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Biomarker presentation, amyloid status, and connectivity between the prefrontal cortex and hippocampus in studies of clinical Alzheimer’s disease/dementia

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BIOMARKER PRESENTATION, AMYLOID STATUS, AND CONNECTIVITY BETWEEN THE PREFRONTAL CORTEX AND HIPPOCAMPUS IN STUDIES OF CLINICAL ALZHEIMER'S DISEASE/DEMENTIA

by

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ABSTRACT

Through rigorous research over the past 100 years, great strides have been made in identifying the neuropathological basis of Alzheimer's disease (AD), its' prognosis, and the onset of cognitive symptoms. Proteins such as amyloid-beta (Aβ) plaques and tau are recognized as primary biomarkers that define AD and neuroimaging methods have been developed to measure these proteins *in vivo* allowing for changes to be monitored in life. These advances have led to the view that AD is a continuum rather than one inclusive state. However, there remains a great deal about AD that is still not understood. Such ambiguities range from an imprecise understanding of the neuropathological events that result in dementia to remaining uncertainty as to why some older adults exhibit symptoms of dementia whereas other retain sharp cognitive abilities in old age despite similar underlying pathology.

In an effort to fill some of these gaps in our knowledge, the overarching goals of the work in this dissertation were to disambiguate some of these nuances by examining neurobiological features of AD in living older adults. Data for the first two studies was collected from older adults enrolled in the nationwide Alzheimer's Disease

Neuroimaging Initiative (ADNI) and data in the third study came from participants aged 50 years and older enrolled in a cohort study at the Boston University Alzheimer's Disease Research Center (BU-ADRC).

In the first study, our objective was to investigate links between brain structure, cognitive performance, and neuropathology in participants within the ADNI who identified as Black or African American (BoAA). Relatively little is known about dementia in this population so our goal was to assess if there would be an increase in AD biomarkers abnormalities between the three clinical syndrome groups: cognitively normal, mildly cognitively impaired (MCI), and dementia. Our findings supported this hypothesis as we found decreased cortical thickness in five of seven selected regions of interest (ROI), decreased hippocampal volume, increased white matter hyperintensities, worsened measures of cognition and function, decreased cerebrospinal fluid (CSF) measures of $A\beta_{1\text{-}42}$, and elevated measures of CSF tau between clinical syndrome groups.

In the next study, we utilized a different subset of ADNI participants to examine how crossing the threshold in Aβ burden from being amyloid negative (A-) to amyloid positive (A+) as measured on positron emission tomography (PET) scans may relate to other neuropathological AD biomarkers and cognition. To make this assessment, we identified two groups of ADNI participants: one who converted in Aβ burden from A- to A+ as measured by amyloid retention on [^{18}F]-labeled florbetapir PET and another who did not convert (e.g. remained A-) over the same follow-up period. We found increased prevalence of *APOE* ε4, greater annualized percent volume loss in selected brain regions, and lower CSF $A\beta_{1\text{-}42}$ in the amyloid converter group compared to the non-converter

group. Amyloid ligand binding on florbetapir PET was also significantly different between the two groups on PET scans taken at two timepoints.

In the last study, we used diffusion tensor imaging (DTI) magnetic resonance imaging (MRI) data from the BU-ADRC to assess the integrity of the cingulum bundle in participants cognitively ranging from normal to amnestic MCI to those with dementia. We found weakened integrity of the cingulum bundle in both dorsal and ventral circuits in participants with worsened cognitive function. Furthermore, we found relationships between diffusion metrics of the cingulum, volume of selected ROI, and measures of executive function and memory. The findings of this study show that age-related changes are likely present in a circuit that connects the prefrontal cortex to medial temporal regions of the brain and that weakening of structural connectivity is related to cognitive decline.

This collection of studies reiterates the complicated nature of AD. Factors such as race, genetics, transitions in biomarker status and perhaps yet to be discovered forms of pathology, all have the potential to modify the clinical presentation of AD. The studies completed in this dissertation help to advance our knowledge but more work is needed to solidify our understanding of the basis of AD.

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LIST OF ABBREVIATIONS

A……Amyloid

Aβ …..…….....Amyloid Beta

$A\beta_{1-40}$ …..……...Amyloid Beta peptide 1-40

$A\beta_{1-42}$ …..……...Amyloid Beta peptide 1-42

AD……Alzheimer's disease

ADNI……Alzheimer's Disease Neuroimaging Initiative

aMCI…….....Amnestic Mild Cognitive Impairment

ANOVA……Analysis of variance

ANCOVA……..Analysis of covariance

APOE…..……...Apolipoprotein

APP…..……...Amyloid Precursor Protein

AT(N)…..……Amyloid, Tau, Neurodegeneration

BoAA…..……...Black or African American

BU-ADRC……...Boston University Alzheimer's Disease Research Center

BUSM …….Boston University School of Medicine

CBI ……..Center for Biomedical Imaging

CD ……...Cingulum - Dorsal

CI……...Confidence Interval

CN…….....Cognitively Normal

CSF……Cerebrospinal Fluid

CST…….Corticospinal Tract

CV ……..Cingulum - Ventral

DTI……...Diffusion Tensor Imaging

EMCI……Early Mild Cognitive Impairment

eTIV ……...Estimated Total Intracranial Volume

FA …….Fractional Anisotropy

FAQ …….Functional Activities Questionnaire

FDR…….....False Discovery Rate

FLAIR…….Fluid Attenuated Inversion Recovery

FOV…….....Field of View

FSL……..... FMRIB Software Library

GM…….....Gray Matter

HARDI…….,,,.High Angular Resolution Diffusion Imaging

HOPE……....Health Outreach Program for the Elderly

HSD…….....Honestly Significant Difference

ID……...… ..Identification

LONI…….....Laboratory of NeuroImaging

LMCI…….....Late Mild Cognitive Impairment

LST…….....Lesion Segmentation Toolbox

MCI..…….....Mild Cognitive Impairment

MD…….....Mean Diffusivity

MRI.…….....Magnetic Resonance Imaging

MTL.…….....Medial Temporal Lobe

N..…….....Neurodegeneration

NACC..……..National Alzheimer's Coordinating Center

NFT.…….....Neurofibrillary Tangles

NHW..…….....Non-Hispanic White

NIA-AA..……National Institute on Aging and Alzheimer's Association

PET..…….....Positron Emission Tomography

PFC ..…….....Prefrontal Cortex

P-TAU……....Phosphorylated Tau

RAVLT..……...Rey Auditory Verbal Learning Test

ROI.…….....Region of Interest

RSC.…….....Retrosplenial Cortex

SD..…….....Standard Deviation

SUVR..…….....Standardized Uptake Value Ratio

T..…….....Tau

TE..…….....Echo Time

TR..…….....Recovery Time

TRACULA..……TRActs Constrained by UnderLying Anatomy

T-TAU…….....Total Tau

WM…….....White Matter

WMH……White Matter Hyperintensities

CHAPTER ONE: INTRODUCTION

Alzheimer's Disease: An Overview

Official sources recognize Alzheimer's disease (AD) as the fifth leading cause of death for Americans aged 65 and older. The prevalence of AD will likely continue to escalate as the oldest members of the baby-boom generation (those born between 1946 and 1964) turned 75 in 2021 (Alzheimer's Association, 2021).

Alzheimer's disease (AD) is a neurological disease characterized by years of changes in the brain which lead a person to develop noticeable symptoms of cognitive decline. Such symptoms are believed to occur as neuronal death takes place. In the healthy brain, neurons are linked to one another through connections called synapses. Information regarding one's internal and external environment is carried as signals through these synapses. This allows different regions of the brain to communicate with one another and these signals form the cellular basis of complex processes like thinking, movement, language, emotions, and memory. Despite early damage to neurons and synapses, individuals may not experience noticeable symptoms right away but the course of AD is progressive. Overtime, these changes often begin to impact one's memory or language abilities. Unfortunately, such damage to the brain can become so devastating that it renders a person unable to carry out basic functions necessary to living.

Through rigorous research over the past 100 years, we have made great strides in obtaining a better understanding of AD, its' prognosis, and the onset of symptoms. We have come to recognize a certain level of cognitive decline is characteristic of aging but in cases of AD, these impairments are compounded. However, our understanding of the

sequence of neuropathological events that result in AD remains imprecise and we have lingering uncertainty as to why these events cause some older adults to exhibit symptoms of dementia whereas other retain sharp cognitive abilities until the age of 100. With these gaps in mind, the goals of the work in this dissertation were to attempt to disambiguate some of these nuances by examining features of AD.

Prior to discussing these studies, this first chapter will present a comprehensive overview of how researchers have come to define AD as both a neuropathologic and clinical diagnosis and the current research framework used to study the progression of AD. Additionally, there will be a description of neuroimaging methods used *in vivo* to diagnose and follow the progression of AD, racial disparities within AD and dementia research and lastly, a description of the study populations that will be examined throughout this dissertation.

Dementia or Alzheimer's Disease?

Aside from being a progressive neurological disease, AD is often referred to as the most common cause of dementia which causes many to wonder what *is* the distinction between dementia and AD? Simply stated, dementia is not specific to any one disease but rather an umbrella term used to describe a particular group of symptoms. Key symptoms of dementia are difficulty with memory, language, and problem-solving that may affect a person's day to day life and the ability to perform everyday activities. Alzheimer's disease is the most common form of dementia *but* there are other forms of dementia. Relatedly, dementia can be caused by other conditions including but not limited to stroke, HIV/AIDS, long-term excessive use of alcohol, and repetitive injury to the brain

(Alzheimer's Association, 2021). What makes the relationship between dementia and AD even more confusing is that the terms are often used interchangeably even in AD literature but thinking of AD as a specific neuropathological disease defined by biological biomarkers and dementia as a syndromic presentation used to describe symptoms that impact memory, performance of daily activities, and communication is likely best way to discern between the two. For the purposes of this dissertation, we will typically use the term "dementia" to describe participants in the studies presented in dissertation since we do not full neuropathologic data for everyone

The diagnosis, or rather naming, of "Alzheimer's disease" traces back to the early 1900s' (Yang et al., 2016). The disease was named after Dr. Alois Alzheimer, a physician in Germany who noticed changes in the post-mortem brain tissue of his patient, Auguste Deter. In the years leading up to her death at age 55 in 1906, Auguste had shown symptoms of amnesia, hallucinations, disorientation, and mania. Upon his patient's death, Dr. Alzheimer received her brain tissue and medical records. He observed thinning of the cerebral cortex in regions thought to be responsible for memory, language and judgement which correlated with Auguste's late life impairments. Along with cortical thinning, Dr. Alzheimer observed extracellular senile plaques and neurofibrillary tangles (NFT) in nerve fibers (Yang et al., 2016). Prior to these findings, such dramatic cognitive changes had very rarely been seen in a patient this young. Likewise, senile amyloid-beta (Aβ) plaques had not been reported in adults under 70 years of age and this was one of the first instances in which NFT were observed (Yang et al., 2016).

Key Biomarkers: Amyloid-Beta (Aβ), Tau, and Neurodegeneration

Amyloid

In the 100+ years since Dr. Alois Alzheimer's discovery, Aβ plaques and NFT have continued to be key biomarkers of AD and a major focus of research studies (Braak and Braak, 1991). Amyloid-beta precursor proteins (APP) are transmembrane proteins found on the surface of cells throughout the human body (Chen & Mobley, 2019). When in the cell membrane, these proteins have many integral physiological functions such as the modulation of synaptic function, facilitation of neuronal growth and survival, and protection against oxidative stress (Bishop & Robinson, 2004).

Amyloid-beta (Aβ) peptides are derived through the cleavage of APP by secretases through two major processing pathways: the nonamyloidogenic and the amyloidogenic pathways (Chen & Mobley, 2019; Jeremic, Jiménez-Díaz, & Navarro-López, 2021). The resulting Aβ peptides produced from these pathways range from 36 to 43 amino acids in length. Upon cleavage, Aβ peptides leave the cell membrane and are processed through the two major pathways (Chen & Mobley, 2019; Lorenzo et al., 2000; Müller et al., 2017). Some peptides can be cleared by cerebrospinal fluid and the vascular system whereas particularly soluble peptides produced by the amyloidogenic pathway can be more difficult to clear due to their "sticky" nature. The sticky nature of these peptides allows for soluble peptides to interact with similar molecules, bond, and aggregate into long fibrils that form β-pleated sheets which are resistant to degradation (Brothers, Gosztyla.& Robinson, 2018; Hensley et al., 1994). These β-sheets eventually form plaques on nerve cells (Lorenzo et al., 2000). Such plaques are initially diffuse accumulations of extracellular Aβ but overtime they progress to being more densely

packed fibrillar deposits located in the interstitial fluid (Chen & Mobley, 2019; Spires-Jones & Hyman, 2014). This sequence of events (e.g. the failure of Aβ clearance and subsequent aggregation which ultimately leads to neurodegeneration) is commonly referred to as the "amyloid cascade hypothesis" (Hardy & Higgins, 1992; Jeremic et al., 2021; Selkoe & Hardy, 2016). The amyloid cascade hypothesis was the prevailing view in AD research in the early 1990's but in recent years, attention has shifted towards examining the role of neurotoxic Aβ oligomers (Brothers et al., 2018; Chen & Mobley, 2019; Spires-Jones & Hyman, 2014).

Tau

The other key protein that is a neuropathological hallmark of AD is tau. Certain isomers of tau are ubiquitous in the healthy human brain and have a significant role in allowing for axonal transport of vesicles and organelles through association with microtubules as well as microtubule stabilization (Buee et al., 2000; Neale et al., 2018). Yet when hyperphosphorylated tau becomes unbound from microtubules, these molecules begin to stick to one another and form aggregates which disrupt normal cell function (Spires-Jones & Hyman, 2014). Overtime, the presence of hyperphosphorylated tau leads to the formation of paired helical filaments and NFT (d'Orange et al., 2018).

The presence of NFT in AD studies has been highly documented. Many of these studies have shown a relationship between the amount and distribution of NFT with the onset of cognitive decline and neuronal loss (Chen & Mobley, 2019; d'Orange et al., 2018). This led many to believe NFT were the primary drivers of subsequent neurodegeneration. However, this view has come into question as other studies have

shown tau in NFT are **not** directly linked to deficits in axonal trafficking and synapse loss, but instead can compromise intracellular transport and other cellular functions that later manifest issues as disease progresses (Chen & Mobley, 2019; Spire-Jones & Hyman, 2014). Due to these findings, focus from NFT has shifted to tau oligomers. Still others believe it is the interaction of Aβ and tau that synergistically leads to toxicity in the brain (Chen & Mobley, 2019; Jeremic et al., 2021; Spire-Jones & Hyman, 2014). These mechanisms will continue to be explored as both Aβ and tau proteins remain key targets in clinical trials and drug therapy research.

Neurodegeneration

Another key biomarker in the diagnosis of AD is neurodegeneration. Unlike changes in amyloid and tau which are believed to be specific indicators of AD, neurodegeneration can result from AD but is not specific to AD alone (Albert et al., 2011; Jack et al., 2018). In cases of AD, the process of neurodegeneration occurs as a result of both synapse and neuronal loss which leads to cortical atrophy (Chen & Mobley, 2019). Neurodegenerative changes have been documented using a wide range of methods. Previous studies using synaptic protein markers and electron microscopy to measures microscopic changes such as synapse and neuronal loss led to the use of magnetic resonance imaging (MRI) to capture more macroscopic changes in regions of interest such as the entorhinal cortex and hippocampus. Many of these studies have further examined how degeneration of synapses and neurons as well as cortical atrophy correlates with cognitive dysfunction and have found striking links (Chen & Mobley, 2019; Morrison and Hof, 1997). Specifically, these studies have found that even in

individuals that have significant amyloid and/or tau pathology, the onset of dementia-like syndromes is often dependent on neuronal and synaptic loss as well as volumetric changes to regions within the temporal lobe (Jeremic et al., 2021; Morrison & Hof, 1997).

The Development of a Research Framework: AT(N)

In 2011, the National Institute on Aging and Alzheimer's Association (NIA-AA) created a workgroup to revise the previous criteria for AD dementia (Jack et al., 2011; McKhann et al., 2011). Notable updates to the 1984 criteria included the incorporation of biomarkers, such as measures of cerebrospinal fluid (CSF), for underlying disease state and the formalization of different stages of disease. The characterization of different phases of AD stemmed from research studies conducted in the 1990s' which had shown a growing number of people were experiencing gradual impairments in cognitive function that exceeded age related decline, yet were not severe enough to impede daily life (Albert et al., 2011; Jack et al., 2011). These findings led to the idea that AD should be recognized as a continuum or continuous process rather than one inclusive disease state (Jack et al., 2011; Jack et al., 2018). This intermediate state between normal cognitive functioning and the more severe clinical symptomology of dementia was termed "mild cognitive impairment (MCI)," and was added to disease staging criterion (Petersen et al., 2014).

In the years following the revisions by the NIA-AA in 2011, the concept of MCI continued to be formalized and biomarkers derived from positron emission tomography (PET) imaging were validated as appropriate proxies for measuring neuropathologic

changes of AD (Abrahamson et al., 2019; Jack et al., 2018; Selkoe & Hardy, 2016). Additional goals of being able to diagnose AD before the onset of cognitive symptoms also emerged since the current criteria relied on the expression of symptoms which only became apparent once AD pathology had entered very advanced stages (Lee, Kim, Hong, & Kim, 2019). Therefore in 2018, another group was assigned by the NIA-AA leadership group to create a unifying update for the 2011 guidelines. The results were a "research framework" to be primarily used in observation and interventional research, but **not** routine clinical care (Jack et al., 2018). The basis of this framework stems from the previously described biological biomarkers and harmonizes those biomarkers as three categories: Aβ, tau, and neurodegeneration, and is referred to as the AT(N) framework. Measures of cognition are not formally included in this framework since they are not biological in nature, but they remain key for staging the severity of cognitive impairment. The name of this framework is structured as "AT(N)" based on the decision that biomarkers such as Aβ and tau are specific to AD and the presence of both is required to fulfill neuropathologic criteria for AD. Neurodegeneration is denoted in parentheses because it is not specific to AD but more generally used to stage the severity of disease progression (Jack et al., 2018). Altogether, by implementing the AT(N) framework, a key goal is to be able to diagnose AD in living persons based upon biological constructs (Jack et al., 2018).

Neuroimaging Methods Used to Study the AT(N) Framework

Commonly used methods to measure AD pathology *in vivo* include CSF, structural MRI, and PET. The AT(N) framework was designed so that complete

biomarker characterization can be conducted with either CSF or imaging alone depending on what technology is available (Jack et al., 2018). The two most common methods for measuring amyloid ("A") are via CSF samples or PET imaging with an amyloid binding ligand (Jack et al., 2018). There are many isoforms of Aβ peptides but CSF measures of $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ are believed to be the most reliable indicators of AD. $A\beta_{1\text{-}40}$ is the most abundant Aβ peptide in CSF but it the CSF concentration of $A\beta_{1\text{-}42}$ and the ratio of $A\beta_{1\text{-}42/}A\beta_{1\text{-}40}$ that correlates best with amyloid deposition and the progression of AD (Lee et al., 2019). At the time of the 2018 revisions, the CSF concentrations of $A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}42}$ / $A\beta_{1\text{-}40}$ became the accepted "A" AD biomarkers due to studies which showed low readings of $A\beta_{1\text{-}42}$ (or $A\beta_{1\text{-}42}$/ $A\beta_{1\text{-}40}$) correspond best with more advanced disease states (Chen & Mobley, 2019; Jack et al., 2018; Toepper, 2017). Specifically, it was shown that a decrease in $A\beta_{1\text{-}42}$ could be attributed to the fact that $A\beta_{1\text{-}42}$ is most likely to aggregate into fibrils and plaques meaning a lesser amount can diffuse into CSF (Lee et al., 2019).

The second, less invasive means for measuring Aβ is PET imaging, which has been in use since 2002. Commonly used amyloid PET ligands include the carbon-labeled Pittsburgh-Compound B (PiB), and related fluorine-18 amyloid ligands Florbetapir, Florbetaben, and Flutemetamol (Abrahamson et al., 2019). Ligands currently available are only able to detect insoluble, fibrillar amyloid (Mattson et al., 2015; Selkoe, & Hardy, 2016). Ongoing studies continue to assess the mechanics of these tracers and which are most suitable in various scenarios (disease, severity, age, etc).

Like that of amyloid, aggregated tau (or neurofibrillary tangles; "T") can be measured through CSF samples of phosphorylated tau or PET with a tau binding ligand

(Jack et al., 2018). Neurofibrillary tangles are primarily made up of phosphorylated tau (p-tau) proteins (Toepper, 2017). Findings in AD studies have shown that increased levels of p-tau reflect a more advanced disease state (Albert et al., 2011; Chen & Mobley, 2019). Being able to use PET to assess tau accumulation may prove very promising to the field since tau is believed to accumulate intracellularly making it difficult to access *in vivo* (Saint Aubert et al., 2017). The advent of tau binding ligands remains very recent and there are many ongoing studies assessing which ligands have the best specificity and sensitivity with minimal off target binding.

The use of either CSF or PET can be advantageous for different reasons. CSF is a cheaper and the more accessible alternative compared to PET yet the nature of a lumbar puncture required for CSF sampling is invasive (Tosun et al., 2021). Advantages of PET imaging include better spatial representation across the cortex allowing for both localization and measuring the strength of deposition. Still, PET is expensive, requires use of a radioactive tracer, and in studies of amyloid, is limited to measuring solely insoluble, fibrillar peptides whereas CSF can measure both soluble and insoluble isoforms (Mattson et al., 2015). Another important consideration when using and/or interpreting results of the two methods are that changes in CSF are believed to appear prior to PET (Jack et al., 2013). This is true of both amyloid and tau (Albert et al., 2011; Hardy & Selkoe, 2016; Landau et al., 2013; Ossenkoppele et al., 2021). The relationship between these two markers, when changes emerge, and the value of other assays like plasma measures continue to be researched (Chen & Mobley, 2019; Tosun et al., 2021).

Figure 1.1 illustrates a current model of the temporal progression of AD-related biomarker abnormalities.

Neurodegeneration ["(N)"] can be measured through anatomic (structural) MRI, CSF samples of total tau (t-tau), or fluorodeoxyglucose (FDG) – PET (Jack et al., 2018). Atrophy measured through structural MRI is believed to reflect cumulative loss and shrinkage of the neuropil. Structural MRI is commonly used to assess atrophy by measuring the cortical thickness, surface area, gray matter volume, or subcortical volume of identified regions of interest (ROI) (Schwarz et al., 2016). Through the early 2000s', many studies emphasized the use of structural MRI to assess longitudinal change within ROI to best predict who with MCI would convert to having AD (Convit et al., 2000; Fan et al., 2008; Killiany et al., 2003). Current studies rely on both cross-sectional and longitudinal measures of structural MRI and measure the volume or cortical thickness of regions within the medial temporal lobe (e.g. hippocampus and entorhinal cortex), posterior cingulate, in addition to a myriad of cortical regions that are often referred to as the "AD cortical signature" (Dickerson et al., 2009; Gold et al., 2012; Sepulcre et al., 2016; Petersen et al., 2014). The CSF measure of t-tau has also been categorized as neurodegeneration within the AT(N) framework because it encompasses hyperphosphorylated tau concentration in addition to the total impact of neuronal injury that consequently occurs (Toepper, 2017).

Neurodegeneration can be attributed to other comorbid conditions aside from AD. However, it is still considered a valuable piece of the AT(N) framework due to its role in helping predict cognitive decline and staging individuals within the framework (Jack et

al., 2018; Toepper, 2017). When implementing the AT(N) framework, the presence of each biomarker is characterized as (+/-) where "+" indicates the biomarker is present and therefore an abnormal scan whereas "-" indicates a normal scan albeit it does not indicate the biomarker is entirely absent. It is believed that the more biomarker groups that are abnormal, the more advanced the pathologic state (Jack et al., 2018). Though currently at three biomarkers, the AT(N) framework has been designed to incorporate more biomarkers as other validated measures emerge (Jack et al., 2018).

Beyond the Framework: Diffusion Tensor Imaging

For decades researchers have been studying neurodegeneration and age-related neuronal changes in the gray matter (GM). More recently, enthusiasm has shifted towards using MRI to examine changes in the white matter (WM) (Gold et al., 2012; Liu et al., 2017; Luo et al., 2020). This enthusiasm is the result of post-mortem findings that have shown a loss of myelin, axonal degeneration, and gliosis in the brains of people who died from AD as well as an increasing number of MRI studies reporting WM abnormalities in brains of living people with AD (Araque Caballero et al., 2018; Lee et al., 2015; Salat et al., 2009). The de-myelination of axonal fibers and overall reduced integrity is believed to result in less efficient conduction of neural signals and impaired neuronal transmission (Fan et al., 2019). It was initially thought that changes in the WM may be secondary to those in the GM, but recent studies have shown these changes are independent of one another and that alterations to WM may even occur first (Esrael et al., 2021). To further investigate where changes in the WM occur during the progression of AD, researchers

are using a form of MRI known as diffusion tensor imaging (DTI) to quantify these changes in WM pathways (Esrael et al., 2021; Liu et al., 2017; Ouyang et al., 2021).

Similar to structural MRI, DTI relies upon the presence of water throughout living tissue. Because water has the propensity to diffuse differently in various tissue types, DTI is able to measure age related changes in the microstructural diffusion of water (Gold et al., 2012; Liu et al., 2017). In fluid-filled regions such as the ventricles, water is rarely bound and diffuses equally. The diffusion of water is fairly non-directional in GM given the non-uniform structures of neuronal cell bodies and dendrites that restrict water movement. In instances like these where water diffuses equally, this behavior is said to be isotropic. Alternatively, in WM, the speed of water diffusion is dependent on the organization and directionality of the fibers and is referred to as anisotropic. In fibers parallel to WM, diffusion of water occurs quite fast whereas in fibers that run perpendicular to WM, diffusion is slower because of the anatomical restrictions placed upon it by surrounding axonal membranes and myelin sheaths (Liu et al., 2017).

Commonly used metrics to assess the integrity of the WM include fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity, and axial diffusivity. FA represents the degree to which diffusion of water molecules are anisotropic. Stated simply, FA reflects the integrity of the axon (Fan et al., 2019; Luo et al., 2020). FA is measured in the range of 0-1 where 0 can be thought of as representing isotropic motion and 1 indicates anisotropic motion. A small FA thereby indicates axonal damage and decreased integrity. MD is the average rate of diffusion. Thus, higher MD values indicate increased diffusion which is suggestive of greater tissue breakdown (Fan et al., 2019;

Gold et al., 2012; Ouyang et al., 2021). MD is also dependent on the density of physical obstructions and how water is distributed in different tissues (Gold et al., 2011; Yang et al, 2021). Impaired integrity of the WM pathways, as indicated by decreased FA and increased MD, have been reported in studies of people with MCI and AD (Gold et al., 2012; Liu et al., 2017). One can visualize a WM pathway with decreased FA and increased MD as being a tract where water diffuses quickly and with little organization because the fibrous internal structure of these pathways has degenerated and has little remaining integrity. The other two metrics used to assess integrity are radial and axial diffusivity. Both reflect the diffusion of water in respect to the axon direction. Radial diffusivity reflects diffusion of water that is perpendicular to the axon direction and axial diffusivity reflects diffusion that is parallel to axon direction (Luo et al., 2020).

While studies have shown a decrease in FA and an increase in MD are characteristic of AD, how early in the AD continuum these changes become detectable remains less clear (Araque Caballero et al., 2018; Liu et al., 2017). Similarly, understanding what particular fiber bundles show the greatest vulnerability as AD progresses has become a central focus. A range of WM pathways have been reported as being affected in the progression of AD including but not limited to the splenium of the corpus callosum, the superior longitudinal fasciculi, the uncinate fasciculi, inferior fronto-occipital fasciculi, and limbic pathways, such as the cingulum and fornix (Lee et al., 2015; Luo et al., 2020; Pichet Binette et al., 2021; Tucholka et al., 2018; Yang et al., 2021). In Chapter 4 of this dissertation, we plan to investigate implications of AD-related changes in the cingulum as well as relationships between the integrity of the cingulum

with volume of selected ROI and cognitive performance in individuals across the AD continuum.

The Importance of Studying Cognitive Decline in Alzheimer's Disease

Although cognition is not a biomarker, measuring cognitive decline remains essential to AD research. Measures of cognition are used to stage the severity of cognitive impairment in people along the AD continuum because of the visibility of cognitive skills in everyday life and its potential to highlight individuals who may later become biomarker positive (Elman et al., 2020; Jack et al., 2018; Weissberger et al., 2017). Researchers and neuropsychologists commonly assess cognitive performance in older adults through use of standardized batteries of neuropsychological tests which allow them to measure cognitive decline over time. Paired with imaging and/or CSF, cognition can be used to stage the progression of AD from cognitively normal to MCI to AD in a manner similar to how that of staging with biological biomarkers is conducted (Jack et al., 2018).

As is true of biological biomarkers, inter-individual variability also exists in cognitive decline based on factors such as cognitive reserve, quality and years of education, pre-existing health conditions, socioeconomic status and other social determinants of health (Werry et al., 2020). However, extensive research examining AD related cognitive decline has shown the onset and type of AD-related changes often follow a pattern (Joubert et al., 2016; Toepper, 2017). Among the first cognitive symptoms of AD are episodic memory deficits, particularly the ability to learn new information. Participants with AD often show poor retrieval or recognition on learning

tasks because they are no longer able to encode new information into long term memories. Additionally, semantic memory is often affected in early stages of AD and is reflected as word-finding difficulty or poor performance on word naming neuropsychology tasks (Joubert et al., 2016; Toepper, 2017). Other domains often affected are those pertaining to orientation and executive functioning, specifically processes that require abstract thinking, cognitive flexibility, and inhibition of irrelevant information (Joubert et al., 2016). As AD progresses, other symptoms may include impaired attention, visuospatial impairments, delayed processing speed, and lastly, behavioral symptoms such as changes in mood and increased agitation. This progression can vary from person to person and often times, which deficits are most pronounced can be indicative of where the most degeneration and/or cerebral changes have occurred (Toepper, 2017).

Measures of cognition are commonly divided into specified cognitive domains such as memory, executive function, visuospatial/motor function, language and attention. Neuropsychological tests are used to assess all of these domains in order to identify deficits. A selection of these tests with brief descriptions are shown in Figure 1.2 It should be noted that while these tables are listed by cognitive domain, the ability to do any of these tests correctly requires skills in multiple cognitive domains. Although not a measure of cognition, another clinical measure commonly used to assess functional capacity in daily life is called the Functional Activities Questionnaire (FAQ) (Pfeffer, Kurosaki, Harrah, Chance, Filos, 1982). An example of the FAQ survey is shown in Figure 1.3.

Given the affordability, high diagnostic value, and non-invasive nature of neuropsychological testing, many argue that using measures of cognition coupled with biological biomarkers will yield the greatest sensitivity in distinguishing changes across the AD continuum (Elman et al., 2020; Jack et al., 2018; Weissberger et al., 2017). Additionally by pairing biological constructs with measures of cognition, it will allow us to see how changes in structure may be related to changes in function. For these reasons, all studies within this dissertation will assess biological aspects of the aging brain, such as biomarkers, atrophy, and changes in WM, alongside measures of cognition such as those listed in Figure 1.2

Racial Disparities in Alzheimer's Disease Research

Research using the AT(N) framework has been continually applied in AD research studies since its inception (Dodich et al., 2020; Kern et al., 2018; Wei et al., 2018). As these studies are published, a growing concern in the field stems from the very small number of people enrolled in these studies that identify as part of racial minority groups (Fleishman et al., 2022; Shin & Doraiswarmy, 2016). In many long-standing AD research initiatives, such as the National Alzheimer's Coordinating Center (NACC), Harvard Aging Brain Study, the Framingham Heart Study, and the Alzheimer's Disease Neuroimaging Initiative (ADNI), 80-90% of all participants racially identify as non-Hispanic White (NHW) (Ashford et al., 2022; Dagley et al., 2017; Tsao et al., 2015).

The underrepresentation of racial minority groups in research studies of medical conditions is not isolated to AD and has been documented for years in conditions such as cardiovascular disease, HIV/AIDS, cancer, and others (Canevelli et al., 2019). The basis

of this disparity likely stems from years of mistrust between racial and ethnic minority populations and research studies led by hospitals, universities and clinics (Shin & Doraiswamy, 2016; Stout et al., 2020). Likely the most documented example of such mistreatment is the Tuskegee experiment conducted on Black and African Americans between 1932-1972 yet other incidents of forced withdrawal from prescribed medications, administration of fenfluramine, and altered (restricted) diet have been documented even in the years following the Tuskegee experiment (Scharff et al., 2010). This history has made recruiting and retaining racial and ethnic minority participants in research studies quite challenging. Yet as racial and ethnically diverse populations in this country continue to grow, understanding the interactions between race, neuropathological burden, and other risk factors in living older adults has emerged, and will hopefully endure, as a priority in AD research studies (Fleishman et al., 2022; Stout et al., 2020). This understanding is imperative in order to guide treatment and diagnosis of AD in racial and ethnic minority groups.

This pattern of underrepresentation of racial minority groups in AD research studies is also reflected in many pharmaceutical trials as estimates show only 3.2% of individuals in drug trials are non-White participants (Gottesman & Hamilton, 2021). This low percentage is partially because the participants in many AD research initiatives (such as the ADNI) are the basis of clinical drug trial populations in addition to the previously mentioned issues with participant recruitment and retainment. This disparity may become particularly problematic as other drugs emerge on the market because it will remain unclear how these drugs will perform in underrepresented groups (Barnes, 2022).

The work in Chapter 2 will focus on understanding biomarkers in Black or African American (BoAA) participants although the breadth of disparities in AD research extends to many other racial and ethnic groups. Our work focuses on these two racial groups due to the large prevalence of BoAA individuals in the total US population (roughly 13%) and the high incidence of dementia/AD reported in this group (Barnes, 2022; Gottesman & Hamilton, 2021; Raman et al., 2021; Shin & Doraiswamy, 2016).

The Alzheimer's Disease Neuroimaging Initiative (ADNI)

Data for participants in Chapters 2 and 3 was collected from older adults enrolled in the Alzheimer's Disease Neuroimaging Initiative (ADNI). The ADNI has been in existence since 2003 and consists of 59 sites across North America. The primary goal of ADNI has been to test whether serial MRI, PET, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD. Upon obtaining informed consent and initial screening, participants undergo a number of tests that are repeated over their course of enrollment in the ADNI. Such tests include a clinical evaluation, neuropsychological tests, genetic testing, lumbar puncture (to obtain CSF), and MRI/PET scans.

A key strength of the ADNI is that it is a nationwide initiative, which means people from all over North America can enroll and participate in clinical evaluations, testing, and imaging at ADNI sites located in their general geographic area. Each of these ADNI sites have a Principal Investigator and follow the same protocols for data collection. This ensures that data collection and processing remain uniform throughout the many study sites. Additionally, the ADNI is constituted of various "cores," each led

by researchers that are experts in their fields (e.g. clinicians, geneticists, biostatisticians, neuropathologists, and neuroimagers who specialize in MRI and PET). These cores regularly meet to update and maintain the protocols and integrity of ADNI data.

Since the ADNI was formed in 2003, thousands of participants have enrolled in the study. Participants are between the ages of 55 – 90 and enrollment of participants has been carried throughout recruitment cycles while new participants are added within each cohort. The ADNI has consisted of four cohorts to date (ADNI 1, ADNI GO, ADNI 2, and ADNI 3) and the fifth (ADNI 4) begins recruitment in fall 2022. A primary goal of this new cohort will be recruiting participants from racial and ethnic minority groups. A schematic of the ADNI clinical schedule timeline is shown in Figure 1.4. Continued participant in the ADNI has allowed researchers to study participants cross-sectionally, but also follow them longitudinally as many participants have now been enrolled in the ADNI for almost 20 years.

Inclusion criteria for the ADNI includes having a study partner, a minimum of 6 grades education or work history, and good general health with no diseases precluding enrollment. Participants are thoroughly screened for medication use and certain medications are considered exclusion criteria for participation to avoid extraneous confounding factors. Following each ADNI visit, participants are evaluated and diagnosed by the ADNI Clinical Core and diagnosed as cognitively normal, MCI or AD. These diagnoses are a primary measure used to track both biomarker and cognitive progression through the AD continuum. It should be noted that in the ADNI GO and ADNI 2 cohorts, participants diagnosed with MCI were further separated into those with

early MCI (EMCI) or late MCI (LMCI). However, these diagnostic criteria were changed back to a sole description of MCI for both subgroups during ADNI 3. For the purposes of our studies, we used MCI throughout this dissertation to describe everyone in this group. Additionally in Chapter 2, we referred to BoAA ADNI participants diagnosed by the ADNI Clinical Core as having AD as the "dementia clinical syndrome group" since we did not have biomarker data for all 85 participants.

The Health Outreach Program for the Elderly (HOPE)

In addition to multisite AD research cohorts, many local Alzheimer's Disease Research Centers (ADRC) exist across the country. These centers are funded by the NIA and contribute data to the NACC, a multisite initiative much like the ADNI. There are currently 37 ADRC within the US; one of which is located at Boston University and is formally called the Boston University Alzheimer's Disease Research Center (BU-ADRC). The BU-ADRC was established in 1996 and services the greater Boston area with an emphasis on advancing AD research and related conditions through ongoing research studies.

Data in Chapter 4 of this dissertation utilizes data from the Health Outreach Program for the Elderly (HOPE), a cohort study conducted at the BU-ADRC with locations both in Boston and Needham, MA. The HOPE study is a community-based resource intended for people with and without memory concerns. Participants enrolled in the HOPE study must be 50 and older and complete annual visits to assess memory and other cognitive domains. All participants must have a caregiver or study partner that can accompany them to annual visits. During study visits, participants are asked questions

about family and medical history before undergoing a full physical and neurological exam with MRI if possible. Participants are also asked to consider donating brain tissue following death. Following annual visits, clinicians, neuropsychologists, psychiatrists, and neurologists review results at consensus diagnosis meetings. Participants and their study partners receive feedback about their health and have the opportunity to speak with clinicians. A major goal of the HOPE study is to advance the diagnosis, prevention and treatment of AD while also allowing participants and their caregivers to have a more active role in their healthcare. Like the ADNI, a major priority for the HOPE study has been the recruitment of racial minority groups, particularly individuals that identify as BoAA.

Goals of the Proposed Research

For many years, studies have repeatedly shown that when people die of Alzheimer's disease their post mortem brain tissue commonly shows deposition of Aβ plaques, the accumulation of tau, and neurodegeneration of brain tissue. What remains unclear is why some people with the accumulation of these *same* biological biomarkers do **not** develop dementia-like symptoms within their lifetime. This principal question has already occupied researchers (and PhD students) for decades and it continues to pose a problem as the baby boomer generation nears 80 years of age. The goals of the work presented in this dissertation were to explore some of the nuances pertaining to what causes certain people to develop AD pathology and associated cognitive decline within their lifetime. Data for the studies in Chapters 2 and 3 was collected from older adults

enrolled in the ADNI and data in Chapter 4 came from participants aged 50 years and older enrolled in the HOPE cohort study at the BU-ADRC.

In Chapter 2, our objective was to investigate links between brain structure, cognitive performance, and neuropathology in participants within the ADNI who identified as BoAA. Relatively little is known about dementia in this population so our goal was to assess if there would be an increase in AD biomarkers abnormalities (as measured by MRI data, cognition and function, and CSF samples of Aβ and tau) between the three clinical syndrome groups: cognitively normal, MCI, and dementia.

Next in Chapter 3, we identified a different subset of participants within the ADNI who showed a change in amyloid burden during enrollment in the ADNI study. We defined a change in amyloid burden in these participants based upon established florbetapir PET standard uptake value ratio (SUVR) thresholds. Sixty-eight participants fit this criterion and we next identified a second group of ADNI participants with similar and representative demographic data (age, years of education, clinical diagnosis) that did **not** show a change in amyloid status. Following the collection of this data, we conducted a comprehensive analysis of other AD biomarkers and cognitive change in the period of time leading up to amyloid conversion.

Lastly, in Chapter 4, we shifted our focus to using a form of DTI to study WM pathway integrity in a large sample of older adults who are part of the BU-ADRC HOPE study. Using segmentations of the cingulum bundle, we first assessed whether diffusion measures (FA and MD) of the cingulum dorsal and ventral pathways weakened between clinical syndrome groups ranging from control participants to amnestic MCI to those with

dementia. We further assessed the relationship between WM integrity of both cingulum pathways with volume of selected ROIs and in relation to executive function and memory. The goal of this series of analyses was to obtain a better understanding of the circuitry of the cingulum and how weakening of structural measures, such as WM integrity and volume of selected ROIs, may be related to cognitive decline.

Through these three studies, we explored how factors such as race, genetics, transitions in biomarker status, and changes to WM integrity relate to the clinical presentation and progression of AD. The studies completed in this dissertation shed light on some of the ambiguities observed in the progression of AD yet much more work is needed to solidify our understanding of a complex disease like AD.

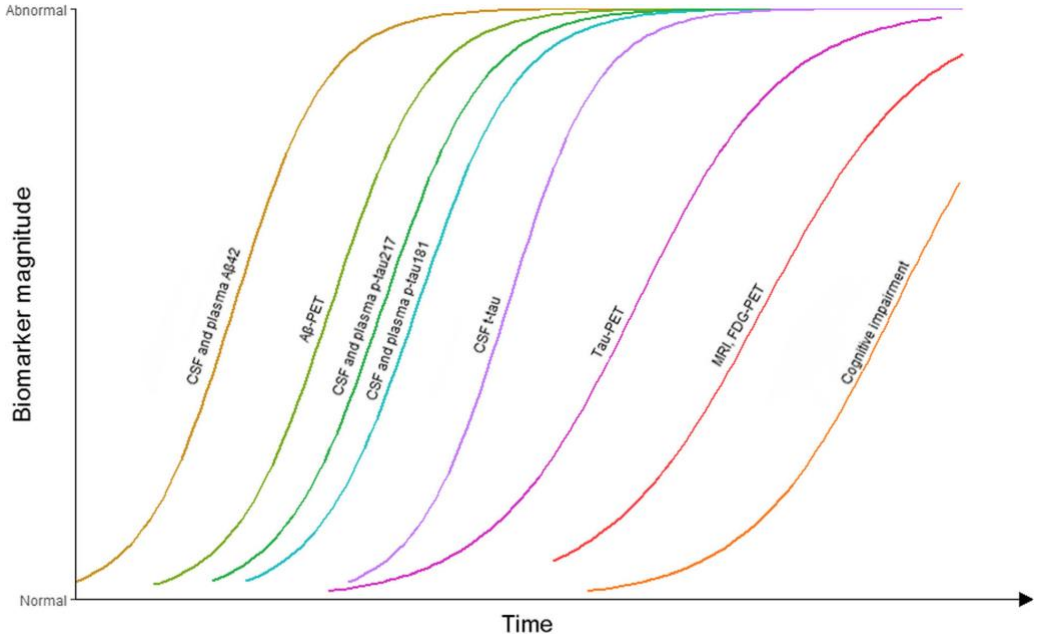

Figure 1.1: A model of the temporal pattern of AD-related biomarker abnormalities
Image reflects the approximate sequence that different biomarkers are believed to change through the progression of AD. Aβ biomarkers become abnormal first, which is shortly followed by alterations of soluble tau. Changes in CSF are shown to occur before changes in PET for both Aβ and tau. Interindividual differences may influence the sequence of these changes. Image from Guo et al. 2021.
Abbreviations: Aβ, amyloid-β; AD, Alzheimer's disease; CSF, cerebrospinal fluid; FDG, fluorodeoxyglucose; MRI, magnetic resonance imaging; PET, positron emission tomography; p-tau181, tau phosphorylated at threonine 181; p-tau217, tau phosphorylated at threonine 217; t-tau, total tau

Logical Memory Recall/Craft Recall (Immediate and Delayed)	Story read to participant and participant is asked to repeat after hearing (immediate) then again after 30-40 minutes (delayed)
Rey Auditory Verbal Learning Test (RAVLT)	Word lists are read to participant and participants has to repeat as many words as they can remember. Interference list introduced to see if participant can correctly remember first list of words

Boston Naming Test	Orally name 30-line drawings
Category Fluency (e.g. Vegetables)	Participants are given category (e.g. vegetables) and must come up with list of vegetables

Clock Drawing Test (Drawing and Copying Administrations)	Participant is asked to draw a standard faced clock with hands correctly set to a given time (Drawing). Participant is shown a picture of a clock and asked to copy it (Copying)

Part B of Trailmaking	Participant is given a piece of paper with 25 circles. Half of the circles have numbers 1-12 and half have A-L. Participant must connect sequential numbers and letters in alternating order (1-A-2-B and so on)

Figure 1.2: Examples of Tasks used to Test Cognition
Tasks used to primarily test memory are shown in purple, language shown in red, visuospatial processing shown in orange, and executive function shown in blue

Functional Activities Questionnaire

Completed by: **Patient** **Family**

1.	Writing checks, paying bills, balancing the checkbook	**0**	**1**	**2**	**3**
2.	Assembling tax records, business affairs or papers	**0**	**1**	**2**	**3**
3.	Shopping alone for clothes, household items, groceries	**0**	**1**	**2**	**3**
4.	Playing a game of skill, working on a hobby	**0**	**1**	**2**	**3**
5.	Heating water, making a cup of coffee, turning off stove	**0**	**1**	**2**	**3**
6.	Preparing a balanced meal	**0**	**1**	**2**	**3**
7.	Keeping track of current events	**0**	**1**	**2**	**3**
8.	Paying attention to, understanding, TV, book magazine	**0**	**1**	**2**	**3**
9.	Remembering appointments, occasions, holidays, medication	**0**	**1**	**2**	**3**
10.	Traveling out of neighborhood	**0**	**1**	**2**	**3**

Rating:

- Dependent = 3
- Requires assistance = 2
- Has difficulty but does by self = 1
- Normal = 0
- Never did the activity but could do it now = 0
- Never did the activity and would have difficulty now = 1

Scoring, cutpoint of 9 is indicative of impaired function.

Figure 1.3: Example of Functional Activities Questionnaire (FAQ)
Form would be filled out by patient and study partner and/or family member.

Figure 1.4: Schematic of ADNI Study Schedule
Image taken from adni.loni.usc.edu

CHAPTER TWO: BIOMARKERS OF ALZHEIMER'S DISEASE IN BLACK PARTICIPANTS OF THE ALZHEIMER'S DISEASE NEUROIMAGING INITIATIVE

Introduction

An overwhelming number of participants in studies of dementia identify as non-Hispanic White (NHW) yet older Black adults (including people of either African/Caribbean descent) are reported to be nearly twice as likely to develop dementia (Canevelli et al., 2019; Mayeda et al., 2016; Raman et al., 2021). This disparity has been attributed to social determinants of health, including factors such as racial inequities, access, quality, and willingness to seek health care, as well as years and quality of education (Canevelli et al., 2019; Carvalho et al., 2015). Other risk factors include the *APOE* ε4 genotype and the prevalence of cardiovascular diseases (Carvalho et al., 2015; Walker et al., 2021). It remains unclear how these factors interact and ultimately lead to dementia (Barnes, 2022; Fleishman et al., 2022; Shin & Doraiswamy, 2016). .

An example of the underrepresentation of Black or African American individuals (BoAA) in dementia research studies is illustrated in the multisite Alzheimer's Disease Neuroimaging Initiative (ADNI), which has been in existence since 2003 and assesses participants at 59 sites across North America. While this initiative has been successful in obtaining data from thousands of older adults, only 5% of this data comes from BoAA participants (Ashford et al., 2022).This discrepancy is not just an isolated problem of the ADNI study. For example, in the aducanumab drug trials, only 0.6% (or 19 individuals)

identified as Black (Manly & Glymour, 2021). Of the 19 Black individuals in the entire study, only 6 were randomized to the treatment dose group.

To enhance our understanding of dementia in BoAA individuals, dementia research studies in this group are desperately needed (Barnes, 2022; Manly & Glymour, 2021). Recruiting and retaining BoAA participants in research studies has become a primary goal of many current studies. As these initiatives gain traction, it is important for us to study the data we have and focus on group specific factors. This type of work may further promote BoAA participation in dementia research (Barnes, 2022; Fleishman et al., 2022). .

This study sought to investigate links between brain structure, cognitive performance, and biomarker correlates of neuropathology in participants within the ADNI who identified as BoAA. Our hypothesis was that we would see an increase in Alzheimer's disease (AD) biomarker abnormalities between the clinical syndrome groups. Differences were assessed using a framework of biomarkers such as MRI data from regions which are affected in AD, white matter hyperintensities (WMH), cognitive and functional measures, CSF measures of amyloid-beta (Aβ), CSF measures of total tau, CSF measures of phosphorylated tau, and *APOE* ε4 carrier status. These measures were assessed across clinical syndrome groups; cognitively normal (CN), mild cognitive impairment (MCI), or dementia defined by the ADNI Clinical Core.

Materials and Methods

Participants

Data used in the preparation of this article were obtained from the ADNI database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial MRI, PET, other biomarkers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD.

After carefully examining all demographic data within the ADNI Image and Data Archives, we identified 85 individuals who self-identified as "Black or African American," "not Hispanic/Latino," and had a 3T MRI scan. Data was downloaded from the ADNI database in August 2020 and CSF data was updated in April 2021. All participants were scanned between 2006 – 2020. Clinical syndromes (CN, MCI, and dementia) were defined by the ADNI Clinical Core (Petersen et al., 1999; Petersen et al., 2014). Demographic and *APOE* ε4 data are listed in Table 2.1. Imaging, cognitive and functional assessments, and CSF data for each participant were obtained within ~3 months of each other.

Standard Protocol Approvals, Registrations, and Patient Consent

Written informed consent or assent was obtained from all participants, and study procedures were approved by the institutional review board at each of the ADNI participating sites. ADNI is listed in the ClinicalTrials.gov registry (ADNI-1: NCT00106899; ADNI-GO: NCT01078636; ADNI-2: NCT01231971; ADNI-3: NCT02854033). All data downloaded from the ADNI website was de-identified and is listed by patient ID number (Supplemental Table 2.1).

Imaging Assessments

The neuroimaging methods and parameters utilized by ADNI for T1 and fluid attenuated inversion recovery (FLAIR) scans have been described previously (Jack et al., 2008; Wyman et al., 2013). Visual inspection for artifact and unexpected neuropathology by the ADNI MRI core was completed at the time of image upload to the ADNI. Upon downloading, we also visually inspected the images for artifacts that could have impaired image processing. All scans downloaded from the ADNI database were from baseline visits except in the case of 7 participants for whom 3T MRI data was not available at baseline and later scans were used. All scans were downloaded in their native DICOM format.

T1 Scans Processing

T1 scans from all 85 participants were processed using Freesurfer version 6.0 (https://surfer.nmr.mgh.harvard.edu/) on a Mac Pro 2013 running OS version 10.14.5 to obtain cortical parcellations and subcortical segmentation of anatomical regions (Desikan et al., 2006; Iglesias et al., 2015). Regions of interest (ROI) were parcellated using the Desikan-Killiany atlas (Desikan et al., 2006; Iglesias et al., 2015) and included the entorhinal cortex, inferior parietal lobule, middle temporal gyrus, parahippocampal gyrus, posterior division of the cingulate cortex, precuneus, and insula. These regions were chosen based on previous studies which have shown these ROI are commonly implicated in the progression of AD (Fennema Notestine et al., 2009; Zhou et al., 2016). The average thickness of each ROI was calculated between the right and left hemispheres and used in further statistical analyses. Hippocampal volume, estimated total intracranial volume (eTIV), and total cerebral white matter volume were also generated by Freesurfer. Total

hippocampal volume was used in statistical analyses by adding the volume of the right and left hippocampi in each participant.

FLAIR Scans

Of the 85 participants in this study, 78 had FLAIR scans available for download from the same imaging session as the T1 scans. Six of the participants without FLAIR scans had imaging conducted prior to when the FLAIR sequence became part of ADNI protocol and images for one participant failed to process correctly and were excluded. Thirty-six participants had FLAIR scans completed with 3D acquisition and the other forty-two were completed using 2D acquisition. Scans were processed using the Lesion Segmentation Tool (LST) running on MatlabR2019b on a Mac Pro 2013 running OS version 10.14.5 (Ribaldi et al., 2021; Schmidt et al., 2012). The Lesion Prediction Algorithm was used to perform a dual channel form of segmentation using both T1 and FLAIR scans. The kappa threshold used was the default measure (0.5) and only lesions > 0.015 mL were identified. The output measure obtained through LST was volume of WMH lesions which were consequently log-transformed to reduce skewness (Barnes et al., 2013; DeCarli et al., 2008).

Assessment of Cognition and Function

All 85 participants reported English as their primary language and performed testing in English at one of the ADNI sites. Details pertaining to ADNI testing procedures have been described previously (Aisen et al., 2010; Aisen et al., 2015). We used ADNI data from eight measures: (1) Functional Activities Questionnaire (FAQ), (2) Logical Memory Immediate Recall (modified from the Wechsler D. Wechsler Memory Scale-

Revised, San Antonio, Texas: Psychological Corporation; 1987), (3) Category Fluency (Animals), (4) Boston Naming Test, (5) Number of Trials Learned on and (6) Sum of Total Trials on the Rey Auditory Verbal Learning Test (RAVLT), (7) Part A and (8) Part B of the Trailmaking Test. None of these measures were used by the ADNI Clinical Core in determining clinical syndrome groups (Petersen et al., 1999; Petersen et al., 2014). To standardize the data, all raw scores were converted to z-scores. Direction of scores was not altered. For some measures, data was not available from all 85 participants due to changes in the ADNI protocols. Assessments with missing data are indicated in Table 2.2.

CSF Sampling and Analysis

Of the 85 participants in this study, 48 had CSF measures generated by ADNI. Data was not available for 37 participants who did not consent to CSF sampling. Standard practice of the ADNI is to measure concentrations of the amyloid beta 1-42 peptide ($A\beta_{1-42}$), total tau (t-tau), and tau phosphorylated at threonine 181 (p-tau) in collected CSF samples. Samples were obtained at the various ADNI sites via lumbar puncture as described in the ADNI procedures manual and previous studies (Shaw et al., 2009).

Statistical Analysis

All analyses were performed in JMP Pro V15.2 on a MacBook Pro 2015 running OS version 10.15.7. Analyses of variance (ANOVAs) were used to assess differences between the CN, MCI and dementia clinical syndrome groups in age and years of education. Chi-square testing was used to assess the distribution in categorical variables

such as sex and *APOE* ε4 carrier status. Statistical significance was set at $p < 0.05$ without correction.

The means and standard deviations of MRI measures, CSF samples, and cognition and functional z-scores are shown in Tables 2.1-2.2. Models assessing the effect of clinical syndrome group were completed within each group of dependent variables (cortical thickness of selected ROI, hippocampal volume, WMH volume, measures of cognition and function, CSF sampling). Prior to creating models, linear regressions were run to determine whether covariates such as age and years of education influenced the measures. For MRI measures of hippocampal volume and WMH, additional linear regressions were run to determine whether covariates such as eTIV, total cerebral white matter, and FLAIR acquisition type influenced these measures. Post hoc Tukey's Honestly Significant Difference (HSD) testing was performed for all significant results. Clinical syndrome group differences, *p* values and 95% confidence intervals are reported (Tables 2.3A-C). For models including measures of cortical thickness, cognition and function, multiple comparisons were corrected for by use of the Benjamini-Hochberg method (Benjamini & Hochberg, 1995). Additional analyses were run assessing the main effect of *APOE* ε4 carrier status and the interaction of clinical syndrome group (CN, MCI and dementia) and *APOE* ε4 carrier status on each set of independent variables. Significant interactions, *p* values and 95% confidence intervals are reported (Table 2.4).

Data Availability

Data used in this article are available for download from the Laboratory of NeuroImaging (LONI; loni.usc.edu). All variables were extracted from spreadsheets posted on LONI.

Results

Demographic Data

Demographic data is listed in Table 2.1. There were no significant differences in age nor years of education between clinical syndrome groups ($p > 0.05$). Chi-square testing showed there were significantly more females (n = 57) than males (n = 28) in the study ($p = 0.002$), but that the number of males and females in the CN, MCI and dementia clinical syndrome groups was not significantly different ($p = 0.36$). Years of education was not significantly different between males and females ($p = 0.50$).

APOE ε4 status

Of the 85 participants in this study, 74 had consented to *APOE* ε4 genotyping (Table 2.1). Those with 1 or 2 ε4 alleles were classified as "carriers" (n = 33) and participants with 0 ε4 alleles were classified as "non-carriers" (n = 41). Chi-square testing revealed a significant difference between the clinical syndrome groups in proportion of *APOE* ε4 carriers (p = 0.018). Follow-up pairwise chi-square testing revealed differences in carrier status between CN and MCI clinical syndrome groups ($p = 0.002$) and CN and dementia clinical syndrome groups ($p = 0.007$). Chi-square testing showed no sex differences in the number of carriers between males and females ($p = 0.23$).

Cortical Thickness

Age had a significant effect on all MRI measures (cortical thickness, hippocampal volume, and WMH volume) and was used as a covariate in subsequent models. ANCOVA models assessing the main effect of clinical syndrome group with age as a covariate showed a significant effect of clinical syndrome group on average cortical thickness of the entorhinal cortex ($F(3,81) = 14.06$, $p < 0.001$), middle temporal gyrus ($F(3,81) = 9.45$, $p < 0.001$), parahippocampal gyrus ($F(3,81) = 4.07$, $p = 0.01$), posterior cingulate ($F(3,81) = 6.48$, $p = 0.001$), and insula ($F(3,81) = 9.20$, $p < 0.001$). The main effect of clinical syndrome group remained significant after correction for multiple comparisons using the Benjamini-Hochberg method in all five regions. Significant clinical syndrome group differences, *p* values, and confidence intervals from Tukey's HSD testing are shown in Table 2.3A. Age had a significant effect in the negative direction on average cortical thickness of the entorhinal cortex ($p = 0.008$), middle temporal gyrus ($p < 0.001$), and insula ($p < 0.001$). Two-way ANCOVAs exploring the interaction of clinical syndrome group and *APOE* ε4 carrier status with age as a covariate on the seven ROIs were not significant.

Hippocampal Volume

Age, but not eTIV, had a significant effect on hippocampal volume so it was included in hippocampal volume models. An ANCOVA model assessing the main effect of clinical syndrome group with age as a covariate showed a significant effect of clinical syndrome group on hippocampal volume ($F(3,81) = 13.38$, $p < 0.001$). Tukey's HSD testing showed the mean value of hippocampal volume was significantly different between CN and dementia clinical syndrome groups and MCI and dementia clinical

syndrome groups. Clinical syndrome group differences, *p* values, and confidence intervals are shown in Table 2.3A. Age had a significant effect ($p < 0.001$) in the negative direction. A two-way ANCOVA exploring the interaction of clinical syndrome group and *APOE* ε4 carrier status with age as a covariate on hippocampal volume was not significant.

White Matter Hyperintensity Volume

Acquisition type and age, but not total cerebral white matter volume, had a significant effect on log-transformed WMH and were included as covariates. An ANCOVA model assessing the main effect of clinical syndrome group with acquisition type and age as covariates showed a significant effect of clinical syndrome group on log-transformed WMH ($F(4,73) = 7.15$, $p < 0.001$). Tukey's HSD testing showed the mean value of log-transformed WMH was significantly different between CN and dementia clinical syndrome groups. Clinical syndrome group differences, *p* values, and confidence intervals are shown in Table 3A. The effect of age ($p = 0.016$) and acquisition type ($p = 0.031$) were also significant. A two-way ANCOVA exploring the interaction of clinical syndrome group and *APOE* ε4 carrier status (with age and acquisition type as covariates) on log-transformed WMH was not significant.

Cognition and Function

Age had a significant effect on cognitive and functional z-scores and was included in cognitive and functional models as a covariate. ANCOVA models using clinical syndrome group as the main effect with age as a covariate showed a significant effect of clinical syndrome group for all eight measures. Results for each model are as follows:

FAQ ($F(3,78 = 74.28$, $p < 0.001$), Logical Memory Immediate Recall ($F(3,81) = 42.19$, $p < 0.001$), Category Fluency ($F(3,80) = 14.48$, $p < 0.001$) the Boston Naming Test ($F(3,43) = 15.65$, $p < 0.001$), Sum of Total Trials on the RAVLT ($F (3, 80) = 19.23$, $p < 0.001$), Number of Trials Learned on the RAVLT ($F(3,80) = 8.39$, $p < 0.001$), Parts A ($F(3,80 = 20.04$, $p < 0.001$) and B ($F(3,76 = 23.02$, $p < 0.001$) of the Trailmaking Test. The main effect of clinical syndrome group remained significant following correction for multiple comparisons using the Benjamini-Hochberg method on all eight measures. Significant clinical syndrome group differences, *p* values, and confidence intervals from Tukey's HSD testing are shown in Table 3B. Age showed a significant effect in the negative direction on performance of three of the cognitive tasks: Parts A ($p = 0.002$) and B ($p < 0.001$) of the Trailmaking Test and the Boston Naming Test ($p = 0.032$).

Two-way ANCOVAs exploring the interaction of clinical syndrome group and *APOE* ε4 carrier status (with age as a covariate) on z-scored measures of cognition and function showed a significant interaction on FAQ ($F(2,66) = 7.09$, $p = 0.002$). Tukey's HSD testing showed the mean value of z-scored performance on the FAQ was significantly different between *APOE* ε4 carriers and non-carriers with dementia (for *p* value and confidence interval, see Table 4). The main effects of clinical syndrome group ($p < 0.001$) and *APOE* ε4 carrier status ($p < 0.001$) were significant for this model. The interaction failed to reach significance on the other seven measures.

CSF Sampling

Neither age nor education influenced CSF measures. One-way ANOVAs showed a significant effect of clinical syndrome group on $A\beta_{1\text{-}42}$ ($F(2, 45) = 3.28$, $p = 0.047$), t-

tau (F (2,45) = 7.25, $p = 0.002$), and p-tau (F(2,45) = 7.44, $p = 0.002$). Tukey's HSD testing showed that the mean value of $A\beta_{1-42}$ was significantly different between CN and dementia clinical syndrome groups and that the mean values of both t-tau and p-tau were significantly different between CN and dementia clinical syndrome groups and MCI and dementia clinical syndrome groups. Clinical syndrome group differences, *p* values, and confidence intervals are shown in Table 3C. Two-way ANOVAs showed a significant interaction of clinical syndrome group and *APOE* ε4 carrier status on t-tau (F(2,38) = 6.90, $p = 0.003$) and p-tau (F(2,38) = 6.16, $p = 0.005$). Tukey's HSD testing showed significant differences between MCI *APOE* ε4 carriers and non-carriers for both t-tau and p-tau (for *p* values and confidence intervals, see Table 2.4). The main effects of clinical syndrome group (t-tau: $p < 0.001$, p-tau: $p < 0.001$) and *APOE* ε4 carrier status (t-tau: $p = 0.029$, p-tau: $p = 0.045$) were significant for this model. The interaction failed to reach significance on CSF $A\beta_{1-42}$.

Discussion

The goal of this study was to examine ADNI participants in varying clinical syndrome groups who identified as BoAA for the purpose of better understanding how biomarkers of disease are impacted within this sample (Babulal et al., 2019; Fleischman et al., 2022; Meier et al., 2012; Shin & Doraiswamy, 2016). Because there is limited literature focused on BoAA participants, it was difficult to place our findings in the context of others. Our hypothesis was that there would be an increase in AD biomarker abnormalities between the clinical syndrome groups. Our findings support our hypothesis as we found decreased cortical thickness in five of seven selected ROIs, decreased

hippocampal volume, increased WMH, worsened measures of cognition and function, decreased measures of CSF $A\beta_{1-42}$, and elevated measures of CSF t-tau and p-tau between the clinical syndrome groups. We also found an interaction between clinical syndrome group and *APOE* ε4 carrier status on the FAQ, CSF t-tau, and CSF p-tau measures.

Analysis of T1 structural images showed that the average thickness of five selected cortical ROI and total hippocampal volume decreased between the clinical syndrome groups. Not surprisingly, the greatest differences in cortical regions and hippocampal volume were found when comparing CN and dementia clinical syndrome groups. Differences in the entorhinal cortex, the parahippocampal gyrus, and the hippocampus were also found between MCI and dementia clinical syndrome groups. Somewhat unexpectedly, we found thickness of the posterior division of the cingulate cortex to be different between CN and MCI clinical syndrome groups.

Using FLAIR images, we assessed the log-transformed volume of WMH between clinical syndrome groups in this population. Our model showed a significant difference between CN and dementia clinical syndrome groups which is consistent with previous findings of others (Carmichael et al., 2012; DeCarli et al., 2008). A study in 2012 by Meier and colleagues examining 112 older Black individuals reported WMH were increased in frontal and parietal lobes in MCI participants and even more so in participants with dementia relative to controls (Meier et al., 2012). Another study by Walker and colleagues in 2021 examining 144 older Black individuals reported increased volume of WMH was strongly associated with greater cortical amyloid burden (Walker et al., 2021).

Our findings examining measures of cognition and function further support our hypothesis as performance on all eight measures declined between the clinical syndrome groups. Post hoc testing in our study revealed significant differences between CN and dementia clinical syndrome groups on all eight measures. Differences between CN and MCI clinical syndrome groups as well as between MCI and dementia clinical syndrome groups were also significant on many of these measures. These findings are consistent with a previous study conducted in a multi-site BoAA cohort which reported performance being worst in participants with dementia followed by MCI and controls on tests such as Parts A and B of the Trailmaking Test, Category Fluency (Animals), Logical Memory Immediate Recall, and the Boston Naming Test (Meier et al., 2012). Similar findings of worsened cognitive performance in BoAA with MCI compared to CN was also found in reports of a study sample (n = 554) drawn from the Baltimore Study of Black Aging (Gamaldo et al., 2010).

Our CSF results in this sample of BoAA ADNI participants are also consistent with our hypothesis that biomarkers of AD would worsen between the clinical syndrome groups. Following Tukey's HSD testing, findings for CSF t-tau and p-tau showed significant differences between CN and dementia clinical syndrome groups and MCI and dementia clinical syndrome groups. Findings for CSF $A\beta_{1\text{-}42}$ also showed significant differences between CN and dementia clinical syndrome groups but not between MCI and either clinical syndrome group.

Questions continue to emerge regarding the effects of *APOE* ε4 carrier status (i.e. carrier vs non-carrier) in terms of race, sex, and the likelihood of this genotype to impact

amyloid burden, hippocampal atrophy, and WMH (Babulal et al., 2019; Cacciaglia et al., 2018; Sudre et al., 2017; Sundermann et al., 2020). In our study, the clinical syndrome group classified as having dementia had a significantly greater frequency of *APOE* ε4 carriers than either the CN or MCI clinical syndrome groups. Within the MCI clinical syndrome group, we found a difference in mean concentration of CSF t-tau and p-tau between *APOE* ε4 carriers and non-carriers with carriers having higher levels of both CSF t-tau and p-tau. Within the clinical syndrome group classified as having dementia, we also found a difference on FAQ between *APOE* ε4 carriers and non-carriers with carriers having increased FAQ compared to non-carriers. To our knowledge, the relationship of *APOE* ε4 carrier status and tau or FAQ has not been highly explored, particularly in BoAA cohorts.

Limitations

Although the study had access to data from 85 BoAA participants, the sample size remains modest. A limiting factor in expanding this sample stems from the underrepresentation of BoAA in dementia research studies (Canevelli et al., 2019; Shin & Doraiswamy, 2016). This is being actively addressed and we anticipate having more data in the future. Furthermore, the use of a cross-sectional design provides insight into differences between clinical syndrome groups, however, it cannot capture the course of a disease progression as well as a longitudinal design.

A strength of using participants within the ADNI sample is that the database is composed of participants across North America as opposed to individuals from one local region. The disadvantage is that the ADNI sample is a clinical trials population which

means not everyone in the general population is eligible to participate. By example, one exclusionary criterion for the ADNI is the presence of cerebrovascular disease.

Conclusion

The results of this study confirmed our hypothesis that there is increase in AD biomarker abnormalities between clinical syndrome groups in the BoAA ADNI sample. We observed that measures of cortical thickness, volume of the hippocampus, volume of WMH, cognition and function, CSF measures of $A\beta_{1\text{-}42,}$ CSF measures of t-tau, and CSF measures of p-tau differed between the clinical syndrome groups. We also found interesting interactions in this BoAA sample between clinical syndrome groups and *APOE* ε4 carrier status in the FAQ, CSF measures of t-tau, and CSF measures of p-tau.

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Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Lumosity; Lundbeck; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Neurotrack Technologies; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Takeda Pharmaceutical Company; and Transition Therapeutics. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Therapeutic Research Institute at the University of Southern California. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California.

	CN (n = 46)	**MCI (n = 27)**	**Dementia (n = 12)**
Female sex, n (%)	33 (71.74)	18 (66.67)	6 (50)
Age, y	70.72 (7.38)	70.99 (7.36)	73.64 (6.97)
Education, y	15.91 (2.74)	15.26 (2.31)	13.92 (3.26)
***APOE* ε4 carrier**, n (%) (n = 74)	12 (29.27)	12 (50)	7(77.78)
Thickness of Entorhinal Cortex, mm	3.39 (0.33)	3.31 (0.35)	2.69 (0.59)
Thickness of Inferior Parietal Lobule, mm	2.35 (0.14)	2.30 (0.15)	2.24 (0.11)
Thickness of Middle Temporal Gyrus, mm	2.74 (0.13)	2.68 (0.16)	2.59 (0.14)
Thickness of Parahippocampal Gyrus, mm	2.69 (0.24)	2.66 (0.23)	2.39 (0.44)
Thickness of Posterior Cingulate, mm	2.38 (0.12)	2.31 (0.14)	2.23 (0.08)
Thickness of Insula, mm	2.80 (0.14)	2.72 (0.18)	2.65 (0.18)
Thickness of Precuneus, mm	2.29 (0.13)	2.25 (0.12)	2.22 (0.11)
Volume of Hippocampus, mm^3	7308.60 (692.98)	6905.53 (923.82)	6032.70 (968.35)
Volume of WMH, mL (n = 78)	6.24 (9.78)	9.57 (16.41)	15.56 (11.82)
Log-transformed Volume WMH (n = 78)	0.30 (0.69)	0.58 (0.66)	1.06 (0.40)
CSF $A\beta_{1-42}$, pg/mL (n = 48)	1325.50 (744.50)	1075.18 (591.82)	583.68 (219.32)
CSF t-tau, pg/mL (n = 48)	193.98 (110.16)	233.61 (111.32)	408.18 (199.76)
CSF p-tau, pg/mL (n = 48)	18.05 (11.43)	22.52 (11.91)	40.69 (20.49)

Table 2.1: Demographic, MRI, and CSF Data Based on Clinical Syndrome Group
All continuous data reported as mean (SD), categorical data (sex, APOE ε4 carrier) reported as number (percent). *APOE* ε4 carriers defined as participants with 1 or more *APOE* ε4 allele.

Cognitive and Functional Measures	CN	MCI	Dementia
FAQ (n = 82)	-0.44 (0.04)	-0.17 (0.60)	2.13 (1.09)
Logical Memory Immediate Recall (n = 85)	0.67 (0.72)	-0.51 (0.48)	-1.37 (0.59)
Category Fluency (n = 84)	0.51(0.80)	-0.39 (0.75)	-1.06 (1.06)
Boston Naming Test (n = 47)	0.46 (0.57)	0.12 (0.74)	-1.43 (1.14)
Sum of Total Trials on RAVLT (n = 84)	0.55 (0.87)	-0.41 (0.68)	-1.16 (0.66)
Number of Trials Learned on RAVLT (n = 84)	0.42 (0.96)	-0.26 (0.89)	-0.96 (0.52)
Part A on Trailmaking Test (n = 84)	-0.42 (0.42)	0.13 (0.84)	1.37 (1.63)
Part B on Trailmaking Test (n = 80)	-0.47 (0.58)	0.26 (0.99)	1.30 (1.13)

Table 2.2: Assessment of Cognition and Function (z-scores)
Values shown are mean (SD). All raw scores were converted to z-scores prior to any analyses. Number of participants with available data for each measure is indicated.

		t ratio, *P* value 95% Confidence Interval Range		
Variables	**ANCOVA Model *p* value**	**CN-Dementia**	**CN-MCI**	**MCI-Dementia**
Thickness of Entorhinal Cortex	<0.001	-5.42, <0.001 [-0.94 - -0.37]	-	-4.51, <0.001 [-0.89 - -0.27]
Thickness of Middle Temporal	<0.001	-2.89, 0.013 [-0.22 - -0.02]	-	-
Thickness of Parahippocampal	0.01	-3.18, 0.006 [-0.50 - -0.07]	-	-2.75, 0.020 [-0.49 - -0.03]
Thickness of Posterior Cingulate	0.001	-3.45, 0.003 [-0.23 - -0.04]	2.49, 0.039 [0 – 0.14]	-
Thickness of Insula	<0.001	-2.71, 0.022 [-0.24 - -0.02]	-	-
Volume of Hippocampus	<0.001	-4.65, <0.001 [-1750.17 - -563.04]		-2.88, 0.014 [-1398 - -130.6]
Log-transformed Volume of WMH	<0.001	2.84, 0.016 [0.10 – 1.13]	-	-

Table 2.3A: Post Hoc Tukey's HSD Testing for MRI Measures
Only regions with significant clinical syndrome group differences ($p < 0.05$) as reported by post hoc Tukey's HSD testing shown. Age was added as a covariate in all models. Acquisition type was also added as a covariate in volume of WMH model.

		t ratio, *P* value 95% Confidence Interval Range		
Variables	**ANCOVA Model p value**	**CN-Dementia**	**CN-MCI**	**MCI-Dementia**
FAQ	<0.001	14.46, <0.001 [2.12– 2.96]	-	12.22, <0.001 [1.83 – 2.72]
Logical Memory Immediate Recall	<0.001	-9.71, <0.001 [-2.51 - -1.52]	7.66, <0.001 [0.81 – 1.55]	-3.78, 0.001 [-1.37 - -0.31]
Category Fluency	<0.001	-5.45, <0.001 [-2.18 - -0.85]	4.50, <0.001 [0.42 – 1.37]	-
Boston Naming Test	<0.001	-6.16, <0.001 [-2.54 - -1.10]	-	-4.41, <0.001 [-2.17 - -0.63]
Sum of Total Trials on RAVLT	<0.001	-6.20, <0.001 [-2.27 - -1.01]	5.02, <0.001 [0.50 – 1.40]	-2.46, 0.0418 [-1.36 - -0.02]
Number of Trials Learned on RAVLT	<0.001	-4.46, <0.001 [-2.08 - -0.63)	3.11, 0.007 [0.16 – 1.20]	-
Part A on Trailmaking Test	<0.001	6.43, <0.001 [1.05 – 2.29]	-2.91, 0.013 [-0.98 - -0.10]	4.09, <0.001 [0.47 – 1.79]
Part B on Trailmaking Test	<0.001	6.11, <0.001 [0.97 – 2.22]	-3.74, 0.001 [-1.12 - -0.25]	3.32, 0.004 [0.26 – 1.58]

Table 2.3B: Post Hoc Tukey's HSD Testing for Cognitive and Functional Measures
Only significant clinical syndrome group differences ($p < 0.05$) as reported by post hoc Tukey's HSD testing shown. Age was added as a covariate in all models.

		t-ratio, *P* value 95% Confidence Interval Range		
Variables	**ANOVA Model *p* value**	**CN-Dementia**	**CN-MCI**	**MCI-Dementia**
CSF $A\beta_{1-42}$	0.047	-2.50, 0.042 [-1460.52 – 23.12]	-	-
CSF t-tau	0.002	3.81, 0.001 [77.87– 350.55]	-	2.97, 0.013 [32.16 – 316.99]
CSF p-tau	0.002	3.86, 0.001 [8.41 – 36.86]	-	2.96, 0.013 [3.31 – 33.03]

Table 2.3C: Post Hoc Tukey's HSD Testing for CSF Measures
Only measures with significant clinical syndrome group differences ($p < 0.05$) as reported by post hoc Tukey's HSD testing shown. No additional covariates added to models.

		t-ratio, *P* value 95% Confidence Interval Range		
Variables	**ANOVA Model *p* value**	**CN** ***APOE* ε4 carriers/non-carriers**	**MCI** ***APOE* ε4 carriers/non-carriers**	**Dementia** ***APOE* ε4 carriers/non-carriers**
FAQ	0.002	-	-	-4.14, <0.001 [-2.58 - -0.44]
CSF t-tau	0.003	-	-3.07, 0.04 [-352.76 - -3.89]	-
CSF p-tau	0.005	-	-3.05, 0.04 [-37.17 - -0.32]	-

Table 2.4: Post Hoc Tukey's HSD Testing for Interaction of Group and *APOE* ε4 status

Only measures with significant interactions between clinical syndrome group and APOE ε4 carrier status ($p < 0.05$) as reported by post hoc Tukey's HSD testing shown. Age added as covariate in ANCOVA model for FAQ.

Supplementary Table 2.1: List of ADNI patient IDs in Chapter 2

All participants enrolled within the ADNI are asked to classify their ethnicity as either "Hispanic or Latino," "not Hispanic or Latino," or "Unknown." Participants are further asked to classify their race as "American Indian or Alaskan Native," "Asian," "Native Hawaiian or Other Pacific Islander," "Black or African American," "White," "More than one race," or "Unknown."

All participants (n = 85) included in Chapter 2 identified as "Black or African American" and "not Hispanic or Latino." IDs for these participants are listed below.

003_S_4441
006_S_6291
007_S_1222
009_S_5037
010_S_4442
010_S_6748
011_S_0021
011_S_0023
011_S_6714
013_S_4395
013_S_4985
013_S_5071
014_S_4576
014_S_4577
014_S_6765
014_S_6831
016_S_6834
016_S_6853
018_S_0406
021_S_4419
021_S_4558
021_S_4633
022_S_6796
022_S_6822
024_S_4169
027_S_1385
031_S_4021

032_S_4277
032_S_4921
032_S_6699
032_S_6700
032_S_6701
032_S_6709
032_S_6717
032_S_6804
035_S_6730
035_S_6739
036_S_4820
036_S_4878
041_S_4427
041_S_4877
041_S_6136
041_S_6785
041_S_6786
041_S_6801
051_S_5005
052_S_1250
057_S_5199
067_S_0056
067_S_0059
068_S_5206
070_S_5040
072_S_2070
072_S_4610
082_S_6563
082_S_6564
082_S_6629
100_S_5075
100_S_5280
109_S_2200
109_S_2237
109_S_2278
109_S_4260

109_S_6218
109_S_6220
109_S_6300
114_S_5047
114_S_6063
114_S_6597
114_S_6813
116_S_4855
127_S_4843
127_S_4844
128_S_4607
129_S_4287
129_S_6304
129_S_6621
129_S_6852
130_S_0956
133_S_0912
135_S_5015
137_S_6794
153_S_6450
168_S_6843
341_S_6820

CHAPTER THREE: AMYLOID CONVERSION IS RELATED TO RISK FACTORS FOR DEMENTIA

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*Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

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Introduction

The amyloid precursor protein (APP) is an essential element of the cell membrane in many tissues and is concentrated in the synapses of neurons. It plays a role in protecting the cell from infection, repairing leakage in the blood brain barrier, promoting recovery from injury, and contributing to the regulation of synaptic function (Bishop & Robinson, 2004; Brothers et al., 2018). Cleavage of APP results in amyloid-beta (Aβ) peptides leaving the cell membrane, changing shape, and aggregating into long fibrils that form β-sheets (Brothers et al., 2018; Chen & Mobley, 2019; Hensley et al., 1994). The progression of insoluble fibrillar Aβ plaque deposition in the brain has been linked to age and increasing severity of Alzheimer's disease (AD) (Braak & Braak, 1991).

Soluble and insoluble Aβ burden can be measured *in vivo* in the cerebrospinal fluid (CSF). Insoluble Aβ can also be measured using radioactive ligands, such as the [^{18}F]-labeled florbetapir ligand, with positron emission tomography (PET) (Landau et al., 2013; Mattsson et al., 2015). Using established florbetapir PET standardized uptake value

ratio (SUVR) thresholds, the presence of Aβ can be categorized as amyloid negative (A-) or amyloid positive (A+) (Jack et al., 2018; Landau et al., 2013).

Studies have begun investigating how AD biomarkers and cognition are modified in people with changing Aβ burden (Dubois et al., 2018; Harrison et al. 2021; Landau et al., 2018; Sperling et al., 2020). In the present study, we sought to longitudinally examine participants in the Alzheimer's Disease Neuroimaging Initiative (ADNI) who underwent a conversion in amyloid status from negative (A-) to positive (A+) in comparison to a group of participants who remained A- over the same follow-up period. Conversion from A- to A+ was based upon florbetapir PET SUVR thresholds. Our hypothesis was that the amyloid converter group would show an increased frequency of *APOE* ε4 alleles, greater annualized percent volume loss in selected magnetic resonance imaging (MRI) regions, and lower CSF $A\beta_{1\text{-}42}$ in comparison to the non-converter group. Based on previous studies in the literature, we did not expect to see differences in CSF measures of tau or annualized change in cognitive performance (Gordon et al., 2018; Harrison et al., 2021; Ossenkoppele et al., 2019).

Materials and Methods

Participants

Data used in the preparation of this article were obtained from the ADNI database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial MRI, PET, other biological markers, and clinical and

neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early AD.

This study utilized data from 136 ADNI participants. Selection criteria was at least two florbetapir amyloid PET scans. All florbetapir PET data used in this study was downloaded in July 2021. All 136 participants were A- at the first scan yet 68 were classified as amyloid converters meaning they later became A+. Amyloid positivity (A+) on florbetapir PET was defined as retention above the established SUVR threshold of 1.11 (Landau et al., 2013; see Jagust et al., 2015 for additional details on ADNI PET protocols). The SUVR was calculated by combining binding measures from the frontal, angular/posterior cingulate, lateral parietal, and temporal cortices then dividing by the mean uptake value of the cerebellum. For participant selection, we extracted data from the first visit SUVR became > 1.11 and the visit prior. The average time between visits was 26.65 months.

Using the same florbetapir SUVR threshold, we identified 68 ADNI participants who were non-converters. Non-converting participants were chosen based upon 2 criteria: (1) that they maintained A- on *all* existing ADNI PET scans (through time of data collection) and (2) that they had representative (comparable) demographic data of the 68 amyloid converters. For instance, if we identified PET data from a male participant with MCI and 18 years of education who converted from A- to A+ at age 70, we looked for PET data from a 70 year old male participant with MCI and 18 years of education who remained A- on all existing ADNI PET scans. PET data for non-converters were downloaded from two visits which best corresponded to the timeframe of scans

downloaded in the amyloid converter group. The average time between visits was 30.88 months.

In addition to having at least two florbetapir PET scan, all participants had to be diagnosed by the ADNI Clinical Core as cognitively normal (CN) or MCI (Petersen et al., 1999; Petersen et al., 2014). Other variables of interest downloaded from the ADNI repository included demographics (years of education, sex, race), medical history such as cardiovascular health and smoking status, and *APOE* ε4 carrier status. Available T1 MRI, CSF, and cognitive data were also downloaded from the time of both PET visits.

Standard Protocol Approvals, Registrations, and Patient Consent

Written informed consent or assent was obtained from all participants, and study procedures were approved by the institutional review board at each of the ADNI participating sites. ADNI is listed in the ClinicalTrials.gov registry (ADNI-1: NCT00106899; ADNI-GO: NCT01078636; ADNI-2: NCT01231971; ADNI-3: NCT02854033). All data downloaded from the ADNI website was de-identified and is listed by patient ID number (Supplemental Table 3.1).

Imaging Assessments

The neuroimaging methods and parameters utilized by ADNI for T1 scans have been described previously (Jack et al., 2008). Visual inspection for artifact and unexpected neuropathology by the ADNI MRI Core was completed at the time of image upload to the ADNI. After downloading and throughout image processing, we also visually inspected the images for artifacts that could have impaired image processing. All scans downloaded were in their native DICOM format.

T1 Scan Selection and Processing Pipeline

Two T1 scans from 129 participants were downloaded and analyzed. T1 scans from seven participants were not used because the timing of scans did not correspond to the time of amyloid conversion and/or other data used in this study. The first T1 scan for amyloid converters was selected to be from the ADNI visit (or within six months after) the participant became A+. The second T1 scan for amyloid converters was chosen to come from a visit prior to conversion that best coincided with the timeframe of PET, CSF, and cognitive data. T1 scans from non-converters were selected to be from the same visits that PET data were collected.

All 258 T1 MRI scans were processed on a Mac Pro 2013 running OS version 10.14.5 using Freesurfer version 7.2 (Desikan et al., 2006; Iglesias et al., 2015). Scans were processed cross-sectionally followed by the longitudinal stream to extract reliable volume and thickness estimates in FreeSurfer (Reuter et al., 2012). An unbiased within-subject template space and image was created using robust, inverse consistent registration (Reuter et al., 2010; Reuter & Fischl, 2011). Several processing steps, such as skull stripping, Talairach transforms, atlas registration, spherical surface maps and parcellations were then initialized with common information from the within-subject template. This processing stream significantly increases reliability and statistical power (Reuter et al., 2012).

Volumes of regions of interest (ROI) from the Desikan-Killiany atlas were extracted and estimated total intracranial volume (eTIV) was generated by Freesurfer (Desikan et al., 2006; Iglesias et al., 2015). Nineteen cortical parcellations (Table 3.1A)

were selected as ROI based upon prior studies which have shown amyloid accumulation in these regions in early phases of AD through use of florbetapir PET (Guo et al., 2017; Palmquist et al., 2017; Villemange et al., 2017). Seven subcortical ROI were also examined (Table 3.1B). Total volume (right and left hemispheres added together) was calculated for both cortical and subcortical ROI. For analyses, the rate of volume change in each ROI was annualized then converted to percent change.

CSF Sampling and Analysis

One hundred and twenty-six participants had CSF measures generated by the ADNI Biomarker Core. CSF data was used if it was acquired within ~24 months of the PET data of interest. CSF data acquired outside this time frame were not included in analyses (n = 26). This resulted in CSF data from 36 amyloid converters and 64 non-converters. Standard practice of the ADNI Biomarker Core is to measure concentrations of amyloid beta 1-42 peptide ($A\beta_{1-42}$), total tau (t-tau), and tau phosphorylated at threonine 181 (p-tau) in collected CSF samples. Samples were obtained at the various ADNI sites via lumbar puncture as described in ADNI procedures and previous studies (Shaw et al., 2009).

AT(N) (Amyloid, Tau, Neurodegeneration) Classification

A total of 94 participants (31 amyloid converters and 63 converters) had the necessary data available to create full amyloid, tau, neurodegeneration [AT(N)] biomarker profiles per Jack et al. (Jack et al., 2018). Criteria for A+ on florbetapir PET is described above. Threshold levels for tau and neurodegeneration continue to evolve in the literature. We elected to use threshold values presented in recent studies assessing

AT(N) biomarkers. Tau positivity (T+) was defined as CSF p-tau ≥ 22 pg/mL (Guo et al., 2020). Neurodegeneration positivity (N+) was defined as total hippocampal volume as measured on structural MRI being less than 1.5 standard deviations below that of the sample's mean total hippocampal volume (Ingala et al., 2021).

Cognitive Evaluation

All participants in the study completed cognitive evaluations in English at the ADNI sites. Details pertaining to ADNI cognitive testing have been described previously (Aisen et al., 2010; Aisen et al., 2015). Cognitive data downloaded from the ADNI included performance on Logical Memory Delayed Recall (modified from the Wechsler D. Wechsler Memory Scale-Revised, San Antonio, Texas: Psychological Corporation; 1987), the Rey Auditory Verbal Learning Test (RAVLT; immediate recall), the Clock Drawing Test (Drawing Administration and Copying Administration), and Part B of the Trailmaking Test. These tests were chosen because none were used previously by the ADNI Clinical Core in determining clinical syndrome groups and they represent a multitude of cognitive domains (e.g. memory, executive function, and visuospatial functioning).

Data from amyloid converters were collected from the visit prior to and at conversion. Data from non-converters was matched to that of the amyloid converter group so that comparisons were based on a similar length follow-up period. Cognitive scores were standardized using z-scores and the rate of decline was annualized between timepoint 1 and 2. Rates of annualized change were calculated so that negative numbers

denoted worsening scores, 0 indicated no change, and positive numbers indicated improvement.

Statistical Analysis

All analyses were performed in JMP Pro V15.2 on a MacBook Pro 2015 running OS version 10.15.7. One-way analyses of variance (ANOVAs) were used to assess differences in age and education between amyloid converters and non-converters. Age was that recorded at the time of most recent MRI (closest to time of conversion). For the seven participants who did not have usable MRI data, age at time of conversion on PET and/or cognitive testing was used. Chi-square testing was used to assess differences between groups in categorical variables such as sex, diagnostic status (CN or MCI), race, history of cardiovascular health, smoking status, and *APOE* ε4 status. Statistical significance was set at $p < 0.05$ without correction.

Models were used to assess the effect of group on SUVR from the two florbetapir PET scans, annualized percent change in volume measured from MRI data, CSF data, and rate of annualized cognitive change. For PET data, we also assessed the effect of group on annualized rate of change (or amyloid accumulation) as measured by SUVR on florbetapir PET. For MRI and cognitive data, we assessed the effect of group on measures from both timepoints (pre and post-conversion). Prior to creating models, linear regressions were run to determine whether covariates such as age, sex, years of education, and diagnosis influenced these measures. For MRI data, we also used linear regressions to assess the influence of eTIV. Multiple comparisons were corrected for by use of the Benjamini-Hochberg method (Benjamini & Hochberg, 1995).

Data Availability

Data used in this article are available for download from the Laboratory of NeuroImaging (LONI; loni.usc.edu). All variables were extracted from spreadsheets posted on LONI.

Results

Demographics

One-way ANOVAs showed no significant differences in age nor years of education between groups ($p > 0.05$). Chi-square testing showed no differences between groups in sex ($p = 0.73$) nor in diagnostic status ($p = 0.86$). Chi-square testing further showed no differences between groups in race, history of cardiovascular health, or smoking status ($p > 0.05$). Data reported in Table 3.2.

SUVR Uptake on Florbetapir PET Scans

Linear regressions showed that sex, but not age, years of education or diagnosis, had a significant effect on PET SUVR and was used as a covariate in subsequent models. Analyses of covariance (ANCOVA) models assessing the main effect of group with sex as a covariate showed a significant effect of group on the SUVR for both florbetapir PET scans. On the first florbetapir PET scan when both groups were A-, the ANCOVA showed a significant effect of group ($F(2,133) = 58.6$, $p < 0.001$) (Table 3.2). On the second scan when the amyloid converter group had become A+, the ANCOVA also showed a significant effect of group ($F(2,133) = 307.6$, $p < 0.001$) (Table 3.2). The effect of sex was significant on both scans ($p < 0.021$). An ANCOVA assessing the main effect of group with sex as a covariate on annualized rate of change in SUVR on florbetapir

scans also showed a significant effect of group ($F(2, 133) = 75.39$, $p < 0.001$), but the effect of sex was not significant ($p = 0.13$). Two-way ANCOVAs exploring the interaction of sex with age and sex with diagnosis (CN/MCI) both with and without group as a covariate were not significant.

APOE ε4 status

All 136 participants had *APOE* ε4 data (Table 3.2). Those with 1 or 2 ε4 alleles were classified as carriers and those with 0 ε4 alleles were classified as non-carriers. Chi-square testing revealed a significant difference between groups in proportion of *APOE* ε4 carriers ($p < 0.001$) and in frequency of ε4 alleles ($p < 0.001$) with the amyloid converter group having more carriers and greater frequency of ε4 alleles than the non-converter group.

MRI Analyses: Annualized Percent Change in Volume

Linear regressions showed covariates including age, sex, years of education, diagnosis, and eTIV had no effect on annualized percent volume change. One-way ANOVAs showed a significant effect of group (converter vs non-converter) on annualized percent change in total volume of 8 of the 19 cortical ROI. These regions included: frontal pole ($F(1,127) = 7.65$, $p = 0.007$), caudal anterior cingulate ($F(1,127) = 7.54$, $p = 0.007$), lateral occipital cortex ($F(1,127) = 6.19$, $p = 0.014$), medial orbitofrontal gyrus ($F(1,127) = 5.21$, $p = 0.024$), pars orbitalis ($F(1,127) = 4.88$, $p = 0.029$), superior temporal gyrus ($F(1,127) = 4.85$, $p = 0.029$), posterior cingulate cortex ($F(1,127) = 4.76$, $p = 0.031$), and the middle temporal gyrus ($F(1,127) = 4.65$, $p = 0.033$). These ROI are illustrated on a pial surface of the right hemisphere in Figure 3.1A-B.

One-way ANOVAs showed a significant effect of group on annualized percent change in total volume of three of the seven subcortical ROI. These regions included: pallidum ($F(1,127) = 5.73$, $p = 0.019$), amygdala ($F(1,127) = 5.12$, $p = 0.025$), and hippocampus ($F(1,127) = 4.35$, $p = 0.039$). The main effect of group remained significant for all cortical and subcortical regions after correction for multiple comparisons using the Benjamini-Hochberg method. One-way ANOVAs used to assess the main effect of group on total volume at each MRI scan (timepoints 1 and 2) showed no effect of group on total volume in 18 of the 19 cortical ROI and all 7 subcortical ROI on either scan. We saw a significant difference between groups in total volume of the superior parietal gyrus at the first ($p = 0.023$) and the second ($p = 0.041$) scans, but neither difference survived correction for multiple comparisons.

CSF Sampling

Linear regressions showed that age, sex, years of education, and diagnosis had no effect on CSF measures. One-way ANOVAs showed a significant effect of group on CSF $A\beta_{1\text{-}42}$ ($F(1, 98) = 42.46$, $p < 0.001$) (Table 3.2). There was no effect of group on CSF t-tau or CSF p-tau.

AT(N) Framework

We found 0 out of 31 amyloid converters were A+T+(N)+. Eight amyloid converters were A+T+(N)- and two were A+T-(N)+. The remaining 21 amyloid converters were A+T-(N)-. All 63 non-converters were A- and none were found to be A-T+(N)+. Of the 63 non-converters, 18 were A-T+(N)- and 4 were A-T-(N)+. AT(N) profiles for these 94 participants are shown in Table 3.3.

Annualized Cognitive Change

Linear regressions showed that age, sex, years of education, and diagnosis had no effect on rate of annualized cognitive change. One-way ANOVAs showed no significant effect of group on rate of annualized cognitive change in performance on any of the five measures ($p > 0.05$). The effect of group on rate of annualized change in performance on Clock Drawing Test (Copying Administration) approached significance ($F(1,134 = 3.81$, $p = 0.053$). One-way ANOVAs further showed no significant effect of group on performance of the five measures at either ADNI cognitive testing visit (timepoint 1 or 2).

Discussion

Our study examined what changes occur while ADNI participants are converting from A- to A+. In comparison to the non-converter group, the amyloid converter group had an increased prevalence of *APOE* ε4, greater annualized percent volume loss in 8 cortical and 3 subcortical brain regions, and lower CSF $A\beta_{1-42}$. Previous studies have taken a similar approach to the present study by examining participants with changing Aβ status and found mixed results. A study by Dubois and colleagues found amyloid status alone did not predict progression to dementia (Dubois et al., 2018). Similarly, Harrison et al. found that relationships between amyloid status and either cortical thickness or memory are dependent on the presence of tau (Harrison et al., 2021). Many existing studies that have examined participants with varying Aβ status have analyzed participants who are *already* A+ in comparison to participants who are A- (Dubois et al., 2018; Harrison et al., 2021; Sperling et al., 2020; Tosun et al., 2021). Therefore, a goal of the

present study was 1) to expand upon previous literature by examining changes in Aβ status with an emphasis on the period *leading* up to amyloid conversion and 2) to investigate the less-explored relationship of changes in Aβ with features such as cortical volume.

The groups in this study did not differ in demographic factors or medical history such as cardiovascular health or smoking status. We expected amyloid converters to show significantly larger SUVR values at the second florbetapir PET scans since amyloid converters must exceed the 1.11 SUVR threshold to become A+. Interestingly, we saw significantly larger SUVR in amyloid converters at the first PET scan as well. This suggests that the increase in amyloid may be a slowly accumulating process that potentially begins some time (years) before conversion takes place. This was also reflected in findings which showed the annualized rate of change was significantly greater in the amyloid converter group whereas the average SUVR of the non-converter group between the two PET scans showed remarkably no change. On a broader scale, these findings reiterate the work of others which has emphasized that the magnitude of amyloid change (as measured by SUVR) rather than baseline Aβ burden alone may be an equally or more effective measure for assessing progression of AD (Farrell et al., 2018; Landau et al., 2018). Our findings of higher average SUVR in females were somewhat unexpected as previous studies have not shown any associations between sex and amyloid positivity (Jansen et al., 2022).

Our *APOE* ε4 findings support the hypothesis that presence of the ε4 allele is a risk factor for amyloid accumulation. This finding is consistent with studies which have

shown the presence of the ε4 allele modifies the ability of Aβ to be cleared by the brain, resulting in ε4 carriers having greater cortical Aβ deposition (Chen & Mobley, 2019; Fouqet et al., 2014; Selkoe & Hardy, 2016), Our findings of the amyloid converter group having more participants with the ε4 allele than the non-converter group is also supported by previous studies which have examined the prevalence of *APOE* ε4 in participants who are A+ or A- (Dubois et al., 2018; Jansen et al., 2022; Sperling et al., 2020; Tosun et al., 2021).

To examine the relationship between Aβ burden and neurodegeneration, we assessed annualized percent change in regional brain volume. These analyses revealed greater annualized percent volume loss in eight cortical ROI in the amyloid converter group compared to non-converter group. Interestingly, there were no group differences in these eight regions when we assessed total volume at the first or second MRI scan. The 19 cortical ROI examined in this study were chosen based upon previous literature which had suggested these regions show amyloid accumulation on florbetapir PET in early phases of AD (Guo et al., 2017; Palmquist et al., 2017; Villemange et al., 2017). In contrast to tau which has a staged deposition pattern, the temporal accumulation of amyloid continues to be a key area of research, particularly with regards to how patterns of amyloid accumulation reflected on PET imaging relates to volume loss and neurodegeneration. Many previous studies that have examined differences in cortical thickness in A- and A+ participants did not find any relationships unless they further accounted for tau (Aschenbrenner et al., 2018; Harrison et al., 2021; Ossenkoppele et al., 2019).

Of the subcortical ROIs we assessed, we found greater annualized percent volume loss in the hippocampus, amygdala, and pallidum in the amyloid converter group. None of these regions showed group differences at the first or second MRI scans. Few studies have assessed Aβ burden and subcortical ROI outside of the hippocampus. Similar to the present study, Morar and colleagues reported a steeper decline in average hippocampal volume in the A+ group in comparison to the A- group (Morar et al., 2022). In their study, they also calculated within-person annual rate of change in hippocampal volume. On the contrary, many studies that have examined hippocampal volume at one timepoint have shown no difference when comparing participants who are A+ and A- (Chen et al., 2021; Dubois et al., 2018). For instance, Dubois and colleagues initially found total hippocampal volume between A- and A+ groups was significantly different but this significance did not survive correction for age, sex, and multiple comparisons (Dubois et al., 2018). Likewise, Chen and colleagues found no difference between amyloid groups when using a different Aβ binding ligand to define A+ (Chen et al., 2021). Notably, both these studies that reported no group difference in hippocampal volume were only examining participants at one timepoint. When we compared hippocampal volume between our converter and non-converter groups at the first or second MRI scan alone, we also saw no differences between groups in hippocampal volume. The difference between groups was only detectable when we measured annualized percent volume loss. Altogether our significant between-group cortical and subcortical findings could indicate that 1) cortical volume is a more sensitive measure than cortical thickness in detecting

disease progression and 2) assessing percent change over a period of time rather than use of a static measure may also be a better way to detect subtle changes.

Our CSF finding of lower $A\beta_{1-42}$ in the amyloid converter group also supports our hypothesis and is consistent with previous studies that have examined differences in CSF $A\beta_{1-42}$ between participants who are A- and A+ on florbetapir PET (Dubois et al., 2018; He et al., 2021). We further examined whether a change in amyloid PET status had any relation to CSF measures of p-tau and t-tau but saw no significance. This finding may be reflective of our choice to examine the time period prior to amyloid conversion whereas changes in both CSF p-tau and t-tau are believed to follow changes in CSF $A\beta_{1-42}$ and amyloid PET (Guo et al., 2021; Jack et al., 2013; Selkoe & Hardy, 2016).

Since the nature of this study already categorized participants as A+/A-, we were curious to categorize full AT(N) AD biomarker profiles. Per the AT(N) framework, the presence of Aβ biomarkers alone are enough to categorize whether or not someone is in the AD continuum but the additional presence of tau is required to make a neuropathologic diagnosis of AD (Jack et al., 2018). Of the 31 amyloid converters (A+ participants) with full AT(N) biomarkers available, we found none were A+T+(N)+ but 8 were A+T+(N)- meaning their neuropathologic profile could be interpreted as consistent with AD (Jack et al., 2018). Of these 8 A+T+(N)- participants, 4 had been classified as MCI and 4 as CN by ADNI Clinical Core guidelines. Two other amyloid converters were A+T-(N)+ and both were CN. None of the 63 non-converters were A-T+(N)+ but 18 were A-T+(N)- and 4 were A-T-(N)+. These profiles indicate non-AD related pathologic changes which means that while these 22 non-converters have neuropathology, they are

not on the AD continuum (Jack et al., 2018). The remaining 41 non-converters were A-T-(N)- meaning their biomarker profiles were “normal.”

Lastly, we found no difference between the two groups (amyloid converters and non-converters) in annualized rate of cognitive change or in cognitive performance at either cognitive testing visit. Other studies have shown disparate findings when comparing cognition between participants who are A- and A+. Findings by Sperling and colleagues showed A+ participants performed worse on all four components of the Preclinical Alzheimer Cognitive Composite (which includes the Logical Memory Delayed Score and other measures of verbal memory used in the present study) than their A- counterparts (Insel et al., 2020; Sperling et al., 2020). Meanwhile, other findings by Dubois and colleagues and Rentz et al. are consistent with ours and showed no difference in time to complete the Trailmaking Test and performance on Logical Memory Delayed Recall (respectively), in participants who are A- and A+ (Dubois et al., 2018; Rentz et al., 2021). It is unclear why some studies have observed a difference in cognitive function between A- and A+ groups whereas others, like the present study, show no difference in annualized rate of change or performance at either cognitive visit.

Limitations

Although we had access to 68 participants who showed conversion on florbetapir PET from A- to A+, this sample size remains modest. While some of these participants also had tau PET scans, we elected to only focus on amyloid PET since there is currently little consensus as to how to interpret tau PET positivity. Once consensus is reached, it would be interesting to see what including tau PET data adds to our findings.

Using the ADNI population allows us to examine data longitudinally and in participants across North America, but certain limitations exist when using a clinical trials population which has selection criteria that restricts many in the general population from study entry. Furthermore, scheduling protocols for ADNI visits have varied over time (and between enrollment cohorts).

Conclusion

The results of this study confirmed our hypothesis that the amyloid converter group would show an increased prevalence of *APOE* ε4, greater annualized percent volume loss in selected MRI regions, and lower CSF $A\beta_{1\text{-}42}$ in comparison to the non-converter group in this ADNI sample. These results provide compelling evidence that important neuropathological changes are occurring prior to amyloid conversion.

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Fujirebio; GE Healthcare; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Lumosity; Lundbeck; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Neurotrack Technologies; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Takeda Pharmaceutical Company; and Transition Therapeutics. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Therapeutic Research Institute at the University of Southern California. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California.

(A) Cortical Regions of Interest
Banks of superior temporal sulcus
Caudal anterior division of cingulate cortex*
Inferior parietal cortex
Inferior temporal gyrus
Isthmus division of cingulate cortex
Lateral occipital cortex*
Lateral division of orbitofrontal cortex
Medial division of orbitofrontal cortex*
Middle temporal gyrus*
Paracentral lobule
Pars orbitalis*
Pars opercularis
Pars triangularis
Posterior division of cingulate cortex*
Precuneus
Rostral anterior division of cingulate cortex
Superior parietal
Superior temporal*
Frontal pole*

(B) Subcortical Regions of Interest
Thalamus
Caudate
Putamen
Pallidum*
Hippocampus*
Amygdala*
Accumbens

Table 3.1: MRI Regions of Interest
Table 3.1A shows 19 selected cortical ROI and Table 3.1B shows 7 selected subcortical ROI. Significant ROI ($p < 0.05$ following Benjamini-Hochberg method) from one-way ANOVAs denoted with (*).

	Amyloid Converters (n = 68)	**Non-Converters (n = 68)**	***p* value**
Age mean (SD), y	76.41 (7.24)	76.01 (7.38)	0.75
Education mean (SD), y	16.56 (2.40)	16.84 (2.80)	0.53
Sex (M/F)	40 M 28 F	42 M 26 F	0.73
Diagnostic Status (CN/MCI)	41 CN 27 MCI	42 CN 26 MCI	0.86
Race	65 White 1 Black 2 More than One	67 White 1 Black	0.36
History of Cardiovascular Health	40 with history 27 without^	42 with history 26 without	0.81
Smoking Status	25 Smokers 42 Non-Smokers	32 Smokers 36 Non-Smokers	0.25
APOE ε4 carrier status n (%) **0 ε4 alleles** **1 ε4 alleles** **2 ε4 alleles**	28 (41.18) 40 (58.82) 22 (32.35) 6 (6.82)	8 (11.76) 60 (88.24) 8 (11.76) 0 (0)	<0.001 <0.001
SUVR PET Scan #1	1.07 (0.03)	1.01 (0.05)	<0.001
SUVR PET Scan #2	1.16 (0.04)	1.01 (0.04)	<0.001
CSF $A\beta_{1-42}$ mean (SD), pg/mL	1003.31 (415.06) (n = 36)	1472.26 (299.85) (n = 64)	<0.001
CSF t-tau mean (SD), pg/mL	242.91 (90.86) (n = 36)	226.26 (64.74) (n = 64)	0.29
CSF p-tau mean (SD), pg/mL	21.29 (7.88) (n = 36)	19.62 (5.64) (n = 64)	0.22

Table 3.2: Demographic, APOE ε4, SUVR, and CSF Data
One-way ANOVAs used for continuous data, chi-square testing used for categorical data. Sex was added as a covariate to the ANOVA models for SUVR on PET scans. Response for medical history recorded as "yes" or "no." ^Medical history not available for one converter. APOE ε4 carriers are participants with 1 or 2 ε4 alleles. Scan #1 indicating the earlier scan and scan #2 indicating the scan that followed.

	Amyloid Converters (n = 31)	Non-Converters (n = 63)
A+T+(N)+	-	-
A+T+(N)-	8 (25.81)	-
A+T-(N)+	2 (6.45)	-
A+T-(N)-	21 (67.74)	-
A-T+(N)-	-	18 (28.57)
A-T-(N)+	-	4 (6.35)
A-T+(N)+	-	-
A-T-(N)-	-	41 (65.08)

Table 3.3: AT(N) Profiles of Participants
Values reported as number (percent). Participants without complete AT(N) biomarker data were excluded.

Supplementary Table 3.1: List of ADNI patient IDs in Chapter 3

IDs for all ADNI participants (n = 136) used for analysis in Chapter 3 are listed below.

002_S_0685
002_S_1261
002_S_1280
002_S_4229
002_S_4654
002_S_5178
003_S_0981
003_S_2374
003_S_4119
003_S_4350
003_S_4441
003_S_4555
003_S_4872
005_S_0602
005_S_4168
005_S_4185
006_S_0498
006_S_4357
006_S_4485
006_S_4960
007_S_2394
007_S_4272
007_S_4620
007_S_4637
009_S_0751
009_S_4543
009_S_4741
009_S_5176
011_S_4105
012_S_4012
012_S_4094
014_S_4401
016_S_4121

018_S_4313
019_S_4285
019_S_4293
019_S_4367
019_S_4835
020_S_5140
020_S_6185
021_S_4254
022_S_0096
022_S_2379
022_S_4173
022_S_5004
023_S_0042
023_S_0926
023_S_4115
023_S_4448
024_S_2239
024_S_4084
027_S_0120
027_S_2183
027_S_2245
027_S_5079
027_S_5083
027_S_5118
027_S_5169
027_S_5288
029_S_5219
031_S_0618
031_S_2233
031_S_4218
032_S_0677
032_S_2119
033_S_1016
033_S_4177
033_S_4505
033_S_5259
035_S_2061

035_S_4464
035_S_4784
037_S_4706
037_S_5126
041_S_0679
041_S_4200
041_S_4271
041_S_4427
041_S_4513
041_S_5097
041_S_5100
041_S_5253
052_S_2249
053_S_2357
057_S_2398
067_S_4767
067_S_4782
068_S_0127
068_S_2187
068_S_4424
072_S_4383
072_S_4445
073_S_0089
073_S_4300
073_S_4552
082_S_4090
082_S_4208
082_S_4224
082_S_4428
094_S_2238
094_S_2367
098_S_0896
098_S_2052
098_S_4050
100_S_4469
109_S_4499
114_S_0416

116_S_4043
116_S_4453
126_S_2360
126_S_5243
127_S_0260
127_S_1427
127_S_4604
128_S_0272
128_S_2036
128_S_2220
128_S_4653
128_S_4842
129_S_2332
129_S_2347
129_S_4369
129_S_4396
130_S_4352
130_S_4417
130_S_4817
130_S_5175
131_S_0123
135_S_4598
135_S_5113
137_S_0722
137_S_4466
137_S_4520
941_S_4036
941_S_4292
941_S_5193

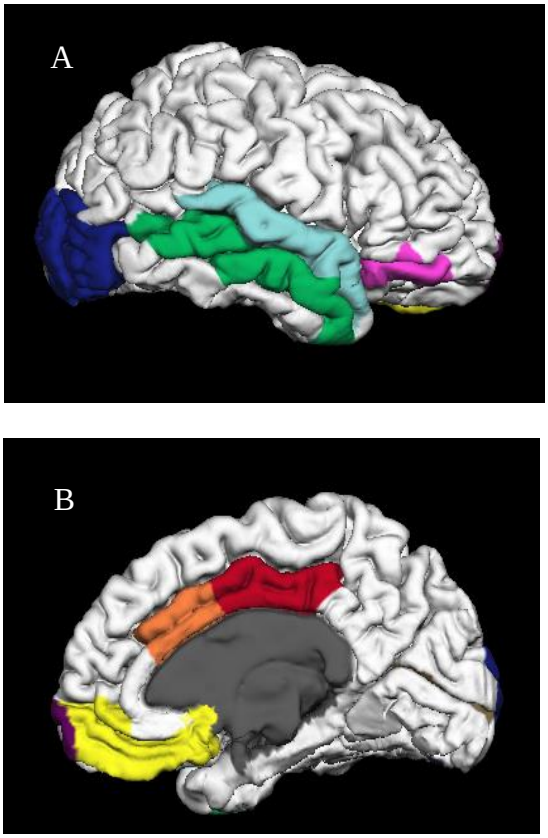

Figure 3.1: Cortical ROI on Pial Surface of Right Hemisphere
ROI that showed significant between group differences shown above ($p < 0.05$ following Benjamini-Hochberg method).

Lateral surface (Figure 3.1A) illustrates lateral occipital cortex, superior temporal gyrus, middle temporal gyrus, and pars orbitalis. Medial surface (Figure 3.1B) illustrates caudal anterior division of cingulate cortex, posterior division of cingulate cortex, medial division of orbitofrontal cortex, and frontal pole.

CHAPTER FOUR: AGE-RELATED CHANGES TO INTEGRITY OF THE CINGULUM, SURROUNDING REGIONS OF INTEREST, AND COGNITIVE FUNCTION

Introduction

Degeneration of both the gray and white matter of the brain is characteristically found in Alzheimer's disease (AD) (Araque Caballero et al., 2018; Fan et al., 2019; Gold et al., 2011). For years, researchers believed that changes in the white matter (WM) were secondary to changes in the gray matter (GM) but recent studies have provided evidence that these changes are independent of one another and that alterations to the WM may even occur first (Esrael et al., 2021; Mayo et al., 2019). Such WM changes often include myelin loss, axonal injury, and edema (Shao et al., 2019). Overtime, this pathology leads to reduced integrity of WM pathways in the brain, less efficient conduction of neural signals, and impaired neuronal transmission (Fan et al., 2019). For more details on how diffusion tensor imaging (DTI) and diffusion measures such as fractional anisotropy (FA) and mean diffusivity (MD) are used to study AD, refer to Beyond the Framework: Diffusion Tensor Imaging in Chapter 1.

Previous studies have shown a decrease in FA and an increase in MD in AD yet how early in the AD continuum these changes become detectable and which WM pathways show the greatest vulnerability remains less clear (Araque Caballero et al., 2018; Liu et al., 2017). A pathway of interest that has received particular attention in studies of AD is the cingulum bundle (Fan et al., 2019; Gold et al., 2011; Jacobs et al., 2018; Mito et al., 2018; Shao et al., 2019). The cingulum bundle has dense projections to

the hippocampus and other regions in the medial temporal lobe (MTL), as well as projections which extend through the retrosplenial cortex, anterior cingulate and into the frontal lobe (Bubb, Metzler-Baddeley, & Aggelton, 2018; Shao et al., 2019). Due to its complexity, many tractography toolboxes further divide the cingulum into "ventral" and "dorsal" pathways with the goal of being able to better localize where and when age-related changes within this region occur (Bubb et al., 2018). The cingulum-dorsal (CD) pathway extends from the frontal lobes into the parietal lobes whereas the cingulum-ventral (CV) pathway extends from the parietal lobes through the temporal lobe and into the hippocampus (Figure 4.1A-B, Bubb et al., 2018, Fan et al., 2019). Another region of interest (ROI) that continues to be explored in AD research due to its role in navigation, spatial and episodic memory is the retrosplenial cortex (RSC) (Vann, Aggleton & Maguire, 2009). The RSC was of particular interest to the present study because of its neuroanatomical location within the posterior cingulate and proximity to both cingulum pathways.

Studies have begun studying the relationship between WM integrity of the cingulum and cognitive functions such as executive function and memory, but few studies have assessed how cognition and diffusion measures are also related to the volume of neighboring ROIs (Bubb et al., 2018; Jacobs et al., 2018; Mayo et al., 2019; Shao et al., 2019). The goals of this study were to define relationships between measures of integrity and regional volume with cognitive function. By assessing participants across the cognitive spectrum (cognitively normal, amnestic mild cognitive impairment (aMCI), and dementia) enrolled in a study led by the Boston University Alzheimer's Disease

Research Center (BU-ADRC), we examined three primary hypotheses. (1) Participants with dementia will show lower FA and greater MD than participants with aMCI or controls in both cingulum tracts. Between groups, we expected to see greater differences in diffusion measures between control (cognitively normal) participants and participants with aMCI in the CV pathway and greater differences between participants with aMCI and dementia in the CD pathway because the volume and connectivity between MTL regions are often affected in earlier stages of AD than frontal regions (Tucholka et al., 2018). To assess whether these differences in WM integrity are ubiquitous or specific to these tracts of interest, we further assessed FA and MD in a control region, the corticospinal tract (CST, see Figure 4.1C). (2) Next, we examined the relationships between WM integrity of the CD and CV to the volume of selected ROI. Based upon its projections to the frontal lobe, we expected the FA and MD of the CD to show relationships with volume of regions in the prefrontal cortex (PFC) and we expected the FA and MD of the CV to show relationships with volume of regions in the MTL. We expected both pathways to show relationships with volume of the RSC. (3) Lastly, we explored relationships between WM integrity and volume with measures of cognition. Our final hypothesis was that in the CD pathway, measures of FA would show a positive relationship and measures of MD a negative relationship with executive function. Volume of PFC regions would show a positive relationship with executive function. Similarly, in the CV pathway, we expected that measures of FA would show a positive relationship and measures of MD a negative relationship with memory function. Volume of regions within the MTL would show a positive relationship with memory function.

Materials and Methods

Participants

The study had access to MRI data from 306 participants within the Health Outreach Program for the Elderly (HOPE), a cohort study led by the BU-ADRC. The BU-ADRC is a center (of 30) funded by the National Institute on Aging that contributes data to the National Alzheimer's Coordinating Center (NACC). The BU-ADRC registry, including subject recruitment and inclusion/exclusion criteria, has previously been described (Ashendorf et al., 2017; Galetta et al., 2017). Participants (n = 306) provided the BU-ADRC with written consent to participate in all aspects of this study. The BU-ADRC executive committee approved of the sharing of these data.

Of the 306 participants within the HOPE study, 286 had a clinical NACC diagnosis. All NACC diagnoses were made at BU-ADRC consensus conferences, following presentation and discussion of all medical history and evaluation results, such as clinical interview, informant input, neuropsychological test scores, and MRI scans viewed for clinical criteria (hippocampal atrophy, WM signal abnormalities, and evidence of microbleeds). For the purposes of this study, only participants with the diagnosis of "control," "aMCI" (with either single or multiple cognitive domains affected), or "dementia" were included (n = 241). Participants with missing diagnoses (n = 5) or diagnoses such as "non-amnestic MCI" (in either single or multiple domains, n = 10) and "cognitively impaired, not MCI" (n = 35) were excluded. Other demographic data collected for the 241 HOPE participants included in this study are listed in Table 4.1.

Clinical Evaluation

In addition to demographic and imaging data, the HOPE study also collects neuropsychological data either at the time of the MRI visit or at a visit shortly thereafter. We used performance on the Craft Delayed Recall (verbatim) as our measure of memory and (time to complete) Part B of the Trailmaking Test as our measure of executive function. Raw scores for both measures were converted to z-scores for statistical analysis. Z-scores were calculated so that positive numbers indicated performance was above the mean and negative numbers below the mean for both measures.

Imaging Assessments

All participants in this study were scanned at the Center for Biomedical Imaging (CBI) at Boston University School of Medicine (BUSM) between 2015-2020. T1 and high angular resolution diffusion images (HARDI) sequences were acquired for all participants on the 3T Philips Acheiva scanner in the CBI. For T1 scans, a 32-channel headcoil and sense factor of 2 was used with the following imaging parameters: TR = 6.8 ms, TE = 3.1 ms, flip angle = 9°, reconstructed and acquisition voxel size = .98 mm x .98 mm x 1.2 mm , FOV = 250 mm x 250 mm x 180 mm, 150 sagittal slices. For HARDI scans, a 32-channel headcoil and sense factor of 2 was used with the following imaging parameters: TR = 10,153 ms, TE = 97 ms, flip angle = 90°, reconstructed and acquisition voxel size = 2 mm x 2 mm x 2 mm, FOV = 224 mm x 224 mm x 128 mm, 64 transverse slices.

T1 scans were processed with Freesurfer version 7.2 on a Mac Pro 2013 running OS version 11.6 to obtain cortical parcellations and subcortical segmentations of anatomical regions (Desikan et al., 2006; Iglesias et al., 2015). The three PFC ROI were

the rostral middle frontal gyrus, caudal middle frontal gyrus, and superior frontal gyrus. The two MTL ROI were the hippocampus and entorhinal cortex. We also examined volume of the isthmus cingulate because this region is most representative of the RSC (Desikan et al., 2006; Vann et al., 2009). Total volume was used in statistical analyses by adding the right and left volume of each ROI. Estimated total intracranial volume (eTIV) was also generated by Freesurfer.

HARDI scans were processed with TRActs Constrained by UnderLying Anatomy (TRACULA), an updated toolbox available as part of FreeSurfer version 7.2 (Maffei et al., 2021; Yendiki et al, 2011). Such processing includes eddy-current compensation via FMRIB Software Library (FSL), intra- and inter-subject registration, ball-and-stick modeling, and tensor fitting (Vipin et al., 2019). Intra-subject registration was completed using boundary-based cost (bbregister) and inter-subject registration was completed using systemic normalization from Advanced Normalization Tools. TRACULA is a novel method for automated global probabilities reconstruction of 42 major WM pathways (https://dmri.mgh.harvard.edu/tract-atlas/). In order to reconstruct these WM tracts, TRACULA utilizes prior information on anatomy from a set of training participants where the tracts of interest were labeled manually. This prior information is the likelihood of each tract to travel through or next to each of the cortical and subcortical segmentation labels previously generated from processing T1 scans through Freesurfer. Our tracts of interest were the CD and CV and our control tract was the CST. Figure 4.2 shows a visual reconstruction of both the CD and CV tracts in Freeview. Following reconstruction of all three tracts, our primary diffusion measures, FA and MD, were

extracted from both right and left pathways. Average FA and MD were calculated between the right and left tracts of interest and used in statistical analyses.

Statistical Analysis

All analyses were performed in JMP Pro V15.2 on a MacBook Pro 2015 running OS version 10.15.7. Analyses of variance (ANOVAs) were used to assess differences between the control, aMCI, and dementia clinical syndrome groups in age and years of education. Chi-square testing was used to assess for differences in the distribution of categorical variables such as sex, race, and *APOE* ε4 carrier status. Statistical significance was set at $p < 0.05$ without correction.

To standardize the data, all raw values (diffusion measures, volume, and cognitive scores) were converted to z-scores for statistical analyses. Mean raw values and z-score values for measures of cognition and diffusion are shown in Tables 4.2-4.3. Next, models were created assessing the effect of clinical syndrome group on FA and MD of the tracts of interest. Prior to creating models, linear regressions were used to determine if age or education influenced the measures. Post hoc Tukey's Honest Significant Difference (HSD) testing was performed for all significant results. Clinical syndrome group differences, *p* values and 95% confidence intervals are reported (Table 4.4). Once initial group differences were established, multiple linear regression models were created to assess the relationship between diffusion measures (FA/MD of CD and CV) and volume of selected ROI such as those within the PFC, MTL, and RSC. Lastly, we conducted multiple linear regressions to assess the relationship between diffusion measures, volume of selected ROI, and performance on the cognitive measures. Age and

eTIV were added as covariates in all models. Models with significant effects that survived false discovery rate (FDR) correction ($p_{FDR} < 0.05$) are reported.

Results

Demographic Data

ANOVAs showed no significant differences between groups in age nor years of education (Table 4.1). Chi-square testing showed no significant difference in race between the clinical syndrome groups, but a significant difference in sex between clinical syndrome groups ($p < 0.001$). Follow-up pairwise chi-square testing revealed differences in sex distribution within the control ($p < 0.001$, significantly more females*)* and dementia groups ($p = 0.008$, significantly more males). Chi-square testing showed proportion of *APOE ε4* carriers between clinical syndrome groups was trending towards significance ($p = 0.06$). Follow-up pairwise chi-square testing revealed differences in proportion of *APOE ε4* carriers between control and aMCI groups ($p < 0.001$) and between control and dementia groups ($p = 0.018$). Performance on measures of cognition were significantly different between clinical syndrome groups as expected ($p < 0.001$, Table 4.2).

FA and MD Between Groups

Linear regressions showed age had a significant effect on diffusion measures (FA/MD) and was added as a covariate in subsequent models. Analyses of covariance (ANCOVA) models assessing the main effect of group with age as a covariate showed a significant effect of group on the FA and MD of both cingulum pathways (Table 4.3). Clinical syndrome group differences, *p* values, and confidence intervals from Tukey's

HSD testing are shown in Table 4.4. Age had a significant effect on all four measures ($p < 0.001$). ANCOVAs assessing the main effect of group with age as a covariate for the CST did not show differences in either FA (p =0.36) or MD (p = 0.97).

Relationships between Tracts and Volume of ROI

The following analyses were conducted between FA and MD of both cingulum tracts and volume of ROI as follows: (1) CD and MTL, (2) CD and PFC, (3) CV and MTL, (4) CV and PFC. The volume of RSC was examined in relation to both tracts. Z-scored diffusion measures and volume of ROI were used in all analyses.

Cingulum Dorsal - MTL

Of the four multiple regression models assessing the relationship of FA and MD of the CD with volume of the two regions in the MTL (with age and eTIV as covariates), both relationships with the hippocampus were significant. The relationship between MD of the CD and volume of the hippocampus was ($F(3,237) = 38.28$, $p < 0.001$) and the effects of age ($p_{FDR} < 0.001$), eTIV ($p_{FDR} = 0.004$), and volume of the hippocampus ($p_{FDR} = 0.014$) were all significant. The relationship between FA of the CD and volume of the hippocampus was ($F(3,237) = 26.94$, $p < 0.001$) and the effects of age ($p_{FDR} < 0.001$), eTIV ($p_{FDR} <0.001$) and volume of the hippocampus ($p_{FDR} = 0.012$) were all significant.

Cingulum Doral - PFC

Of the six multiple regression models assessing the relationship of FA and MD of the CD with volume of the three regions in the PFC (with age and eTIV as covariates), only the relationship between MD of the CD and volume of the caudal middle frontal gyrus was significant ($F(3,237) = 38.69$, $p < 0.001$). The effects of age ($p_{FDR} < 0.001$),

eTIV (p_{FDR} = 0.001), and volume of the caudal middle frontal gyrus (p_{FDR} = 0.009) were all significant.

Cingulum Ventral - MTL

Of the four multiple regression models assessing the relationship of FA and MD of the CV with volume of regions in the MTL (with age and eTIV as covariates), only the relationship between MD of the CV and volume of the hippocampus was significant (F(3,237) = 29.67, $p < 0.001$). The effects of age ($p_{FDR} < 0.001$), eTIV ($p_{FDR} < 0.001$), and volume of hippocampus ($p_{FDR} = 0.006$) were all significant.

Cingulum Ventral – PFC

Of the six multiple regression models assessing the relationship of FA and MD of the CV with volume of regions in the PFC (with age and eTIV as covariates), four models were significant: the model between FA of the CV and volume of the caudal middle frontal gyrus was significant and all three MD models were significant. The relationship between FA of CV and volume of caudal middle frontal gyrus was (F(3,237) = 14.20, $p < 0.001$) and the effects of age (p_{FDR} = 0.004), eTIV ($p_{FDR} < 0.001$), and volume of caudal middle frontal gyrus (p_{FDR} = 0.014) were all significant. For MD of the CV and volume of the rostral middle frontal gyrus, the model was (F(3,237 = 27.12, $p <$ 0.001) and the effects of age ($p_{FDR} < 0.001$), eTIV (p_{FDR} = 0.003), and volume of rostral middle frontal gyrus (p_{FDR} = 0.007) were all significant. For MD of the CV and volume of the caudal middle frontal gyrus, the model was (F(3,237) = 27.65, $p < 0.001$) and the effect of age ($p_{FDR} < 0.001$), eTIV (p_{FDR} = 0.004), and volume of caudal middle frontal gyrus (p_{FDR} = 0.004) were all significant. For MD of the CV and volume of superior

frontal gyrus, the model was ($F(3,237) = 26.63$, $p < 0.001$) and the effects of age ($p < 0.001$), eTIV ($p_{FDR} = 0.005$), and volume of superior frontal gyrus ($p_{FDR} = 0.014$) were all significant.

Retrosplenial Cortex

Of the four multiple linear regression models assessing FA and MD of both cingulum tracts with volume of RSC (with age and eTIV as covariates), only the model assessing FA of the CV was significant ($F(3,237) = 15.40$, $p < 0.001$). The effects of age ($p_{FDR} < 0.001$), eTIV ($p_{FDR} = 0.038$), and volume of RSC ($p = 0.004$) were all significant.

Relationships between Tracts, Volume of ROI, and Cognition

Our third hypothesis was centered upon examining WM integrity of the (a) CD with volume of the three regions in the PFC and executive function and (b) CV with volume of the two regions in the MTL and memory function. We further examined the relationship of both cingulum tracts and cognitive functions with volume of the RSC as well as the remaining relationships between all three measures (WM integrity, volume, and cognitive function). Z-scored diffusion measures, volume of ROI, and cognitive measures were used in all analyses.

Cingulum Dorsal – PFC – Executive Function

Of the six models assessing FA and MD of the CD with volume of the three regions in the PFC and performance on Part B of the Trailmaking Test (with age and eTIV as covariates), none were significant.

Cingulum Ventral – MTL - Memory

Of the four models assessing FA and MD of the CV with volume of the two regions in the MTL and performance on Craft Memory Recall (with age and eTIV as covariates), two were significant. The model for the relationship between FA of the CV with volume of entorhinal cortex and performance on Craft Memory Recall was ($F(4, 119) = 4.76$, $p = 0.001$) and the effects of eTIV ($p_{FDR} = 0.007$), volume of the entorhinal cortex ($p_{FDR} = 0.032$) and FA of the CV ($p_{FDR} = 0.047$) were all significant. The model for the relationship between MD of CV with volume of the hippocampus and performance on Craft Memory Recall was ($F(4, 119) = 11.02$, $p < 0.001$) and the effects of eTIV ($p_{FDR} < 0.001$), volume of the hippocampus ($p_{FDR} <0.001$) and MD of the CV ($p_{FDR} = 0.009$) were all significant.

Retrosplenial Cortex - Executive Function

Of the four multiple regression models assessing FA and MD of either cingulum tract with volume of RSC and performance on Part B of the Trailmaking Test (with age and eTIV as covariates), only the relationship with FA of the CV was significant. The model was ($F(4, 192) = 7.24$, $p < 0.001$) and the effects of age ($p_{FDR} = 0.039$), eTIV ($p_{FDR} = 0.005$), volume of RSC ($p_{FDR} = 0.033$) and FA of the CV ($p_{FDR} = 0.039$) were all significant.

Retrosplenial Cortex - Memory

Of the four multiple regression models assessing FA and MD of either cingulum tract with volume of RSC and performance on Craft Memory Recall (with age and eTIV as covariates), none were significance.

Cingulum Dorsal – MTL – Executive Function

Of the four multiple regression models assessing FA and MD of the CD with volume of regions in the MTL and performance on Part B of the Trailmaking Test (with age and eTIV as covariates), none were significant.

Cingulum Ventral – PFC - Executive Function

Of the six models assessing FA and MD of the CV with volume of regions in the PFC and performance on Part B of the Trailmaking Test (with age and eTIV as covariates), all three models assessing MD of the CV were significant. The model for volume of the rostral middle frontal gyrus was ($F(4, 192) = 9.77$, $p < 0.001$) and the effects of eTIV ($p_{FDR} < 0.001$), volume of the rostral middle frontal gyrus ($p_{FDR} = 0.003$) and MD of the CV ($p_{FDR} = 0.014$) were all significant. The model for volume of the caudal middle frontal gyrus was ($F(4,192) = 11.64$), $p < 0.001$) and the effects of eTIV ($p_{FDR} < 0.001$), volume of the caudal middle frontal gyrus ($p_{FDR} < 0.001$) and MD of the CV ($p_{FDR} = 0.045$) were all significant. The model for volume of the superior frontal gyrus was ($F(4,192) = 9.69$), $p < 0.001$) and the effects of eTIV ($p_{FDR} < 0.001$), volume of the superior frontal gyrus ($p_{FDR} = 0.004$) and MD of the CV ($p_{FDR} = 0.017$) were all significant.

Cingulum Ventral – MTL - Executive Function

Of the four models assessing FA and MD of the CV with volume of regions in MTL and performance on Part B of the Trailmaking Test (with age and eTIV as covariates), the two models assessing volume of the entorhinal cortex were significant. The model for FA of the CV was $F(4, 192) = 9.76$, $p < 0.001$) and the effects of age ($p_{FDR} = 0.046$), eTIV ($p_{FDR} < 0.001$), volume of entorhinal cortex ($p_{FDR} < 0.001$), and FA of CV

(p_{FDR} = 0.04) were all significant. The model for MD of the CV was (F(4,192) = 10.65, p < 0.001), and the effects of eTIV (p_{FDR} < 0.001), volume of entorhinal cortex (p_{FDR} < 0.001), and MD of CV (p_{FDR} = 0.007) were all significant.

Cingulum Ventral – PFC - Memory

Of the six models assessing FA and MD of the CV with volume of regions in PFC and performance on Craft Memory (with age and eTIV as covariates), two of the models assessing MD of the CV were significant. The model for volume of the caudal middle frontal gyrus was (F(4, 119) = 6.82, p < 0.001 and the effects of eTIV (p_{FDR} = 0.003), volume of caudal middle frontal gyrus (p_{FDR} = 0.044), and MD of the CV (p_{FDR} = 0.005) were all significant. The model for volume of the superior frontal gyrus was (F(4,119) = 7.18, p < 0.001) and the effects of eTIV (p_{FDR} = 0.001), volume of superior frontal gyrus (p_{FDR} = 0.022), and MD of the CV (p_{FDR} = 0.009) were all significant.

Cingulum Dorsal – PFC - Memory

Of the six models assessing FA and MD of the CD with volume of regions in PFC and performance on Craft Memory (with age and eTIV as covariates), none were significant.

Cingulum Dorsal – MTL - Memory

Of the four models assessing FA and MD of the CD with volume of regions in MTL and performance on Craft Memory (with age and eTIV as covariates), none were significant.

Discussion

The present study aimed to examine (1) whether clinical syndrome group differences are specific to the CD and CV pathways, but not to the CST, (2) relationships between the WM integrity of the CD and CV pathway with volume of selected ROI in an effort to further develop our understanding of WM circuitry between the PFC and hippocampus, and (3) relationships between WM integrity and regional volumes with measures of memory and executive function. The three clinical syndrome groups (control, aMCI, and dementia) did not differ in age, years of education, or race. Pairwise chi-square testing revealed a greater number of *APOE* ε4 carriers in the dementia and aMCI groups relative to controls which is consistent with a wide breadth of literature studying the *APOE* ε4 allele and lifetime risk of AD (Cacciaglia et al., 2018; Chen & Mobley, 2019; Corder et al., 1993). Performance on Craft Memory Recall and Part B of the Trailmaking Test showed worsening in the direction expected (dementia > aMCI > control).

As predicted, we found clinical syndrome group differences in both the FA and MD of the CD and CV pathways. These changes did not extend to the CST. Like previous studies assessing WM integrity in AD, we saw FA decreased between clinical syndrome groups whereas MD increased (Araque Caballero et al., 2018; Liu et al., 2017). These findings confirmed those of previous studies which have shown between clinical syndrome group differences in FA and MD of the cingulum (Lee et al., 2015; Mito et al., 2018; Shao et al., 2019; Tucholka et al., 2018). As expected, age showed a negative relationship with FA and a positive relationship with MD.

Post hoc testing revealed that the mean value of all four measures (FA of CD, MD of CD, FA of CV, and MD of CV) were significantly different between control and dementia clinical syndrome groups. However, the differences between aMCI and control and aMCI and dementia clinical syndrome groups were not what we expected. Instead, we found differences in the FA of the CD between control and aMCI clinical syndrome groups and differences in the MD of the CV existed between aMCI and dementia clinical syndrome groups. We had hypothesized diffusion measures in the CV would reflect changes earlier in the AD continuum since this is typically when surrounding MTL regions are affected and that changes in the CD would follow in more advanced phases of the continuum when the frontal lobe becomes affected. Therefore, these results do not fully support our hypothesis. Interestingly, a study by Lee and colleagues using a previous release of TRACULA in the ADNI cohort showed changes in FA and MD of the cingulum-angular bundle (which appears analogous to the CV pathway) existed between all three clinical syndrome groups (e.g. controls and AD participants, controls and MCI participants, and MCI and AD participants). Interestingly in this study, no differences were found between the three groups in the cingulum-cingulate gyrus bundle (which appears analogous to the CD pathway) (Lee et al., 2015). Other studies are also assessing clinical syndrome group differences but there does not appear to be a consensus in the literature as to which aspects of the cingulum are most vulnerable through the different phases of AD progression (Mito et al., 2018; Shao et al., 2019; Wang et al., 2017).

For our second hypothesis, we expected that the WM integrity of the CD would show relationships with the volume of regions in the PFC (rostral middle frontal gyrus, caudal middle frontal gyrus, and superior frontal gyrus) because of projections from the CD to the frontal lobe. Likewise, our hypothesis was that the WM integrity of the CV would show relationships with the volume of regions in the MTL, such as the hippocampus and the entorhinal cortex. We expected that FA would have a positive relationship and MD a negative relationship to volume. Our findings widely supported these predictions and actually revealed relationships between diffusion measures and volume were more widespread than what we predicted. In addition to relationships between the CD and regions within the PFC and the CV and regions within the MTL, we saw that both FA and MD of the CD were related to volume of the hippocampus and that FA and MD of the CV were related to the volume of all three frontal ROI. Age showed a negative relationship with FA and volume, and a positive relationship with MD.

Unexpectedly, volume of the entorhinal cortex did not show a significant relationship with diffusion measures of either cingulum pathway. Volume of the hippocampus was related to MD of the CV and both FA and MD of the CD. Interestingly, we found more relationships between volume of PFC ROI with the CV (4 relationships in total) than the CD (1 relationship). We thought that volume of the RSC would show relationships to both cingulum pathways because of its central location but there was only a relationship between volume of the RSC with FA of the CV. It is unclear whether these findings are unique to our study. One interpretation is that the cingulum, although segmented as having distinct “dorsal” and “ventral” pathways, is truly a circuitous

pathway connecting the PFC to the hippocampus through the parietal and temporal lobes. Based on these relationships, it appears many of the ROI we investigated and both cingulum pathways are subject to age-related changes. Another conclusion from our findings is that MD may be a more sensitive measure than FA based upon the number of relationships shown between either pathway and volume of selected ROI. This is interesting because while FA is a more forthright measure for assessing integrity of the WM, others in the field have argued that MD is more sensitive to detecting fiber tract alterations (Araque Caballero et al., 2018; Mayo et al., 2019).

For our last hypothesis, we sought to build upon our findings from the first two hypotheses and extend them to include how WM integrity and regional volume may relate to function. Previous studies have assessed how integrity of the cingulum relates to cognition but fewer studies have added volume to these models (Mayo et al., 2019; Metzler-Baddeley et al., 2012; Shao et al., 2019). We predicted that the WM integrity of the CD would be related to volume of PFC ROI and executive function as assessed by Part B of the Trailmaking Test and that the WM integrity of the CV would be related to volume of MTL ROI memory as assessed by Craft Memory Recall. Interestingly, we found that the WM integrity of the CV had more global relations with cognition as evidenced by relationships with measures of both executive function and memory. On the contrary, the WM integrity of the CD was not related to either cognitive measure. This could indicate that weakened WM integrity of the CV pathway more directly relates to impaired memory and executive function. Similar to our findings for our second

hypothesis, we saw that MD appears to be a more sensitive measure than FA as it showed a greater number of relationships with both executive function and memory.

Executive Function

When assessing the relationships between WM integrity, volume, and executive function, we noticed these relationships were not just limited to volume of regions within the PFC. Rather, these relationships extended to include volume of the entorhinal cortex (but not volume of the hippocampus) and volume of the RSC in addition to all three PFC ROI. In agreement with our hypothesis, we observed that relationships between FA and performance on Part B of the Trailmaking Test were positive whereas those with MD were negative. The relationship between volume of ROI and performance was positive. In instances where age showed a significant effect on performance, this relationship was negative (older age, worsened performance).

Our findings of relationships with performance on Part B of the Trailmaking Test extended to include relationships with volume of the entorhinal cortex and RSC. These relationships between a measure of executive function and regions outside of the PFC are notable because they are somewhat contrary to the historical view that declines in executive function are the direct result of neurodegeneration in the PFC (West, 1996). A study by Bettcher and colleagues assessed gray matter volume of the frontal, temporal, parietal and occipital lobes in relation to FA of the cingulum and found significant relationships between all four regions (Bettcher et al., 2016). Neither volume of the entorhinal cortex nor the RSC were accounted for in this study by Bettcher et al. but taken together with our findings, these studies provide compelling evidence that

relationships between the WM integrity of the cingulum and executive function may be impacted by regions outside of the frontal lobes. Moreover, these findings reiterate our previous interpretation that the cingulum is a circuitous pathway and that degeneration of this pathway may affect many cognitive domains, including executive function.

Memory

When assessing the relationships between WM integrity, volume, and memory, we observed relationships with volume of the entorhinal cortex and hippocampus as well as two PFC ROI: the caudal middle frontal and the superior frontal gyri. Unlike what we observed when examining performance on Part B of the Trailmaking Test, volume of the RSC showed no relationship to performance on Craft Memory Recall. This was somewhat unexpected because damage to the RSC has been highly linked to impairments in both verbal and visual memory function (Maguire, 2001; Vann et al., 2009),

Previous studies have established relationships between WM integrity of the cingulum, volume of the hippocampus, and memory function (Ezzati, Katz, Lipton, Zimmerman, & Lipton, 2016; Jacobs et al, 2018; Kantarci et al., 2011; Wang et al., 2017). These studies showed relationships in the same directions as the present study between diffusion measures, volume, and performance on neuropsychological tests targeting memory function. Unlike the present study, the study by Ezzati et al. did *not* show an association between volume of the entorhinal cortex and recall on the Free and Cued Selective Reminding Test (FCSRT) (Ezzati et al., 2016). Although both tools for memory assessment, the FCSRT and Craft Memory Recall elicit unique cognitive processes so it will be of interest to see if future studies show a relationship between

volume of the entorhinal cortex and memory function. Notably many of these existing studies that have incorporated relationships between WM integrity and memory function with gray matter volume have not assessed volume of the entorhinal cortex or regions outside of the MTL (Ezzati et al., 2016; Jacobs et al, 2018; Kantarci et al., 2011; Pichet Binnete et al., 2021; Wang et al., 2017).

Limitations

Although we had access to a large sample (n = 241), we were unable to control for the uneven sex distribution between clinical syndrome groups in our analyses. The BU-ADRC data we had access to included significantly more females in the control group and significantly more males in the dementia group. Lastly, while we had access to structural MRI, HARDI, and cognitive data, we did not have access to other AD biomarkers such as CSF or PET measures of amyloid-beta (Aβ) and tau. The addition of this data could provide valuable insight as to how the presence of Aβ and tau are potentially impacting WM integrity, volume in selected ROI, and cognitive function.

Conclusion

The results of this study confirmed our hypothesis that diffusion measures in the cingulum would be sensitive to the progression of dementia and that the WM integrity of the cingulum, volume of neighboring ROI, and measures of executive function and memory are related. Our findings provide compelling evidence that volume of regions ranging from the PFC to the hippocampus are linked to the integrity of the cingulum and that age-related changes to these structures may result in cognitive decline, particularly when the ventral pathway is involved.

Acknowledgements

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	Control (n = 141)	**aMCI (n = 59)**	**Dementia (n = 41)**	***p* value**
Age, mean (SD), y	70.88 (8.63)	72 (9.33)	74.12 (8.55)	0.11
Education, mean (SD), y	16.42 (2.25)	16.85 (2.54)	16.17 (2.64)	0.34
Female sex, n (%)	97 (68.79)	32 (54.24)	12 (29.27)	< 0.001
Race	124 White 13 Black 1 American Indian 3 Asian 0 Other	53 White 5 Black 0 American Indian 1 Asian 0 Other	34 White 6 Black 0 American Indian 0 Asian 1 Other	0.46
***APOE* ε4 carrier** n (%)	39 (29.77)	23 (41.82)	20 (50.0)	0.06

Table 4.1:Demographic Data
One-way ANOVAs used for continuous data, chi-square testing used for categorical data. Race is self-reported by participant. No participants identified as “Pacific,” “More than one race,” or “Unknown.”

	Control	aMCI	Dementia	*p* values
Craft Delayed Verbatim (n = 124)	19.49 (0.47) (n = 68)	14.98 (-0.08) (n = 36)	3.8 (-1.46) (n = 20)	< 0.001
Part B of Trailmaking Test (in seconds) (n = 197)	70.61(0.37) (n = 125)	93.78(0.01) (n = 49)	223.91 (-2.02) (n = 23)	< 0.001

Table 4.2: Measures of Cognition and Function
Values shown are raw scores (z-scores). Z-scores for both measures calculated so that positive numbers indicate above the mean and negative numbers indicate below.

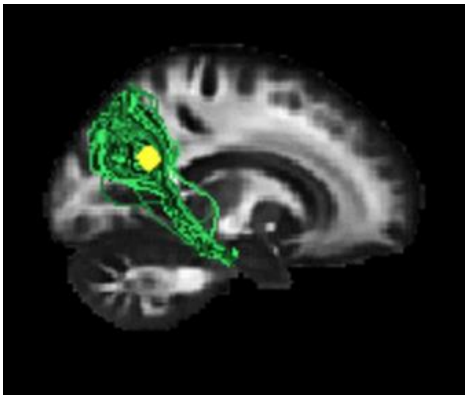
	Control (n = 141)	**aMCI (n = 41)**	**Dementia (n = 59)**	***p value***
Cingulum - Dorsal				
FA	0.423 (0.21)	0.409 (-0.25)	0.406 (-0.36)	0.002
MD	$5.17x10^{-4}$ (-0.18)	$5.26x10^{-4}$ (0.14)	$5.33x10^{-4}$ (0.41)	0.011
Cingulum - Ventral				
FA	0.401 (0.15)	0.396 (-0.02)	0.382(-0.48)	0.008
MD	$5.31x10^{-4}$ (-0.20)	$5.37x10^{-4}$ (0.01)	$5.55x10^{-4}$ (0.68)	< 0.001
Corticospinal				
FA	0.446 (-0.03)	0.445 (-0.07)	0.450 (0.20)	0.36
MD	$4.72x10^{-4}$ (-0.04)	$4.73x10^{-4}$ (0.03)	$4.74x10^{-4}$ (0.09)	0.97

Table 4.3: Diffusion Measures of White Matter Pathways
Values shown are raw scores (z-scores). Age was added as a covariate in ANCOVA models assessing between group differences.

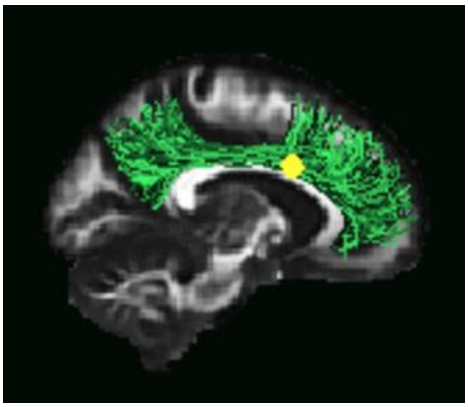
		t ratio, *P* value 95% Confidence Interval Range		
Variables	**ANCOVA Model**	**Control - Dementia**	**Control- aMCI**	**aMCI- Dementia**
MD of Cingulum Dorsal	F(3,237) = 36.77, $p < 0.001$	-2.72, 0.019 [-0.76 - -0.05]	-	-
FA of Cingulum Dorsal	F(3,237) = 25.15, $p < 0.001$	2.61, 0.026 [0.04 - 0.78]	2.99, 0.009 [0.09 – 0.73]	-
MD of Cingulum Ventral	F(3,237) = 32.26, $p < 0.001$	-4.75, <0.001 [-1.08 - -0.36]	-	3.24, 0.004 [0.15 – 0.97]
FA of Cingulum Ventral	F(3,237) = 9.50, $p < 0.001$	3.16, 0.005 [0.14 – 0.94]		-

Table 4.4: Post Hoc Tukey's HSD Testing for Tracts of Interest

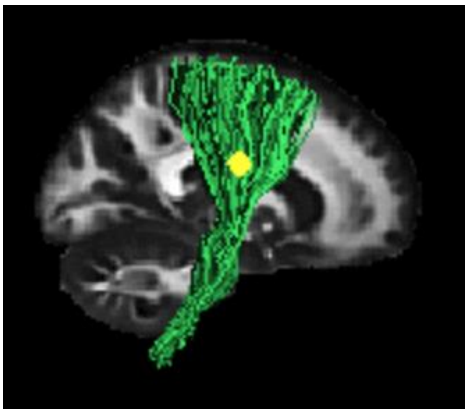
Only tracts with significant clinical syndrome group differences ($p < 0.05$) as reported by post hoc Tukey's HSD testing shown. Age was added as a covariate in all models.



(A) Left Cingulum Ventral (CV)



(B) Left Cingulum Dorsal (CD)



(C) Left Corticospinal Tract (CST)

Figure 4.1: TRACULA Tracts of Interest
Images taken from https://dmri.mgh.harvard.edu/tract-atlas/

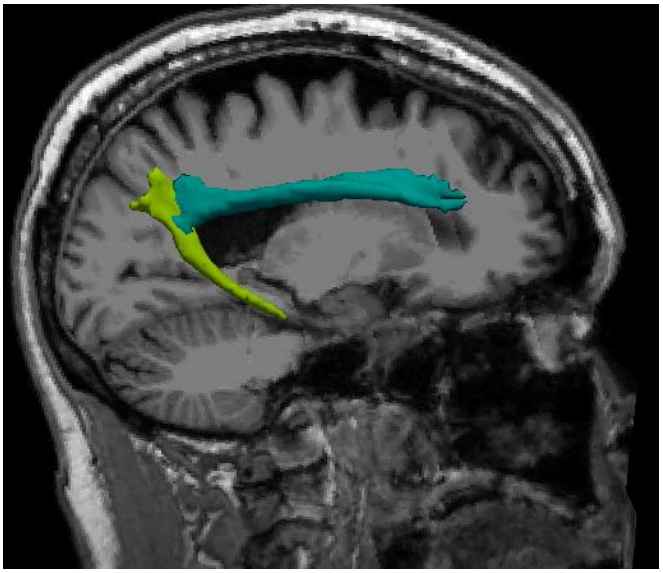


Figure 4.2 Reconstruction of Cingulum Pathways in HOPE Participant
Left Cingulum Dorsal pathway (shown in aqua), Left Cingulum Ventral pathway (shown in light green). Image taken in Freeview.

CHAPTER 5: DISCUSSION

Summary of Key Findings

The neuropathology of Alzheimer's disease (AD) has been established for over 100 years but major gaps in our understanding of this disorder persist. A key question that lingers is why neuropathological changes can be particularly detrimental to some older adults yet *not* to others. The work in this dissertation attempted to disentangle some of the nuances underlying why certain people develop dementia and/or AD over the course of their lifetime while others do not, despite the presence of many of the same neuropathologic biomarkers and subsequent changes.

In Chapter 2, our primary goal was to investigate links between brain structure, cognitive performance, and neuropathology in participants within the ADNI who identified as Black or African American (BoAA). Between 80-90% of participants in AD research studies identify as Non-Hispanic White (NHW). The purpose of our study was to further explore the progression of dementia in the BoAA population. By assessing magnetic resonance imaging (MRI), cognitive, and cerebrospinal fluid (CSF) data, we found decreased cortical thickness in five of seven selected regions of interest (ROIs), decreased hippocampal volume, increased volume of white matter hyperintensity (WMH) lesions, worsened measures of cognition and function, decreased measures of CSF $A\beta_{1-42}$, and elevated measures of CSF t-tau and p-tau between clinical syndrome groups (defined by the ADNI Clinical Core as cognitively normal (CN), mild cognitive impairment (MCI) and AD). We also found an interaction between clinical syndrome group and *APOE* ε4 carrier status on the Functional Activities Questionnaire (FAQ), CSF t-tau, and

CSF p-tau. Altogether these findings supported our hypothesis that there would be an increase in the number of AD biomarkers between clinical syndrome groups as the cognition worsens.

Next in Chapter 3, we identified a different subset of participants within the ADNI who showed a change in amyloid burden during enrollment in the ADNI study. A change in amyloid burden was defined as a conversion in amyloid status from A- to A+ based upon established florbetapir positron emission tomography (PET) standard uptake value ratio (SUVR) thresholds. A second group of ADNI participants with representative demographic data (age, years of education, clinical diagnosis) were also identified but these participants were selected on the basis that they did *not* show a change in amyloid status over their course of enrollment (i.e. remained A- during their ADNI enrollment). The purpose of comparing these two groups was to examine changes in the period of time leading up to amyloid conversion as well as to investigate less-explored relationships between changes in Aβ and features like cortical volume. Our findings showed an increased prevalence of *APOE* ε4, greater annualized percent volume loss in 8 cortical and 3 subcortical brain regions, and lower CSF $A\beta_{1\text{-}42}$ in the amyloid converter group compared to the non-converter group. We saw no differences in annualized cognitive changes or CSF measures of t-tau and p-tau. Interestingly, we also saw a difference between the two groups in SUVR on florbetapir PET at both timepoints and greater annualized amyloid accumulation (as measured by SUVR) in the amyloid converter group. These findings provide evidence that there may be important neuropathological changes occurring in the period of time leading up to amyloid conversion.

Finally, in Chapter 4, we shifted our focus to using diffusion tensor imaging (DTI) to study white matter pathway integrity in a large sample of older adults who are part of the BU-ADRC HOPE study. Using segmentations of the cingulum bundle, we first assessed whether diffusion measures of the cingulum-dorsal (CD) and cingulum-ventral (CV) pathways differed between clinical syndrome groups ranging from control participants to those with aMCI to those with dementia. Following these initial analyses, we explored the relationship of the integrity of these pathways with the volume of selected ROIs, and in relation to executive function and memory. Our findings showed that (1) there were significant clinical group differences in both fractional anisotropy (FA) and mean diffusivity (MD) that were specific to both cingulum pathways, (2) ROIs in both the prefrontal cortex (PFC) and medial temporal lobe (MTL) showed relationships with FA and MD of both cingulum tracts, and (3) the CV tract was related to both executive function and memory performance and that volume of ROI in both the MTL and PFC were influenced by this relationship. Due to the many relationships shown between the CV and CD with the volume of regions throughout the PFC and MTL, these findings provide evidence that age-related changes may occur throughout the cingulum in regions spanning from the PFC to the hippocampus. Furthermore, our findings show that the integrity of the cingulum, particularly the ventral pathway, is related to both memory and executive function.

What Do Our Findings in the BoAA Population tell us about Dementia in this Group?

The reason researchers have begun to focus upon understudied racial groups in AD research studies is multi-faceted. Some of this interest stems from growing awareness that the elderly population is more racially and ethnically diverse than ever before, yet reported data reflects that research studies and drug trials still heavily rely on the recruitment of NHW Americans (Manly & Glymour, 2021; Shin & Doraiswamy, 2016; Stout et al., 2020). This disproportion stands plainly in opposition to estimates reporting that older BoAA and Hispanic/Latino individuals are at a greater risk to develop dementia compared to NHW adults in their lifetimes (Barnes, 2022; Raman et al., 2021). These reports have led many in the field to call for increased inclusion and study of racially and ethnically diverse participants in AD and dementia studies in hopes to better understand what leads to a higher prevalence of disease in this understudied group (Barnes, 2022; Fleishman et al., 2021).

Our study confirmed that AD biomarkers and cognitive impairment seem to worsen across clinical syndrome groups. This observation is similar to what others have observed in research studies using predominantly NHW participants (Fennema-Notestine et al., 2009; Li et al., 2017). Post hoc testing in our study showed these differences in AD biomarkers and cognition were mainly between CN and dementia clinical groups which is to be expected, but some notable differences emerged between MCI and both of the other clinical syndrome group which may be worth further exploring. Likely among our most unexpected findings was the significant difference in thickness of the posterior

cingulate between the CN and MCI clinical syndrome groups whereas other ROI only reflected differences later in the dementia continuum or between control and dementia clinical syndrome groups. Interestingly, a previous study by Fennema-Notestine and colleagues using a larger pool of ADNI data also showed changes in the posterior cingulate to be among some of the earliest changes in the progression of AD (Fennema Notestine et al., 2009). Exploring this region in other studies, particularly those conducted with BoAA cohorts, could help solidify when these changes occur and if this is an area we should pay more attention to in the earliest phases of AD progression. Similarly, many measures of cognition such as Logical Memory Immediate Recall, Category Fluency, both RAVLT measures, and Parts A and B of the Trailmaking Test showed significant differences in performance between CN and MCI clinical syndrome groups. These findings reflect cognitive abilities being affected early in at least three different cognitive domains (verbal memory, language and executive function). Being able to recognize these deficits early could prove significant to being able to detect subtle cognitive deficits earlier in the AD continuum (Weissberger et al., 2017).

As soon as we began assembling the ADNI sample to be used in Chapter 2, the small number of racial and minority participants within the ADNI became readily apparent. Thousands and thousands of participants have enrolled in the ADNI since its inception in 2003, yet we were only able to identify 85 BoAA participants in the LONI database with 3T MRI scans necessary for our analysis. Of these 85 participants, even fewer had CSF data which requires participants to consent to more invasive procedures such as a lumbar puncture to obtain CSF samples. One reason there is a small proportion

of BoAA participants within the ADNI may be the historical mistreatment of racial and ethnic minority groups in research studies which has led many BoAA adults to be hesitant of participating in research studies. Of the 85 BoAA participants used in our study, we also noticed there were significantly more females (57 females; $p = 0.002$) than males. This trend has also been reported in previous studies examining enrollment of BoAA adults in research studies (Stout et al., 2020).

Another significant pattern we noticed when compiling the sample population for Chapter 2 was that the average education level of all participants was 15.42 years (Table 2.1). This finding in addition to the higher proportion of BoAA women in our study is consistent with previous reports. A recent study by Fleishman et al. examining 376 older Black adults reported 82.2% of participants in the study were women that had an education level above high school (average = 15.25 years) (Fleishman et al., 2022). This level of education is notable because 15 years of education entails 3 years of post-high school education. On the contrary, reports from the US Census Bureau in 2020 reflect that only 37.5% of all Americans age 25 and older have a college education. When these reports were restricted to solely Black Americans aged 25 and older, this proportion narrowed to 26%. In comparison, 46 (or 54.12%) of the 85 participants in our study had 16 years of schooling or the equivalent of a Bachelor's degree. This is more than double the population average. These broader reports of the US population should be considered when interpreting the findings of our results in Chapter 2 because it may indicate that the subset of BoAA participants in our study (as well as those in other research studies of dementia) may not be representative of all BoAA in the general population (Fleishman et

al., 2022). Thus, when we are interpreting the findings of our study, we have to be cognizant that really what we have gauged is how biomarkers and dementia present in this small sample of BoAA participants within the ADNI population.

Beyond thinking about how the results of Chapter 2 translate to other BoAA individuals across the US, similar considerations must also be taken when trying to make comparisons of biomarkers between racial groups. For instance, recent studies comparing CSF levels between NHW and BoAA showed BoAA actually had lower levels of CSF p-tau and t-tau than NHW participants despite the reported prevalence of dementia being higher in this racial group (Garrett et al., 2019; Howell et al., 2017; Morris et al., 2019). Other studies report no difference in biomarkers (Barnes, 2022). These mixed results are difficult to interpret. Coupled with the idea that BoAA groups in dementia research studies may not be illustrative of the entire population, these findings raise the important question as to whether the data we currently have is truly representative enough to be answering overarching questions regarding differences between racial groups. Attempting to answer this type of question about race when we have limited data on sociodemographic and socioeconomic status makes these comparisons even more complicated. In future studies, it may be insightful to gather data pertaining to social determinants of health on all participants enrolled in AD research studies. Such data could help us make more informed between-racial group comparisons and better shape our understanding of how these important social factors influence long-term health and cognitive function.

As we continue to try and make AD research studies as inclusive and representative as possible, added emphasis could be placed on strengthening communication between researchers and participants to alleviate concerns about being part of research studies. Similarly, making enrollment and participation more accessible and beneficial to those willing to participate may also be helpful. Lastly, we want to recognize that while the focus of Chapter 2 was isolated to examining BoAA, studies of dementia and AD biomarkers in other racial groups such as American Indian or Native Alaskan, Asian, Native Hawaiian or Pacific Islanders, or people who are biracial, are also limited and equally significant.

AT(N) Biomarker Profiles in ADNI Participants

Another pattern we chose to explore in both Chapters 2 and 3 was the distribution of AT(N) biomarker profiles in the ADNI participants assessed. During literature review for the writing of this dissertation, it was apparent how commonly terms such as "Alzheimer's disease" and "dementia" are used interchangeably when really expressions like "clinical diagnosis of Alzheimer's disease," "dementia," or "dementia due to Alzheimer's disease pathology" are most suitable to describe a clinical diagnosis, and the term "Alzheimer's disease" for describing neuropathology. This distinction was particularly interesting when examining ADNI participants who had been diagnosed as "having Alzheimer's disease" per the ADNI Clinical Core criteria despite whether they had a neuropathologic profile consistent with AD. In Chapter 2 when we discussed the 12 BoAA participants diagnosed with AD by the ADNI, we referred to them as having dementia since we did not know the full neuropathologic profile of all 12 participants.

A complete categorization of the 8 possible AT(N) biomarker profiles and their interpretation per Jack et al. 2018 is illustrated in Table 5.1 (Jack et al, 2018). The threshold values used to create profiles in Chapter 2 and 3 are reported in the Methods: AT(N) Classification section of Chapter 3. It is worth noting that since PET data was not utilized and/or available for all participants in Chapter 2, CSF measures of $A\beta_{1\text{-}42}$ <1000 pg/mL were used instead to define amyloid positivity (A+) (Hansson et al., 2018).

The following can be read with the guidelines of the AT(N) framework kept in mind: the presence of Aβ alone is enough to categorize whether or not someone is on the AD continuum but the additional presence of tau is required to make a neuropathologic diagnosis of AD. Neurodegeneration is further used to measure the severity of disease progression (Jack et al., 2018). Below we discuss the profiles for participants in both Chapters 2 and 3.

BoAA Participants (Chapter 2)

Of the 85 BoAA participants examined in Chapter 2, 48 (25 CN, 17 MCI, and 6 dementia) had the CSF measures needed to complete full AT(N) biomarker profiles (Table 5.2) Three participants were A+T+(N)+ and another seven were A+T+(N)- meaning that a total of **ten** participants had changes consistent with AD. Interestingly, only 6 of these 10 participants were diagnosed by the ADNI as having AD. The other 3 were MCI and 1 was CN. Sixteen more BoAA participants were A+. All 16 were A+T-(N)- and none were A+T-(N)+, yet per the AT(N) framework, the presence of A+ alone is enough to indicate Alzheimer's pathologic change and therefore, these individuals can still be considered on the AD continuum. Interestingly, none were clinically diagnosed as

AD (10 CN, 6 MCI). Of the 22 A- participants, there were 4 with A-T+(N)- pathology and 1 who was A-T+(N)+. Per the AT(N) framework, this group is considered to have "non-AD pathologic changes." The remaining 17 A- participants showed "normal" profiles meaning biomarkers for amyloid, tau, and neurodegeneration were all below thresholds. All 22 A- participants were clinically diagnosed as CN or MCI.

Amyloid Conversion Participants (Chapter 3)

Of the 136 participants examined in Chapter 3, 94 participants (53 CN and 41 MCI) had the data needed to complete full AT(N) biomarkers (Table 5.3). Participants clinically diagnosed by the ADNI as AD had previously been excluded from this study. From creating the study sample based upon florbetapir PET SUVR, we were aware 31 participants were A+ and the remaining 63 were A-. No participants in Chapter 3 were A+T+(N)+ but 8 were A+T+(N)- which means the changes present are considered consistent with AD. Of these 8 participants, none were clinically diagnosed by the ADNI as having AD but 4 were MCI and 4 were CN. Two other amyloid converters were A+T-(N)+. This profile is unique because it is believed to reflect both Alzheimer's pathology as well as concomitant suspected non-Alzheimer's pathologic change. Interestingly, both A+T-(N)+ participants in this study had a clinical diagnosis of CN. The final 21 amyloid converters were all A+T-(N)- and diagnosed as CN (n = 8) or MCI (n = 13) despite the presence of A+ alone being enough to place all 21 participants on the AD continuum. In the non-converter group, no participants were A-T+(N)+ but 22 were positive for either T or N. Lastly, 41 non-converters showed "normal" biomarker profiles [(A-T-(N)-].

The purpose of dissecting some of these diagnostic inconsistencies between clinical diagnoses and AT(N) profiles is not meant to be a criticism of either staging criteria. Both criteria are significant and there are many instances when only one criterion can be used to make a diagnosis (e.g. biomarker data is not always accessible meaning guidelines for consistent clinical diagnosis are necessary and vice versa). Instead, this discussion is meant to stimulate further conversation and considerations as to how these criteria differ and how this may impact how we interpret findings. For instance, not knowing that someone who clinically presents as cognitively normal actually has underlying AD pathology (or that someone who pathologically presents as non-AD but shows significant cognitive decline) could be particularly meaningful in allowing us to identify pathology in someone with no symptoms and could also influence the results of a study trying to assess between clinical syndrome group differences.

Lastly, there are some important limitations to recognize when examining AT(N) profiles. First, AT(N) profiles are typically created on the basis of threshold values and these thresholds continue to evolve. The threshold values chosen for the purposes of this dissertation were gathered based upon recent studies using these thresholds with reports of strong accuracy and other measures of sensitivity when distinguishing between clinical syndrome groups (Guo et al., 2020; Hansson et al., 2018; Ingala et al., 2021).

What does Amyloid Conversion Really Mean?

Emphasis on studying Aβ has been dominant in AD research since the early 1990s' due to the renowned study by Hardy & Higgins when the two researchers proposed that deposition of the Aβ protein is the "causative agent of Alzheimer's

pathology and that the neurofibrillary tangles, cell loss, vascular damage and dementia follow as a direct result of this deposition" (Hardy & Higgins, 1992). In more recent years, the view that amyloid *alone* "causes" AD has become a point of contention, yet Aβ remains a primary protein of interest because many studies have shown evidence that changes in amyloid precede changes in other AD biomarkers (refer back to Figure 1.1; Elman et al., 2021; Jack et al., 2013; Jack et al., 2018).

Similar to our study in Chapter 3, other studies have begun identifying individuals who are "A+" or "A-" in an effort to elucidate the events underlying amyloid conversion. Meanwhile, some have begun to wonder if conversion in amyloid status is really the critical point in entering the AD continuum or if critical changes are occurring even prior (Elman et al., 2021; Farrell et al., 2018; Landau et al., 2018; Young & Mormino, 2022). These skepticisms can be broken down into two primary perspectives. The first is based on the belief that substantial and pathologically significant amyloid accumulation may be present before positive amyloid PET scans occur. This could be the result of current amyloid PET ligands only being able to detect insoluble, fibrillar peptides (Mattson et al., 2015; Young & Mormino, 2022) or it may reflect that many widely used positivity thresholds are not sensitive enough to detect the earliest biological changes in the AD continuum (Elman et al., 2021; Young and Mormino, 2022). The second viewpoint is that more subtle changes in cognition and tau may actually be driving conversion from A- to A+ (Elman et al., 2021; Jagust & Mormino, 2011; Landau et al., 2018). This argument is a bit harder to prove since it is difficult to discern the cause and effect of these changes,

but it raises an interesting point since both cognitive deficits and lower levels of CSF p-tau are associated with a higher risk of becoming A+ (Elman et al., 2021).

Our Findings

With these points in mind, we revisited our findings in Chapter 3. Our results showed significant differences in prevalence of *APOE* ε4, greater annualized percent volume loss in 8 cortical and 3 subcortical brain regions, and lower CSF $A\beta_{1-42}$ in the amyloid converter group compared to the non-converter group. From examining the literature, we noticed that in cases when our results differed from previous studies, it was possible that these differences were the result of our choice to annualize rate of change rather than use data from one definitive timepoint. As discussed in Chapter 3, other studies have suggested that measuring magnitude of change and/or amyloid accumulation might be more sensitive to detecting subtle differences than using baseline measures (Farrell et al., 2018; Landau et al., 2018). It was striking that our findings measuring both the rate of amyloid accumulation on florbetapir PET and annualized percent volume loss on structural MRI supported this viewpoint.

An additional finding that stuck out to us when we examined our results in Chapter 3 was that the amyloid converter and non-converter group showed a significant difference in SUVR at **both** PET scans – including the PET scan that was pre-conversion (taken at timepoint 1). Seeing a difference in amyloid accumulation at the first PET scan poses the question as to whether the amyloid converter and non-converter groups were already on different trajectories of amyloid accumulation and potential AD risk even

before the time of conversion. In order to better understand this process, further studies of amyloid accumulation and peripheral biological processes are needed.

Executive Function Extends Beyond the Frontal Lobe

Our findings in Chapter 4 revealed just how widespread relationships between the WM integrity of the cingulum pathway are with many ROI (spanning from the PFC to the MTL) in addition to executive function and memory. However, there were a few relationships or lack thereof that warrant further discussion. The first being that we did *not* see any relationships between the CD and either of the two cognitive domains. Particularly in regards to executive function, this was unexpected since the CD pathway extends into the frontal lobe and previous studies have shown that the dorsal and/or anterior portions of the cingulum bundle mediate performance in tests of cognitive control and executive function (Betcher et al., 2016; Bubb et al., 2018; Kantarci et al., 2011). Despite our lack of findings surrounding the CD pathway and executive function in Chapter 4, there are many other neuropsychological tests that gauge executive function. In future work, it may be interesting to see if others observe a relationship between WM integrity of the CD and executive function if they use other assessments such as the Stroop Test, the Digit Symbol Subtest, or a composite score of executive function measures.

Previous studies have shown relationships between volume of frontal regions such as the middle frontal gyrus with performance on measures of executive function (Duarte et al., 2006). Our study also showed positive relationships between performance on Part B of the Trailmaking test and volume of the 3 PFC ROI examined. Additionally, we saw

these relationships with executive function extended to include volume of the entorhinal cortex and retrosplenial cortex (RSC) despite the historical view that executive function is a "frontal lobe task" (West et al., 1996).

We are not the first to show evidence that gray matter volume of regions outside the frontal lobe may further support a complex construct like executive function. Previously, Bettcher and colleagues showed that gray matter volume of the temporal and occipital lobes were predictors of executive function performance (Bettcher et al., 2016). Similarly, MacPherson and colleagues showed positive relationships between cortical thickness of regions in the temporal cortex and the isthmus of the cingulate (which corresponds closely to the RSC) with performance on Part B of the Trailmaking Test (MacPherson et al., 2017). Furthermore, as we reflect on the skills necessary to complete Part B of the Trailmaking Test both quickly and with minimal errors, it becomes clear that memory and spatial orientation are also essential to complete this task. To connect letters and numbers in sequential and alternating order, one must remember what circle they just passed, recognize if it was a letter or number, then find the following sequential and alternating letter or number. Thus, if we think about Part B of the Trailmaking Test as more than just a test of executive function, it seems very fitting that volume of the entorhinal cortex and RSC would show positive relationships with performance.

Altogether, the findings from Chapter 4 showed many extensive relationships between WM integrity of the cingulum, volume of selected ROI, and performance on Part B of the Trailmaking Test and Craft Memory Recall. To perform this study, we processed HARDI data with the updated TRACULA toolbox, which was part of the

Freesurfer version 7.2 released in July 2021. Use of this new toolbox was advantageous because it segmented an intricate pathway like the cingulum into two pathways that allowed us to better localize relationships. However, because this toolbox is relatively recent, not many studies using these exact segmentations of the cingulum, CD and CV, have been published yet. As more AD-related studies using this toolbox to examine WM integrity of the cingulum are published, it may be of particular interest to see whether the same between group differences among clinical syndrome groups (control, aMCI, and dementia) are shown or whether different findings emerge.

Conclusion

Because the prevalence of AD will likely continue to grow, understanding the sequence of neuropathological events that result in AD, who is most at risk to develop AD, and how to identify the earliest signs of AD will remain paramount. Through countless studies of AD that were conducted as early as the beginning of the 20th century, we have come to recognize that AD is an enormously complex disease that can hardly be summarized in a simple cause and effect manner. In this dissertation, we explored many of these subtleties and risk factors that have the potential to compound over the course of a lifetime such as race, genetics, transitions in biomarker status, and changes in WM integrity. The ultimate goal of this work was to gain a better understanding of how these factors relate to not only the biological presentation of AD, but also the clinical diagnosis of age-related dementia.

In Chapter 2, we saw that characteristics of dementia present in the BoAA population much like AD biomarkers have been reported to present in the NHW

population. Thus, it remains unclear what other factors are leading to the increased prevalence of dementia in the BoAA group. We are hopeful future studies can build upon our findings in larger and unique samples of BoAA participants. In Chapter 3, we examined conversion in amyloid status. We saw evidence that other biological factors and processes outside of amyloid accumulation are likely concurrent to a change in amyloid status, and that measuring the rate of these changes over a period of time may be the most sensitive way to gauge differences between those who become A+ versus those who stay A-. Lastly in Chapter 4, through use of DTI, we saw that the integrity of the cingulum pathway is widely related to volume of regions spanning from the PFC to the hippocampus, in addition to cognitive functions like executive function and memory. Altogether, this work expands our knowledge of dementia in the BoAA population, provides additional perspective on the factors leading to a conversion in amyloid status, and highlights the utility of the cingulum as a key region in studying age-related decline and neurodegeneration. Moving forward, we are hopeful future work can continue building upon these findings in an attempt to further disentangle the many factors which lead to AD.

AT(N) profile	Biomarker category
A+T+(N)+	Alzheimer's disease
A+T+(N)-	Alzheimer's disease
A+T-(N)+	Alzheimer's and concomitant suspected non-Alzheimer's pathologic change
A+T-(N)-	Alzheimer's pathologic change
A-T+(N)-	Non-AD pathologic change
A-T-(N)+	Non-AD pathologic change
A-T+(N)+	Non-AD pathologic change
A-T-(N)-	Normal

Table 5.1 The 8 AT(N) Biomarker Profiles and Categories
The four profiles indicated within brackets are all considered to profiles within the Alzheimer's continuum.

	CN (n = 25)	**MCI** (n =17)	**Dementia** (n = 6)	**Total ADNI BoAA participants** (n = 48)
A+T+(N)+	-	1 (5.88)	2 (33.33)	3 (6.25)
A+T+(N)-	1 (4.0)	2 (11.76)	4 (66.67)	7 (16.58)
A+T-(N)+	-	-	-	-
A+T-(N)-	10 (40.0)	6 (35.29)	-	16 (33.33)
A-T+(N)-	3 (1.2)	1 (5.88)	-	4 (8.33)
A-T-(N)+	-	-	-	-
A-T+(N)+	-	1 (5.88)	-	1 (2.08)
A-T-(N)-	11 (44.0)	6 (35.29)	-	17 (35.42)

Table 5.2 AT(N) Biomarker Profiles for ADNI BoAA Participants
Data displayed is from participants examined in Chapter 2. Values reported as number (percent). Participants without complete AT(N) biomarker data were excluded.

		CN (n = 53)	**MCI** (n = 41)	**Total ADNI Conversion Participants** (n = 94)
Amyloid Converters (n = 31)	**A+T+(N)+**	-	-	-
	A+T+(N)-	4 (7.54)	4 (9.76)	8 (8.51)
	A+T-(N)+	2 (3.77)	-	2 (2.13)
	A+T-(N)-	8 (15.09)	13 (31.71)	21 (22.34)
Non-Converters (n = 63)	**A-T+(N)-**	14 (26.42)	4 (9.76)	18 (19.15)
	A-T-(N)+	1 (1.89)	3 (7.32)	4 (4.26)
	A-T+(N)+	-	-	-
	A-T-(N)-	24 (45.28)	17 (41.46)	41 (43.62)

Table 5.3 AT(N) Biomarker Profiles for ADNI Amyloid Conversion Participants

Data displayed is from participants examined in Chapter 3. Values reported as number (percent). Participants without complete AT(N) biomarker data were excluded.

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