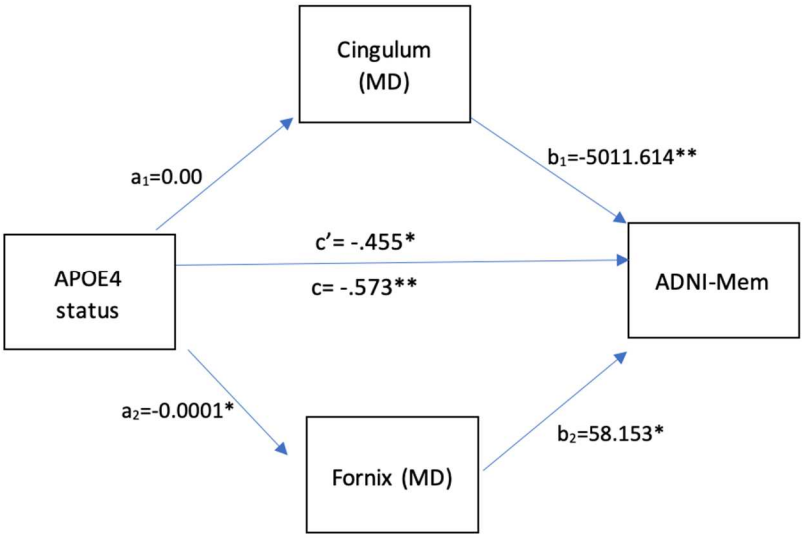

Figure 3.5. A statistical diagram of the mediation model for the relations amongst APOE4 status, MD regions of interests, and memory function controlling for age. Standardized regression coefficients are presented. *p<.05, **p<.001.

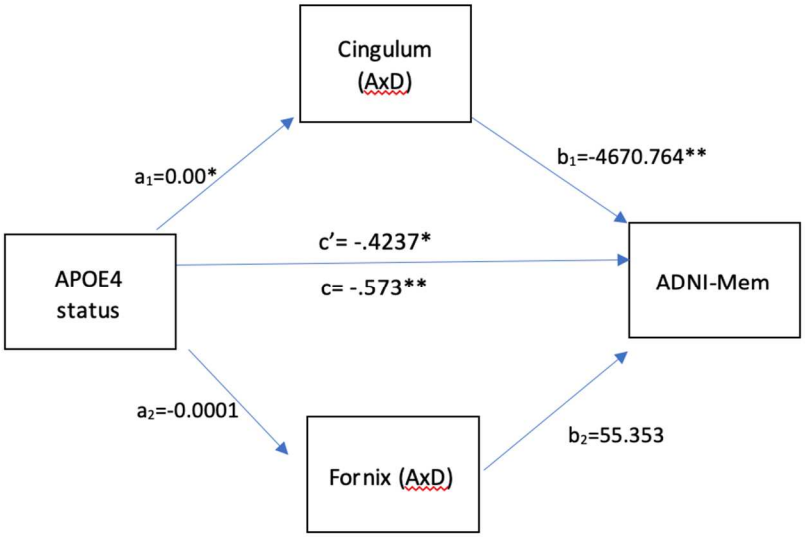

Figure 3.6. A statistical diagram of the mediation model for the relations amongst APOE4 status, AxD regions of interests, and memory function controlling for age. Standardized regression coefficients are presented. *p<.05, **p<.001.

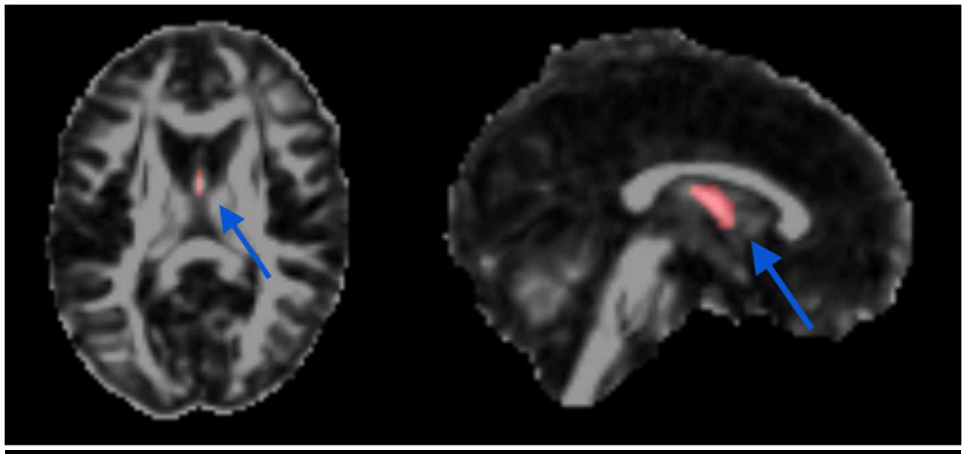

Figure 4.1. FA map of a participant with a superimposed ROI (fornix body column) delineation. Images are shown in the axial and sagittal view.

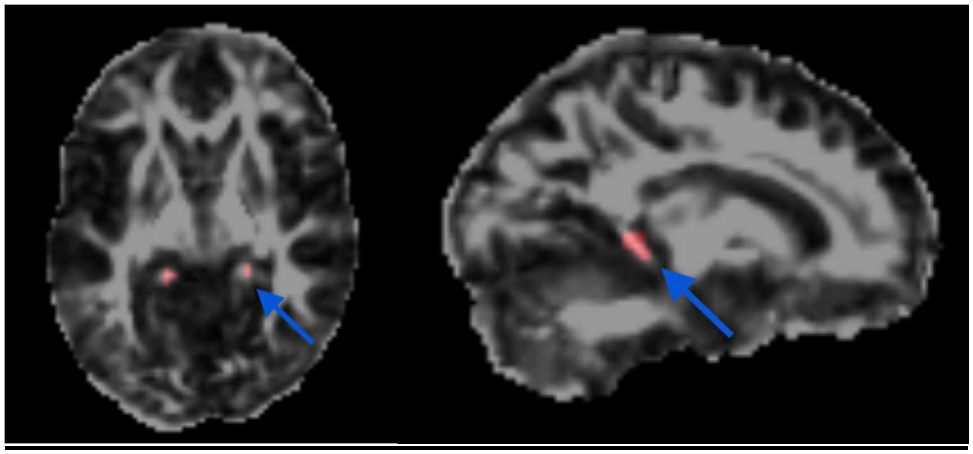

Figure 4.2. FA map of a participant with a superimposed ROI (hippocampal cingulum) delineation. Images are shown in the axial and sagittal view.

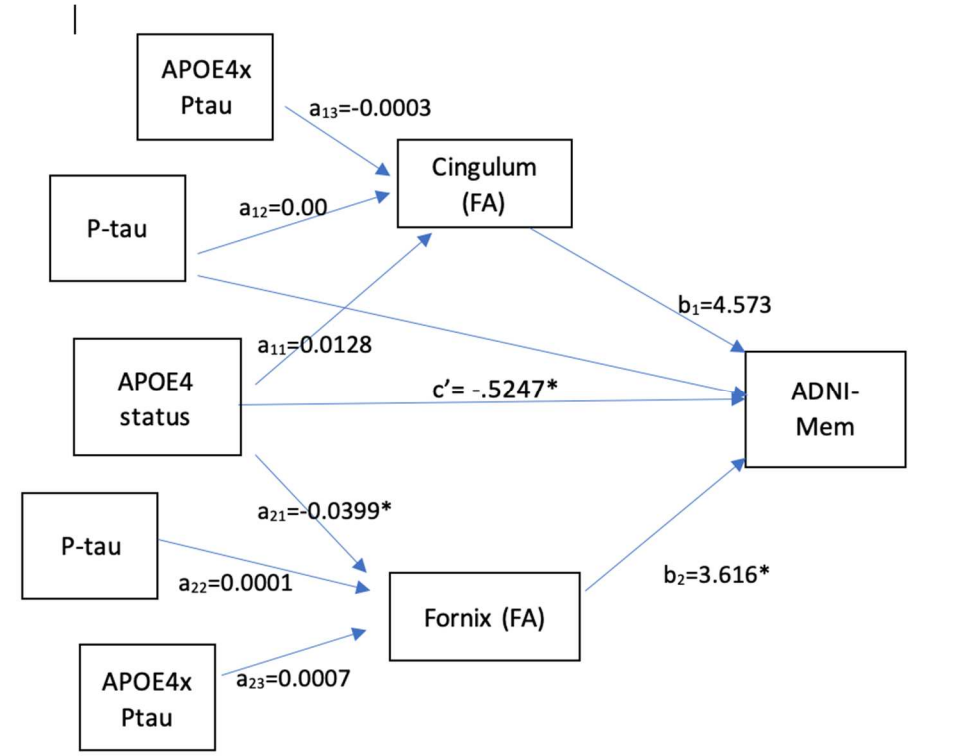

Figure 4.3. A statistical FA diagram of the moderated mediation model for the relations amongst APOE4 status, FA regions of interests, and memory function controlling for age. Unstandardized regression coefficients are presented. *p<.05.