ABSTRACT

Title of Thesis:	COGNITIVE TESTING, NEUROIMAGING, AND BLOOD BIOMARKERS IN THE DEVELOPMENT AND PROGRESSION OF ALZHEIMER'S DISEASE
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Alzheimer's disease (AD) is a progressive neurodegenerative disorder, characterized by significant loss of memory and cognitive dysfunction. It has a significant impact on an individual's health and may financially and socially burden these individuals and their loved ones. Although the disease has been researched extensively, there is still no clear understanding of the proposed mechanisms behind the development of AD and factors aside from genetics which potentially influence the risk of developing AD. The purpose of this research is to compile and analyze data on cognitively healthy participants, participants with MCI, and participants with AD to better understand the importance of genetic risk and changes in cognitive function, bioimaging and biomarker levels, as recorded on the Alzheimer's Disease Neuroimaging Initiative (ADNI) database. There are complex and significant relationships among these three variable groups with genetics and time. Executive function scores in healthy participants and participants with MCI were decreased with age and increased with education. In participants with AD, scores decreased over time. Language scores in healthy participants decreased with

age, increased with education and for women. In participants with MCI, scores decreased with risk and time, and there was an interaction between these two variables. They also decreased with age and increased with education. In participants with AD language scores decreased over time. Memory scores in healthy participants increase with time and education and for women. In participants with MCI, scores increased with education and decreased with risk and time, and there was an interaction between these two variables. For participants with AD, there was a decrease over time. Visuospatial ability scores in healthy participants decreased with education. In participants with MCI, scores decreased with genetic risk and increased with education. In participants with AD, scores decreased over time and increased with age. Left hippocampal volume in healthy participants decreased with time, age, and education, and is increased in women. In participants with MCI, volume decreased with risk, time, age, and education. In participants with AD, volume decreased with time and age. Right hippocampal volume in healthy participants decreased with time, age, and education. In participants with MCI, volume decreased with risk and time, and there was an interaction between these two variables. Volumes also decreased with age. For participants with AD, volume decreased with risk, time, and age. Total hippocampal volume in healthy participants decreased with time, age, and education, and was increased for women. There was also an interaction between risk and time. In participants with MCI, volumes decreased with risk and time, and there was an interaction between these two variables. Volumes also decreased with age and education. For participants with AD, volumes decreased with risk, time, and age. Aβ42 levels in healthy participants decreases with risk and increased with time. In participants with MCI, levels increased with time and age, and were lower in women. In participants with AD, levels increased with time. Aβ40 levels in healthy

participants increased with time and were lower for women. For participants with MCI, levels increased with time and age, and were lower for women. In participants with AD, levels increased over time. The Aβ42/40 ratio in healthy participants decreased with risk and time, and decreased with time in participants with MCI. The findings give insight into AD development and contribute to a greater understanding of longitudinal changes in AD progression. In relation to the study of AD includes the perpetuation of racial inequalities. People of color have an increased risk of developing AD and are disproportionately affected by the disease, yet are severely underrepresented in most research studies, including the research collected in the ADNI database. Racial minorities also often do not have the same access to healthcare as white people, thus contributing to the decreased possibility of early detection and treatment of AD. Black Americans, specifically, often face socio-economic barriers, which further renders the burden of AD development and progression more serious for minority families. In order to promote awareness of AD among underrepresented communities, Team Brain virtually presented to the African American Health Program, a local community of minority elders, via virtual presentations. Overall, this research concluded that hippocampal atrophy and cognitive tests appear to be the most consistent factors in the progression of MCI and AD. The analysis of blood biomarkers produced inconclusive results. This research indicates a clear set of imaging and cognitive factors that can be used to create less invasive and novel diagnostic methods for AD as well as supports the need for further research on blood biomarkers to understand their relationship with cognitive decline and progression of AD.

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Chapter 1: Introduction

1.1 Alzheimer's Disease Overview

Dementia is an "umbrella" term used to describe a range of symptoms associated with cognitive impairment (Cunningham et al., 2015). AD is the most common form of dementia and is an irreversible progressive neurodegenerative disorder that is characterized by significant loss of memory and cognitive dysfunction (Karantzoulis, 2011). AD has become a major global health concern because its impact on the physical, mental, and social health places a significant burden on society. An estimated 5.8 million Americans are currently living with AD and this number is projected to rise to about 13.8 million by the year 2050 (Santhanam, 2019). In the United States, AD is the sixth leading cause of death with a financial burden that exceeds $200 billion annually, spent solely on direct patient care of affected individuals (Santhanam, 2019). The only way to definitively diagnose AD is a post-mortem brain autopsy, wherein medical examiners may identify features such as synaptic/neuronal loss, gliosis, and cerebral amyloid angiopathy (CAA) in patients with AD (Hane et al., 2017). Currently, there are no curative methods of treatment (Perl, 2010), however, there is active research in the development for early detection and treatment.

There are two types of AD: familial AD, occurring in less than 0.5% of the population and characterized by mutations in three specific genes (amyloid precursor protein, presenilin 1, and presenilin 2) and sporadic AD (Crous-Bou et al., 2017). With respect to age of onset, there are two more subcategories of AD: early-onset AD (EOAD) and late-onset AD (LOAD) (Crous-Bou et al., 2017). Early-onset AD occurs in individuals before the age of 65, typically between 30 and 50 years of age, and LOAD which presents typically in individuals older than 65 years of

age. It is believed that the epidemiology of AD is multifactorial, including genetics, lifestyle and environment (Lane et al., 2018).

The preliminary diagnosis of AD is based on the history of the illness, pattern of cognitive deficits, mental status tests, neurological exam, imaging of the brain to rule out structural brain lesions and to exclude nondegenerative causes of the symptoms including depression, nutritional deficiencies, substance abuse, medications and metabolic and endocrine disorders. Striking cardinal features of AD are the presence of beta-amyloid plaques, which are dense deposits of amyloid-beta peptides and cellular material that accumulate outside and around nerve cells and neurofibrillary tangles (NFTs), which are aggregates of twisted fibers of a microtubule-associated protein, tau, which undergoes chemical changes and become hyperphosphorylated (Metcalfe & Figueiredo-Pereira, 2010). The tau proteins tend to detach from microtubules and instead stick together in their phosphorylated form, causing build up of these aggregates called NFTs within the nerve cell (Metcalfe & Figueiredo-Pereira, 2010. These features are the gold standard for the diagnosis and can be seen in autopsy pathological evaluations (Pillai et al., 2018).

AD is a complex and multifactorial disease and the pathophysiology of AD is unable to be explained solely on one theory. There are several competing hypotheses on the causes of AD including the cholinergic, amyloid cascade and tau hypotheses (Du et al., 2018). In the cholinergic hypothesis, synthesis of acetylcholine is reduced and destruction of these neurons causes disruptions in distant neuronal networks (Du et al., 2018). On the other hand, the basis for the amyloid hypothesis is the accumulation of a fragment of the amyloid precursor protein (APP) that leads to the formation of plaques, synaptic failure and neurodegeneration. Once amyloid-

beta is cleaved from APP by secretase enzyme, it is secreted in the interstitial fluid. In healthy patients, excess amyloid-beta is cleared from the brain. In diseased individuals, amyloid-beta misfolds, aggregates and becomes neurotoxic. Amyloid-beta lesions first appear in the neocortex and then later in the hippocampus (Y. Gao et al., 2018). The abnormal aggregation of hyperphosphorylation of tau protein, a microtubule-associated protein that is responsible for stabilizing neuronal microtubules promoting stability of the cytoskeleton, creating neurofibrillary tangles and disintegrating the neuron's transport system leading to cell death is the basis for the tau hypothesis (Y. Gao et al., 2018). Although these are competing hypotheses, some studies have shown some interplay between them. Previous research looked at the relationship between amyloid-beta and tau, concluding that although tau protein may be dependent on amyloid-beta aggregation (Zhong et al., 2014), this protein is needed for the toxic effects of amyloid-beta aggregation due to the observation that no neurodegeneration was observed upon depletion of tau protein.

Biomarkers are measures of what is happening inside the body that can help clinicians make earlier diagnosis, track progression in a disease and tailor treatments to each patient while monitoring for efficacy (Paulsen, 2009). They are an important focal point in past and current research in AD. Numerous studies have highlighted the importance of biomarkers in identifying and diagnosing AD in the early stages. Three cerebrospinal fluid (CSF) biomarkers have been studied: amyloid beta, tau protein and phosphorylated tau. These biomarkers collectively increase the validity for diagnosis by giving results which are sensitive to >95% and specific to >85% (Mulder et al., 2010). CSF biomarkers present some challenges as they are obtained through lumbar punctures which are invasive to the individual and costly. Newer biomarkers

which can be obtained using blood are cheaper, less invasive and easily collectable so could be used to follow the progression of the disease.

1.2 Mild Cognitive Impairment

Mild Cognitive Impairment (MCI) is a syndrome in which a person develops cognitive decline greater than the expected level for the individual's age and education level, but that does not interfere with daily activities in their lives (Gauthier et al., 2006). MCI is prevalent in adults older than 65 years, and majority progress to dementia within five years (Gauthier et al., 2006). Diagnostic criteria of MCI include memory complaints by family, mildly impaired complex daily activities, decreased total recall, and persistence of memory changes (Dubois & Albert, 2004). Since MCI is a significant predictor for AD. Correlations between the progression of MCI and AD may provide further indications as to how these issues relate to each other, as well as how MCI may be predictive of AD through blood biomarkers.

1.3 Blood Biomarkers

Biomarkers are any substance or process in the body that can be measured to predict an outcome, disease, or effectiveness of treatment (Strimbu & Tavel, 2010). Biomarkers can be anything including pulse and blood pressure as well as blood or tissues (Strimbu & Tavel, 2010). Numerous pathological studies have attempted to link certain biomarkers in cerebrospinal fluid as well as the bloodstream. Research at the Alzheimer's Association International Conference (AAIC) suggested that the use of blood serum analysis could possibly be used in order to screen for brain diseases such as AD (Cavedo et al., 2014). Analyzing specific brain biomarkers could help in doing so. Specifically, Plasma/Serum NFL, Aβ42/40 ratio, Sphingolipids, Glycerophospholipids, Amyloid beta (Aβ), Tau, Plasma clusterin, Insulin, and Apolipoprotein J.

All of these have proven to be fairly significant biomarkers when it comes to testing for AD and other cognitive impairments. What appears to be a very significant blood biomarker for neurodegeneration is plasma/serum NFL (Zetterberg, 2019). This biomarker is able to aid in observing the gradual degradation of certain cells in the brain. In addition to this, the plasma Aβ42/40 ratio appears to be useful as a blood biomarker for cerebral Aβ pathology (Zetterberg, 2019). Sphingolipid metabolism is a process that provides us with information on the formation of a number of bioactive metabolites and/or second messengers that tend to be essential in cellular signaling and apoptosis. In the brain, the proper balance of sphingolipids is critical to neurons functioning properly, evidenced by several brain disorders that have come about due to enzyme deficiency present in enzymes responsible for metabolizing sphingolipids. Both studies taking place with animals and in laboratory settings suggest sphingolipids contribute to amyloid-beta production and AD pathogenesis through direct and indirect mechanisms (Mielke & Lyketsos, 2010). Lipids such as sphingolipids and glycerophospholipids are closely correlated with metabolism of the Amyloid Precursor Protein (APP). APP produces Amyloid-beta peptide (Aβ), the main component of senile plaques, which represent the main pathological hallmark of AD (Kosicek & Hecimovic, 2013). Amyloid-beta is produced by the cleaving of amyloid precursor proteins into a monomeric form by β-secretase and γ-secretase, which is then transformed into oligomeric and fiber forms, and then finally into amyloid plaques. Oligomeric amyloid-beta is the major toxic species of Aβ associated with AD disorder pathology as well as dysfunction within the synapse (Youn et al., 2019). Tau is a structural protein in the brain. Tau protein, having many phosphorus groups (P-tau) is capable of producing neurofibrillary tangles, which are twisted protein fragments that develop within nerve cells and disrupt the ability of the

cells to transport signals in the brain (Himmelstein et al., 2012). Clusterin and beta-amyloid (Aβ) are both involved in the pathogenesis of AD. Plasma clusterin could serve as a biomarker for the severity of cognitive decline (Hsu et al., 2017). The most frequent form of amyloidosis and a major cause of dementia stems from the Apolipoprotein J, also known as Apo J. Apo J is found in amyloid plaques and cerebrovascular deposits but rarely seen in NFT-containing neurons (Calero et al., 2000). All of these blood biomarkers are able to provide information on plaque build-up and/or the development of cognitive impairment, proving to be helpful in the detection of cognitive diseases like MCI and Alzeheimer's.

1.4 Cognition

In Alzheimer's patients, cognitive functions such as thinking, reasoning, and remembering decline. Measuring cognition in older adults is difficult as while certain trends are present, cognition is variable among different people and is affected by many factors. Past studies have shown that in older adults, brain structure changes with age, with brain volume and white matter declining, leading to less brain mass (Weintraub et al., 2018). Changes in the brain such as brain atrophy and oxidative stress lead to brain metabolism reduction as well as neuronal dysfunctions such as amyloid deposition and tangle formation related to cerebral blood flow decline. These alterations in the brain all contribute to cognitive impairment and Alzheimer's Disease (Portugal et al., 2015). Cognitive tests can be used to evaluate the progression of AD. Such tests are typically done using paper-and-pencil and assess memory, reasoning, writing, vision-motor control, comprehension, and ability to express ideas. Cognition is linked to deposition of amyloid beta protein, and higher deposition is linked with poorer scores on

memory and cognition tests (Weintraub et al., 2018). The progression of AD can be observed by measuring cognition over a period of time.

1.5 Exploratory Aim: MRI Scans

Magnetic reasoning imaging (MRI) can be used to measure brain activity as well as brain structure, and has been used in previous cognition and AD studies. In older populations, MRIs indicate that brain activity increases in the frontal cortex with age to combat degradation of brain structures, such as decreasing white matter volume and brain thickness (Weintraub et al., 2018). MRIs are useful in evaluating AD because they detect brain abnormalities associated with MCI to indicate which patients are in the initial stages of AD. A study by Washington University School of Medicine found that MRI's can predict what patients get dementia with an 89 to 95% accuracy rate an average of 2.6 years before memory loss is detectable by using diffusion tensor imaging that measures the brain's white matter (Raji, et al., 2018). This method measures movement of water molecules along paths that white matter takes when connecting brain regions. Slower movement of water indicates less white matter, meaning lower cognition, MCI, and AD.

MRIs also serve as a tool for measuring AD progression by visualizing the size of the hippocampus. The hippocampus shrinks in size as the brain loses mass due to MCI and AD. In MCI and AD patients, the hippocampus will be noticeably smaller than in adults that do not experience cognitive decline (Vijayakumar & Vijayakumar, 2012). Future areas of research include measuring brain atrophy with MRI to diagnose AD with more accuracy and differentiate among different forms of dementia.

MRI scanning is a useful biomarker because it allows for almost direct quantification of the progression of AD which is included in the blood biomarker analysis.

Millions of people are affected by AD, taking a significant emotional and financial toll on these individuals and their families. Currently, there are no treatments for AD or definitive predictors of its onset. Additionally, despite the extensive research that has been performed on mechanistic models of Alzheimer's Disease, it still remains unknown which factors, both from a biochemical and environmental perspective, can definitively classify which individuals with Mild Cognitive Impairment will transition into the development of AD.

Team BRAIN is looking to correlate biomarker levels with the progression of Alzheimer's Disease. By analyzing changes in a set of biomarkers characteristic of the progression of Alzheimer's Disease, we hope to contribute to a novel, quantitative method of diagnosing the disease, as well as identify potential drug targets in the future.

Chapter 2: Literature Review

2.1 Biological Overview

AD is a neurodegenerative disorder that only in the 20th century became recognized as a cause of dementia and a major cause of death. It is characterized by memory loss, challenges in solving problems, time and location confusion, visuospatial troubles, poor judgment, more apathetic, anxious, and depressive behavior (Hang, 2019).

There are at least 5 genetic loci on chromosomes 1, 12, 14, 19 and 21, that influence the initiation and progression of AD. Those affected by the disease will have an altered amyloid precursor protein, which leads to deposition and fibrillar aggregation of beta-amyloid. The beta amyloid is a small piece of the amyloid precursor protein that is notorious for being chemically

"stickier" than other fragments of the protein. Beta amyloid first form oligomers, then grow more to form fibrils, then grow more to form beta-sheets. Finally, these beta sheets accumulate to form the plaques that we see as a prime characterization of the manifestation of AD. Healthy patients are able to clear amyloid beta proteins from the brain, however, those who are diseased suffer from amyloid-beta that misfolds, aggregates and becomes neurotoxic (*Beta-Amyloid and the Amyloid Hypothesis*, 2017).

Tau protein is also a known contributor to the manifestation of AD, through its excess accumulation in a phosphorylated form in the cerebral cortex. Normally, tau protein performs the roles of stabilizing microtubules and assisting with axonal transport. The phosphorylation of tau occurs during post-translational modifications, and once phosphorylated, tau has a decreased affinity for microtubules (Guo et al., 2017). Thus, phosphorylated tau loses its ability to assist with microtubule stabilization (Guo et al., 2017).

In healthy individuals, tau manifests in a non-phosphorylated form, and is very soluble (C. M. Gao et al., 2010). It is in individuals with AD that phosphorylated tau proteins arise, leading to more aggregation and formation of neurofibrillary tangles, which are clumps of the tau proteins that cause synaptic dysfunction (Jouanne et al., 2017). The mechanism of tau-mediated neurodegeneration is unknown, although there is a strong correlation between the accumulation of hyperphosphorylated tau in AD neurodegeneration.

2.2 Onset Types

The onset of AD can be categorized into two types: Early-onset AD (EOAD) and Late-onset AD (LOAD) (Tellechea et al., 2018). EOAD is characterized as the development of AD before 65 years of age, while LOAD occurs after 65 years of age (Tellechea et al., 2018). Rather

than limiting the differences to merely the age cut-off, EOAD and LOAD differ in various areas (Mendez, 2017).

EOAD is the most common type of early onset neurodegenerative disorder, and it can be familial or sporadic, meaning that individuals who are diagnosed with it may or may not have family members with the disease as well (Mendez, 2017). EOAD tends to have a faster rate of progression and a shorter duration of disease as compared to LOAD. Despite its atypical characteristics, EOAD affects approximately 10% of those affected by AD, however diagnosis is delayed by an average of 1.6 years, leading individuals in the age range from 45-64 years to potentially lose their lives and/or productivity (Mendez, 2017). Studies have indicated that the decreased connectivity with frontoparietal networks, which affect working memory, language, and higher visual networks, seem to drive EOAD (Zhao et. al., 2018).

On the other hand, LOAD is a more common diagnosis among elderly individuals, and research indicates that the numbers will only increase in the human population over time. LOAD has no specific gene mutations that are correlated with its inheritance yet; however, its inheritance is sporadic and not based on familial presence (Isik, 2010).

Research comparing the two types of onsets has indicated that EOAD tend to have better memory recognition scores and semantic memory, however, they have worse attention, worse executive functions, worse visuospatial skills, and more ideomotor apraxia compared to those who have LOAD (Awada, 2015). While both types of onsets are detrimental to an individual, we can see that the complexity of AD can also lie in the different ways it manifests in individuals.

2.3 Risk Assessment Methods

Assessing one's risk for presenting with AD is important for a potential patient to be prepared and pursue a possible course of action. The current primary method for determining an individual's risk for developing AD during their lifetime is genetic analysis, but there are other promising methods that may provide a greater understanding of AD development and progression.

2.3.1 Blood

A recent study demonstrated a new way of assessing risk for AD through a blood test. Using mass spectrometry on blood samples, the study determined two different forms of amyloid beta: Aβ42 and Aβ40. The ratio between the amounts of these two forms decreases when amyloid beta deposits start to form. This ratio can be measured in blood, and positive plasma Aβ42/Aβ40 corresponds to having a positive amyloid PET scan in the future, not necessarily at the time of the draw, and therefore can be used to predict the development of brain amyloidosis (Schindler et al., 2019). Though not available to the public as of August 16, 2019; this datum suggests that the proposed blood test is more sensitive and accurate than brain scans in determining risk and predicting the development of AD. Using tests such as this, those at risk can start to implement lifestyle changes early as prevention from developing the disease in the future.

2.3.2 Genetics

Genetics is an important component in assessing risk for AD development, and thus the current approach is to look at family history or to do a genetic screening for particular genes (Reitz, 2015). The most common gene associated with late onset AD is apolipoprotein E, or APOE. Within the gene there are three common variations, APOE e3, APOE e2 and APOE e4.

APOE e3 is the most common variation and it does not have an effect on the development of AD. APOE e2 is the rarest form with protective effects against developing the disease. APOE e4 is less common than APOE e3 and increases the risk of developing AD (Liu et al., 2013). Individuals with only one copy of the APOE e3 gene have a lower risk than those who have two copies of the variant. Other gene variants that increase risk for late onset AD include ABCA7, CLU, CR1, PICALM, PLD3, TREM2 and SORL1 (Reitz, 2015). Conversely, early onset AD is more accurate to predict using three genetic variants: APP, PSEN 1 and PSEN2. These variants cause an increase of production of amyloid- beta. Individuals with one of these genes will most likely develop early onset AD. However, many people that develop AD do not have any variants increasing their risk, indicating that genetics is not the only variable influencing development.

2.4 Prevention Methods

Although genetics attributes to developing AD, further lifestyle factors can prevent the early onset of this disease. The best way to treat AD is to start early, before it develops. Lifestyle factors such as diet, exercise and social engagement, as well as health factors including the development of vascular and metabolic conditions can influence the development of AD and increase risk (Flicker, 2010). Therefore, determining one's risk for the disease is crucial to preventing it, and alleviating these risks may be a way to delay onset.

2.4.1 Diet

The MIND diet (Mediterranean-DASH Intervention for Neurodegenerative Delay) has been highlighted as a framework for organizing eating patterns with the potential to slow mental degradation (Morris et al., 2015, p. 1008). The MIND diet rules includes eating: three servings of whole grains a day, leafy green vegetables six times a week, other vegetables at least once a day,

berries two times per week, red meat four times a week or less, fish once a week, poultry twice a week or more, beans over three times a week, nuts more than five times a week, less than a tablespoon of margarine/butter per day, less than one serving of cheese a week and less than five pastries/sweets a week; as well as limiting fried and fast food to once a week and using olive oil for cooking (Morris et al., 2015, p. 1009). This 15-point diet is a more flexible version of the Mediterranean diet, and it does not have to be followed exactly. In addition, healthy eating can benefit cardiovascular and metabolic health, which also affects AD development.

2.4.2 Exercise

Physical activity is one of the factors that help reduce the risk of AD or slow down the progression of the disease. Studies show that those who are more physically active, especially with aerobic physical activities, are less likely to develop mild cognitive impairment (MCI) in their later ages for those without the APOE gene, the greatest genetic factor of later onset AD (Wu et al., 2007). also show that exercise improves the plasticity of the brain, specifically, the hippocampus, which leads to improved cognitive functions and other functions of the hippocampus. In addition, production of some growth factors such as the brain-derived neurotrophic factor (BDNF) are exercise-induced and have shown to improve neurogenesis and cognitive functions. Exercise is also shown to increase proliferation of multipotent and neural stem cells which are related to improvement in memory and learning (Hoveida et al., 2011). Other studies also show that physical activities help deplete the beta-amyloid load. In AD, the collection of beta-amyloid leads to high uptake of a radiotracer, PIB, but physically trained elderly patients had low PIB uptake (Liang et al., 2010). The mechanisms that are affected by the physical activities are not clear, but there are some possible mechanisms that are possibly linked

to reduced risk of cognitive impairment. Physical activities can increase blood flow to the brain which allow blood supply for more brain cells and help remove metabolic waste. Moreover, physical activities help with the prevention and treatment of depression, insomnia and other sleep disorders; the risk of developing dementia can be reduced (Gallaway et al., 2017). Gait, the manner of walking, is another way of detecting mild cognitive impairment. Disturbances in gait have been linked to AD progression, and thus should be a good predictor of what stage the participants are in (Muir et al., 2012).

2.4.3 Social Engagement

Though results have been varied, social engagement has been found to decrease the risk of AD in older adults. A study using data from the Kungsholmen Project in Sweden found that having a developed social network resulted in decreased risk for developing AD by looking at participants' engagement in activities 6.4 years before being diagnosed with dementia. This suggests that social engagement has protective properties stemming from social interaction and intellectual stimulation (Wang et al., 2006, p. 1081). A similar study found that having poor social connections and rare participation in social activities are linked to the risk of developing dementia, however, they also found that engagement with friends had a protective effect against dementia for women only (Zunzunegui et al., 2003, p. S96).

2.5 Methods of Assessing AD Progression

One reason for the high mortality rate associated with AD is the difficulty of diagnosis and measurement of progression. Antemortem measurements of AD pathology would allow researchers to study the effects of novel treatments and therapies on the disease itself, rather than to just combat dementia symptoms. A comprehensive system of methodology for assessing AD

progression is a key component to the development of any prevention and treatment methods. This section will review current blood biomarkers, imaging methods, cognitive assessments, and physical function testing, all integral to a well-rounded understanding of Alzheimer's Disease.

2.5.1 Blood Biomarkers

Blood biomarkers are a good way of determining AD progression because of the numerical evidence they present. There are multiple molecules in the blood in which abundance is determined by processes impacted by AD. By doing a blood sample and measuring the levels of these molecules, the progression of the disease can be quantified without bias introduced by interpretation of tests like self-report or a dementia battery.

2.5.1.1 Amyloid-β (Aβ)

One of the main characteristics of AD is the development of amyloid plaques. These amyloid plaques are tough deposits of fibrils that impede normal brain function and lead to dementia when enough has accumulated (Lane et al., 2018b). Extracellular β-amyloid peptide (Aβ) plaque deposits are one of the pathologies required for an AD diagnosis (Murphy & LeVine, 2010).

The Amyloid-β precursor protein is anchored to the cell membrane and is normally integral in neuronal growth and repair (Small et al., 2006). Later in life, the protein sometimes is released from the cell membrane and floats as a free peptide; in this form, it is susceptible to conformational changes that cause it to aggregate, forming clumps on nerve cells (Johansson, 2005).

Amyloid-β starts to accumulate in the brain 10-15 years before the first clinical symptoms start to appear (Bateman & Chakrabartty, 2004). Some recent studies even show that

Aβ shows up 15-20 years before symptoms. This period of preclinical AD is now characterized by amyloidosis in the brain as shown by PET scans and samples of CSF from people with normal cognitive abilities (Qi et al., 2018). Finding signs of amyloid-β and corresponding biomarkers in the blood is an extremely promising area for Team BRAIN.

2.5.1.2 Tau Protein

The excess accumulation of phosphorylated tau protein in the cerebral cortex is another characterizing feature of AD. Under normal conditions, tau protein is present in neurons and serves the purpose of regulating the stability and assembly of microtubules (Wu et al., 2017). Additionally, tau protein serves an important role in axonal transport (Wu et al., 2017).

The ability of tau to perform its function of regulating microtubules is regulated by its phosphorylation. Excess phosphorylation, or hyperphosphorylation, is seen in individuals with AD. Tau hyperphosphorylation in AD is a result of the combination of increased phosphorylation by kinase enzymes and decreased dephosphorylation by phosphatase enzymes (Jouanne et al., 2017). It is likely a result of a combination of both processes being deregulated, resulting in an imbalance that favors hyperphosphorylated tau (Duan et al., 2012).

In its non phosphorylated form, tau is actually a highly soluble protein and is unlikely to aggregate (Jouanne et al., 2017). On the other hand, phosphorylated tau isoforms promote more aggregation than non phosphorylated tau. These phosphorylated tau proteins tend to form clumps known as neurofibrillary tangles. Neurofibrillary tangles may cause synaptic dysfunction, but the degree to which they actually contribute to memory loss and behavioral changes in AD remains uncertain. In vivo models have shown that neurofibrillary tangles are not the main contributors to memory loss and tissue degeneration during the early stages of AD (Jouanne et al., 2017). This

suggests that amyloid-beta is the primary pathological protein causing AD Although, there is also evidence to support that phosphorylated tau aggregations, or neurofibrillary tangles, do contribute to worsening the behavioral changes associated with AD. Transgenic mice were created with reduced levels of tau, and without any changes being made to their amyloid-beta levels, these mice showed decreases in behavioral deficits (Duan et al., 2012).

Although the exact mechanism of tau-mediated neurodegeneration is unknown, it is apparent that the accumulation of hyperphosphorylated tau along with the accumulation of amyloid-beta contributes to neurodegeneration. Examining how levels of hyperphosphorylated tau change in the blood as AD progresses may be an important step towards earlier diagnosis of AD, as well as provide more insight into the mechanism behind hyperphosphorylated tau's role in the deterioration of brain tissue that seen in individuals with AD.

2.5.1.3 Blood Acetylcholinesterase

Acetylcholinesterase (AChE) is one of the enzymes that exist in the brain and focuses on breaking down esters at the postsynaptic membrane. Because it also exists on the cell membrane of red blood cells, it may serve as a promising biomarker that can be taken from the blood, as opposed to the CSF (Xiang et al., 2017)).

A study performed by Han et al. (2019) shows that blood AChE is associated with deposits of Aβ in the brain. The study was done on CN, MCI, and AD patients and data was collected using the Pittsburgh compound B positron emission tomography for amyloid imaging and analyzed five plasma biomarkers using mass spectrometry. They concluded that AChE is not an automatic blood biomarker for AD, making it a promising area for Team BRAIN to study.

2.5.1.4 Neurofilament Light

Neurofilament light (NfL) is a measure of the amount of neurofilament polypeptides in the blood. Neurofilaments consist of three different types of protein chains: the light, intermediate, and heavy chain (Lewczuk et al., 2018). Each subunit is made of an alpha helical core, an N and C terminus (Lewczuk et al., 2018). To determine neurofilament levels, the levels of neurofilament light chain is used. Neurofilaments are integral in the structure of the neuronal cytoskeleton and regulate the function of microtubules, and injured cells release it. Thus, large quantities of this polypeptide in the blood can indicate axonal damage to brain tissue due to AD (Lewczuk et al., 2018). This makes it an ideal candidate as a blood biomarker. Baseline levels of NfL in healthy controls, mild cognitive impairment and AD dementia have been determined and have been found to differ between individuals from the three conditions. The healthy control condition had the lowest NfL levels (32.1ng/L), followed by the mild cognitive impairment condition (37.9ng/L) (Mattsson et al., 2019). The AD dementia condition had the highest concentration at 45.9ng/L (Mattsson et al., 2019). Furthermore, until recently NfL has been determined only through cerebrospinal fluid and has not been studied to the same degree. It has been found to be a promising, novel blood biomarker in recent research and could be analyzed with amyloid beta protein and tau protein to determine AD progression (Hu et al., 2019). Not only are NfL levels important, the change in NfL levels can also be a good predictor of dementia progression (Mattsson et al., 2019). Because of its close relation to AD, novelty and fit with our project we plan on using plasma Neurofilament Light as one of our blood biomarkers.

2.5.1.5 Aβ42/40 ratio

The Aβ42/40 ratio is the ratio between the amyloid-beta 42 protein and amyloid-beta 40 protein, and is currently being used in clinical trials to detect AD (Lehmann et al., 2018). The concentration of Aβ42, which can be collected as a blood sample or in cerebrospinal fluid, decreases as amyloid accumulates in brain tissue, but is not a concrete indicator of AD (Lehmann et al., 2018). However, when the ratio of Aβ42/40 is evaluated, the results are more congruent and can be compared to control groups, which reduces variability in measurement and bias in the analysis. The cross-sectional analysis of the Aβ42/40 ratio in blood plasma and the cortical Aβ burden, which is the concentration of amyloid-beta deposition in the cerebral cortex of the brain, showed an inverse relationship, and analyzing the Aβ42/40 ratio is 81% predictive of high amyloid beta burden in the brain (Fandos et al., 2017). This indicates that the Aβ42/40 ratio is a good biomarker of AD because it quantifies the buildup of amyloid in the brain and is more reliable as a biomarker than just Aβ42 concentration.

The Aβ42/40 ratio can also be used as a biomarker indicator of AD decades before the clinical onset, but has not been actively studied because of invasiveness and cost of taking cerebrospinal fluid samples. Cerebrospinal fluid samples are commonly collected by a lumbar puncture (LP), in which a needle is inserted into the spinal canal to draw the surrounding fluid from the brain and spinal cord. This procedure is both costly and complicated. Many risks include a persistent headache status post LP, bleeding, infection, and much more. Although multiple factors are evaluated, a lumbar puncture can typically range from $400 to about $4000. As a blood biomarker, measuring the Aβ42/40 ratio is affordable and less invasive, and numerous studies have reported that a lower ratio in blood is correlated with a higher risk of AD

and greater buildup of amyloid beta cerebral fillary in the brain (Fandos et al., 2017). Most current clinical studies examine the Aβ42/40 ratio collected from CSF and PET scans, so using the Aβ42/40 ratio as a blood biomarker is a relatively novel idea. We will analyze the Aβ42/40 ratio in AD patients and control groups to compare how the ratio changes as the disease progresses and amyloid accumulates. It is expected that the Aβ42/40 ratio will decrease for patients with AD and dementia.

2.5.1.6 Apolipoprotein J (Apo J)

Apolipoprotein J, also known as clusterin, is a multifunctional glycoprotein that is able to link and interact with various molecules (Calero et al., 2000). Apolipoprotein J has been linked to AD on many occasions, due to previous studies finding strong relationships that suggest a correlation. Previously, Apo J has shown to be significantly higher in levels with those that have mild cognitive impairment or AD (Gupta et al., 2015). Apo J is known for limiting the formation of amyloid-beta deposits (Gupta et al., 2015) . This then leads to its toxicity, as it interacts with other prefibrillar species, and keeps fibrils from being formed (Gupta et al., 2015). In addition to this, the clusterin gene has been identified as one of the strong genetic loci of AD, making it worthwhile to study (Gupta et al., 2015). Studies show high levels of Apo J have been linked to an increase in hippocampal volume and higher white matter lesion volume (Koch et al., 2018). This aids in making this biomarker indicative of AD pathology (Koch et al., 2018). It is expected that Apolipoprotein J levels will be higher amongst those that show evidence of cognitive impairment as opposed to controls. This will connect to the overall study by allowing Team BRAIN to be able to analyze Apo J's effect on hippocampal volume. This will allow the team to analyze any progression in MCI or AD based on changes in hippocampal volume size.

2.5.1.7 Glucose

While many studies have shown a correlation between insulin resistance and AD, there is also a relationship between how the brain breaks down glucose and the severity/expression of AD (specifically, brain tissue glucose concentration, glycolytic flux, and GLUT3) (An et al., 2018). This study, conducted by the National Institute on Aging's Laboratory of Behavioral Neuroscience, measured the ratios of glycolytic amino acids to glucose in autopsy patients as well as the protein levels of neuronal (GLUT3) and astrocytic (GLUT1) protein transporters (An et al., 2018). These measurements are a direct reflection of abnormalities in the way that the brain breaks down glucose.

The NIA study is groundbreaking and abnormalities in glucose-homeostasis may become an important indicator in the future, but for the constraints of this study, Team BRAIN is unable to take autopsies or otherwise measure glucose dysregulation.

2.5.1.8 HbA1c

HbA1c, also called Hemoglobin A1c and glycohemoglobin, measures glucose levels not only at the time of the blood draw but for two to three months prior to the blood draw (Significance of HbA1c citation). This is a useful tool because it can depict if a participant has been regularly keeping glucose low or high. Elevated HbA1C levels can suggest diabetes or poor glycemic control, which increases glucose hypometabolism in areas of the brain affected by AD (Roberts et al., 2014). This biomarker is a link between AD and diabetes, and will be used in the study.

2.5.1.9 Insulin

In an effort to obtain some more concrete results when it comes to how insulin levels in the blood may be correlated with the progression of AD, an assay will be used to test insulin levels in participants.

Insulin has been found to be positively associated with the degradation of amyloid-beta (Byun et al., 2017). This indicates that individuals with AD may be more likely to have lower levels of insulin, which has also been found in some studies. For instance, one study showed that lower insulin levels are associated with increased accumulation of amyloid-beta and a decreased glucose metabolism in the regions of the brain that are most affected by AD (Byun et al., 2017). However, another study proved the opposite to be true, finding higher levels of insulin in the blood of individuals with AD.

Despite the variance in the findings of studies of insulin as an AD blood biomarker, insulin is an important biomarker to consider since glucose metabolism is largely related to the disease. AD is very similar to Type II Diabetes Mellitus, in the sense that the same resistance to insulin is characteristic of both (Calsolaro & Edison, 2016). In both diseases, insulin receptors are less sensitive than normal, leaving to impaired glucose metabolism (Calsolaro & Edison, 2016). In fact, this type of diabetes is well-established as a risk factor for dementia (Marden, Mayeda, Tchetgen, Kawachi, & Glymour, 2017). Although the concrete mechanism behind this phenomenon is unknown, one possibility is that an increase is glycosylated hemoglobin (chronic hyperglycemia) is responsible for the relationship between the two diseases (Marden, Mayeda, Tchetgen, Kawachi, & Glymour, 2017).

Although the link between AD and insulin levels remains unclear, it has been chosen as a blood biomarker for our study in hope that it can contribute to the clarification of the insulin/Alzheimer's association.

2.5.1.10 Homocysteine

Development of AD dementia has been linked to many cardiovascular diseases (Seshadri et al., 2002, p. 476). Because cardiovascular problems can increase risk for the development of AD, quantifying this risk is useful in determining the major cause of dementia development. High homocysteine levels have been associated with deaths caused by cardiovascular causes, coronary heart disease, carotid atherosclerosis and stroke (Seshadri et al., 2002, p. 476). The development of these cardiovascular problems can induce the development of AD, and homocysteine has been associated with cognitive decline due to this association. By looking at 1092 people with an average follow up period of eight years, a study found that 111 people with elevated homocysteine developed dementia, and 83 of those people had AD (Seshadri et al., 2002, p. 478). The risk of developing AD was 1.9 with an increase of 1SD from the baseline test, and 1.6 with an increase of 1SD eight years before the baseline was done. If the concentration of homocysteine was greater than 14 μmol/L, the risk doubled (Seshadri et al., 2002, p. 478). Though this could be an interesting biomarker to look at, there is not enough evidence supporting its reliability so it may not be used.

2.5.1.11 Glycerophospholipid

Glycerophospholipid is a glycerol based phospholipid, which means it plays a major role in the structure and permeable characteristics of the cellular membrane. Its many functions include being a major source of fatty-acid derived lipid mediators, contributing to cell signalling,

creating surfactant, and assisting in mitochondrial membrane protein activity and stability. Neural membranes contain several classes of glycerophospholipids that not only provide necessary backbone structure, but provide the membrane with a suitable environment and fluidity, with selective permeability (Frisardi & Farooqui, 2011).

The degradation of glycerophospholipids occurs with the assistance of phospholipase A2, and in the process, it releases two important brain polyunsaturated fatty acids, known as PUFAs (Proitsi & Legido-Quigley, 2017). These PUFAs can be oxidized through non-enzymatic and enzymatic oxidation, therefore producing several lipid mediators. These lipid mediators are what correlates glycerophospholipids with neuronal pathways involved in the neurobiology of AD, however, there is no clear mechanism indicating the relationship between lipid mediators in the pathogenesis of AD. The implications of this correlation are that it suggests that there is a function of lipids in brain tissue, potentially paving the way for new preventative or therapeutic options to regress the development of AD.

In Team BRAIN's study, glycerophospholipid will be observed as a blood biomarker, because of its correlation with AD. The hypothesis is that, as AD progresses over the course of the six months of a patient's life, the levels of glycerophospholipid will increase. Because it contributes to the creation of lipid mediators that in turn contribute to the neuronal pathways associated with AD, it is predicted that as time progresses, these glycerophospholipids will increase, as will the presence of lipid mediators, and lead to the progression of the disease (Hishikawa et al., 2014).

2.5.1.12 Alpha sheet

Much of AD research has been focused on preventing the buildup of mature amyloid fibrils, assuming the buildup is responsible for the effects of AD. However, treatments for lowering the deposition of amyloid fibrils in the brain have proven ineffective and recent research suggests an alternative theory in which soluble oligomers are responsible for the toxicity of AD along with other diseases (Bi & Daggett, 2018). While traditional methods for identifying the oligomer have been of limited use due to the ease with which they dissociate into monomers or aggregate into fibrils, computer modeling methods have led to the alpha-sheet hypothesis, which proposes that a secondary structure between the alpha helix and beta sheet, termed the alpha sheet, is responsible for the toxicity of amyloid (Bi & Daggett, 2018). To test the theory, Bi and Daggett (2018) designed alpha-sheet peptides which would be complementary to the proposed oligomer structures and studied the resulting inhibition of amyloid beta aggregation. As a control, they also tested the effect of two random coil peptides on inhibition of amyloid beta aggregation. The study found that all of the alpha-sheet compounds inhibited aggregation while the controls had very little effect, thereby agreeing with the hypothesis (Bi & Daggett, 2018).

The promising results linking the alpha-sheet structure of amyloid with the toxicity of AD make it a possible biomarker for the disease. The viability of the soluble oligomer (though not alpha-sheet) as a biomarker was tested with a novel ELISA assay for detecting oligomers in cerebrospinal fluid (C. M. Gao et al., 2010). Given a patient population of 26 patients with mean age 71.8±7.3 years and a control group of 10 individuals with mean age 69.4±9.7 years, the group found a correlation between AD and Aβ40 oligomers, therefore encouraging further study

on the utility of soluble oligomers as biomarkers (C. M. Gao et al., 2010). Thus, alpha sheets may be chosen as one of the biomarkers tested in this study in order to provide more information about how alpha sheet levels change in response to dementia progression, as well as how lifestyle factors impact alpha sheets.

2.5.1.13 Sphingolipids

Sphingolipids are a class of lipids derived from the aliphatic amino alcohol sphingosine Peripheral sphingolipid levels and clinical outcomes across a range of AD severities have resulted promising results for ceramides. Many current studies are attempting to analyze the findings in humans by examining postmortem tissue, cerebrospinal fluid (CSF) and peripheral sphingolipid levels, primarily focusing on the levels of ceramides and sphingomyelins in the blood (Mielke et al., 2012). Results are difficult to compare, as there are differences in brain regions examined as well as the clinical and pathological severity of the AD brains. Mass spectrometry techniques have been used to quantitatively measure the individual sphingolipid species in the blood mostly in longitudinal studies (Mielke et al., 2012). There is insufficient development of high-throughout sphingolipid assays that can be used in this study, so sphingolipids will not be used as a biomarker (Mielke et al., 2012).

2.5.1.14 Human Serum Albumin

Human Serum Albumin (HSA) is the most abundant protein found in human blood, and plays an important role in preventing the formation of the Aβ peptide (Milojevic & Melacini, 2011; Stanyon & Viles, 2012). A study that aimed to characterize the stoichiometry and affinities of the albumin-Aβoligomer interactions found that certain albumin domains can recognize Aβ oligomers, and can inhibit fibril formation (Milojevic & Melacini, 2011). NMR experiments

revealed that once HSA binds to the Aβ oligomers, the kinetics of Aβ fibrillization is inhibited, and the peptides become trapped in a nonfibrillar form. This in turn reduces the total concentration of Aβ fiber that is generated. Because the buildup of Aβ is associated with the development of AD, levels of HSA could be indicative of AD progression (Milojevic & Melacini, 2011).

A study investigated the effect of HSA on both Aβ42 and Aβ42 fibril growth, and found that the amount of Aβ fibers generated directly correlates with the proportion of Aβ not competitively bound to albumin (Stanyon & Viles, 2012). Aβ40 and Aβ42 fibrils showed similar fibril growth kinetics, and that the overall fibril growth is reduced in the presence of albumin (Stanyon & Viles, 2012). HSA has been known to bind to many different hydrophobic molecules, such as pharmaceuticals like diazepam and warfarin, and endogenous fatty acids. Because HSA has an affinity for many different molecules, these molecules may compete with Aβ binding to HSA (Stanyon & Viles, 2012). Because of this property, human serum albumin will not be used as a blood biomarker in this project.

2.5.2 Brain Scanning

Brain scans are used to show the brain architecture and brain activity in MCI and AD patients. Structural magnetic resonance imaging (MRI) and functional magnetic resonance imaging (fMRI) show the structure of the brain and the connectivity of different structures in the brain, respectively. Positron Emission Tomography (PET) can provide clear visualizations of Amyloid-β plaques.

2.5.2.1 Structural MRI

Structural MRI scans are the most widely used method to visualize the morphology of the brain. These scans are important in examining the patterns of brain atrophy. Hippocampal atrophy is considered one of the core biomarkers of AD (Apostolova et al., 2006; Shi et al., 2009). A meta-analysis study found that hippocampal volume is greatly reduced in patients with MCI or AD compared to the hippocampal volume of healthy controls : results from previous research that conducted MRI studies on MCI and AD patients were synthesized, and it was found that compared to the healthy controls, the extent of bilateral hippocampal atrophy is the greatest in AD patients and less in MCI patients (Apostolova et al., 2006; Shi et al., 2009). Additionally, the hippocampal volume deficits have been found to be correlated with cognitive disorder development and episodic memory deficits (Pini et al., 2016). In this study, MRI scans of MCI patients and healthy controls were taken, once at baseline and another 24 months later. These results were analyzed with patients' performance on cognitive tests. The researchers concluded that regional atrophy is associated with cognitive impairment (Pini et al., 2016). It should be noted that hippocampal atrophy is evident in not only AD, but other non-AD forms of dementia such as vascular dementia, semantic dementia, and Parkinson's dementia (Bastos-Leite et al., 2007; Chan et al., 2001; Laakso et al., 1996).

2.5.2.2 Functional MRI

Functional MRI (fMRI) scans show activity across different regions of the brain. By measuring the blood flow in the brain, the connectivity between certain regions can be determined. Resting state fMRI scans of AD patients show that hippocampal connectivity is disrupted, which in turn affects the default mode network (Wang et al., 2006). These scans show

that the functional connectivity between the right hippocampus and surrounding areas is decreased, while the functional connectivity between the left hippocampus and the right lateral prefrontal cortex is increased (Wang et al., 2006)). Additional research has suggested that these increases in connectivity may be the brain's way to compensate for decreased connections in other areas such as those involving cognitive functions (Zhong et al., 2014). Researchers identified eight regions of the brain, conducted fMRI scans on AD patients and healthy controls, and analyzed the changes in the levels of activity in each of them. It was found that compared to healthy controls, AD patients have increased prefrontal region interactions and decreased cognitive region interactions (Zhong et al., 2014). While fMRI imaging is a valuable tool for analyzing the progression of AD, it is rather expensive and will only be done if there are necessary funds.

2.5.2.3 Positron Emission Tomography

Positron Emission Tomography (PET) uses a radioactive tracer to detect the location in the body using a higher level of energy. Researchers have used an amyloid-binding radiotracer to tag Amyloid-β proteins for disease staging and early detection. In this study, patients were classified as having AD, MCI, or no cognitive impairment, and PET scans were conducted. The binding of the radioactive tracer to Aβ proteins in each brain region was quantified and averaged. It was found that the tracer-Aβ binding was lower in the control group than the MCI group, and the tracer-Aβ binding was lower in the MCI group than the AD group (Small et al., 2006). It should be noted that while it is useful in determining the risk a patient has in developing dementia, PET scans of Amyloid-β are insufficient as the only tool in diagnosing AD or staging AD because Aβ plaques themselves are insufficient for a positive diagnosis of AD. An additional

observation of tau-pathologies is also necessary to confirm the diagnosis. Researchers created a PET tau tracer that can bind to tau tangles. It was found that worse memory performance was associated with greater PET tau, and the combination of PET tau and PET Aβ tracers yield more accurate diagnoses of AD (James et al., 2015). While PET scans are a promising way to measure the progression of AD, they require another method of confirmation and it is ideal to use one method to save time and money on this project.

2.5.3 Cognitive Testing

Series of tests have been used to assess patients' cognitive impairment. The most widely used method of assessing cognitive function by clinicians and researchers is the Mini Mental-State Exam (MMSE), which consists of eleven questions focused on memory, attention, orientation, visuo-spatial skills, and the patient's ability to follow verbal and written commands. A total score on a scale of 0-30 is assigned, in which a score below 24 indicates that the patient is cognitively imparied (Folstein et al., 1975). However, it is important to note that there is no single test that is best suited for all patients with AD or those presenting MCI. The following paragraphs summarize other tests that have been used as screening techniques.

To detect MCI and AD in a population-based sample, researchers have used the Montreal Cognitive Assessment (MoCA). This test, also on a 30-point scale, was created to screen patients with MCI that fall in the normal range on the MMSE. The questions are more specific, have fewer learning trials, and give subjects a longer delay before recall (Nasreddine et al., 2005). The MoCA was found to be the most comprehensive, as it covers all seven cognitive domains commonly encountered in MCI: memory, orientation, language, executive function, visuo-spatial abilities, praxis, and attention (De Roeck et al., 2019). The Memory Impairment Screen is

another method used by researchers. While it only measures subjects' episodic memory, it is still considered a useful method for measuring cognitive impairment in older patients (Lin et al., 2013).

If tests were to be conducted in a memory clinic, the Quick Mild Cognitive Impairment (Qmci) test was found to be the most suitable (De Roeck et al., 2019). The Qmci screen, consisting of six subtests, was developed to differentiate subjective memory complaints, MCI, early dementia, and normal cognition. The cut-off scores for this test are adjusted for level of education and age, and this test is a quick 3-5 minute screen, and can be very effective in busy clinical settings (O'Caoimh et al., 2017).

2.6 Aims and Hypotheses Review

The purpose of this study is to understand how the progression of AD can be monitored by the analyses of biological biomarkers. These biomarkers include: amyloid-beta, tau protein, blood acetylcholinesterase, neurofilament light, amyloid-beta 42/20 ratio, apolipoprotein J, HbA1c, insulin, p-tau (phosphorylated form of tau), glucose, homocysteine, glycerophospholipid, alpha sheet, sphingolipids, and human serum albumin.

Neurofilament light is expected to increase from the neurotypical control condition, to the mild cognitive impairment condition, to AD dementia. NfL levels are expected to increase from the baseline measurement to the six month measurement with the progression of the disease. Insulin levels are expected to differ from the neurotypical control condition and mild cognitive impairment condition. It is also expected that insulin levels will change from the neurotypical control in the same manner after the six month period. Like insulin, p-tau levels are expected to increase from the neurotypical control in the progression of the six month period and better

detect AD from other non-dementing neurodegenerative disorders. If homocysteine is used, its levels are expected to increase with dementia progression and will increase from the baseline blood draw to the blood draw after six months, as well as from a healthy neurotypical control to a person with MCI or AD. HbA1C levels are expected to increase with the presence of diabetes and hypoglycemia in the brain, which can be found in those with AD.

Chapter 3: Methods

3.1 Alzheimer's Disease Neuroimaging Initiative

The data for this analysis was acquired from the Alzheimer's Disease Neuroimaging Initiative (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 magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD). For up-to-date information, see www.adni-info.org. ADNI contains imaging from MRI and PET scans, cognitive testing data, diagnosis data, biomarkers, cerebrospinal fluid (CSF) biomarkers, and genetic data for cognitively healthy participants, participants with Mild Cognitive Impairment (MCI), and participants with Alzheimer's disease (AD). The data for ADNI's biomarker analysis is composed of samples of cerebrospinal fluid (CSF), plasma, and serum from subjects in the ADNI-1 dataset. The serums are housed at -80 degrees Celsius at the University of Pennsylvania with constant surveillance. Roche Elecsys cobas e 602 immunoassays were used for the standardization of CSF Aβ42, Aβ40, t-tau and p-tau181. ADNI variables analyzed include: APOE status, ADNI Memory, ADNI Language,

ADNI Executive Function, ADNI Visuospatial (VS), Aβ40, Aβ42, left and right hippocampal volume.

The ADNI database provides an opportunity for analysis to be done with large datasets, with a large sample of individuals with different backgrounds and histories. ADNI has a set of research standards for the stakeholders involved in the partnership between pharmaceutical companies, non-profit organizations, and research institutions in the field. This influences the standards for data collection and operating procedures in each site affiliated with ADNI. A steering committee consisting of the ADNI principal investigator, core leaders from the eight ADNI cores, all of the site principal investigators, and representatives from the National Institutes of Health and Food and Drug Administration. The high standards for operations and data acquisition allow ADNI to be a valid and reliable source for data collection for this study.

3.2 Variables

3.2.1 Cognitive Testing

Cognitive test variables include ADNI Memory Score, ADNI Language Score, ADNI Executive Function Score, and ADNI Visuospatial Ability Score. This data is from the UWNPSYCHSUM ADNI dataset. The scores for each of the aforementioned variables were compiled by ADNI from various tests used to assess that cognitive ability. The final model for ADNI Executive Function includes Category Fluency- animals, Category Fluency- vegetables, Trials A and B, Digit span backwards, WAIS-R Digit Symbol Substitution, and 5 Clock drawing items (circle, symbol, numbers, hands, time). Category Fluency- animals, Category Fluency-vegetables, and WAIS-R Digit Symbol Substitution scoring is determined by counts in a pre-specified time span. Trials A and B scoring is determined by times to completion. Digit span

backwards scoring is determined by the number of items completed correctly. Clock drawings scoring is dichotomous, either correct or incorrect. The ADNI Executive Function measure has been validated by Gibbons et al. and Crane et al (1,2). The final model for ADNI Memory includes Trial 1, Trial 2, Trial 3, Trial 4, Trial 5, Inference, Immediate recall, 30 minute delay, and Recognition scores from the RAVLT, Trial 1, Trial 2, Trial 3, Recall, Recognition present, and Recognition absent scores from the ADAS-Cognitive Behavior assessment, Immediate and Delay scores from the Logical Memory assessment, and Ball recall, Flag recall, and Tree recall scores from the MMSE. The ADNI Memory measure has been validated by Gibbons et al. and Crane et al(Gibbons et al., 2012),(Crane et al., 2012). The final model for ADNI Language includes Category Fluency- animals, Category Fluency- vegetables, and Boston Naming (Total) scores from the Neuropsychological Battery, Following Commands, Object Naming, and Ideational Practice scores from the ADAS-Cognitive Behavior assessment, Naming an Object- Watch, Naming an Object- Pencil, Repeating a Sentence, Reading a Sentence, Writing a Sentence, and Following a Series of Instructions scores from the MMSE, and Letter F Fluency scores from the MoCA. The ADNI Language measure has been validated by Choi et al (Choi et al., 2020). The final model for ADNI VS includes Clock copy- Circle, Clock copy- Symmetry, Clock copy- Numbers, Clock copy- Hands, Clock copy- Time scores from the Neuropsychological Battery, Constructional praxis scores from the ADAS- Cognitive Behavior assessment, and Copy design scores from the MMSE. The ADNI VS measure has been validated by Choi et al (Choi et al., 2020).

3.2.2 Blood Biomarkers

Aβ40 and Aβ42 data is from the UPENN- Plasma Biomarker Data set. This is part of the Proteomic Analysis ADNI method, which analyzed plasma samples from the cohorts in a 190 analyte multiplex immunoassay panel. The multiplex immunoassay panel was developed on the Luminex xMAP platform by Rules-Based Medicine (*Biomarker Consortium Project*, 2010).

3.2.3 Imaging

Left and right hippocampal volume data is from the ADNI University of California, San Francisco Surgical Navigation Technologies (SNT) Hippocampal Volumes dataset. The data was collected by using SNT software designed to assist in tracing the human hippocampus through the following procedures: patient MRI data, landmarks around the hippocampus of patient MRI data, a brain atlas with the hippocampus defined, and SNT algorithms. For ADNI 1, the MRI protocol "focused on consistent longitudinal structural imaging on 1.5T scanners using T1- and dual echo T2- weighted sequences. One fourth of ADNI 1 subjects were also scanned using essentially the same protocol on 3T scanners." (*ADNI | MRI Analysis*, n.d.). The total hippocampal volume variable was obtained by adding the left and right hippocampal volume data for each participant.

3.2.4 Genetic Testing

ADNI's genetic analyses consist of generated SNP genotypes using Illumina BeadStudio 3.2 Software. This Illumina method was used to ensure consistency in the identification of genotypes. Genomic sequencing was performed to generate SNP genotypes using the Illumina BeadStudio 3.2 software. The genetic analysis resulted in a designation of APOE2, APOE3 or APOE4.

3.2.5 Data Analysis

For the demographics analysis, both chi square tests and t tests were used. A chi square test was used for the categories: male, female, and total participants. A homoscedastic t test was used for the categories: age, years of education, systolic blood pressure, diastolic blood pressure, and mean arterial pressure. A linear mixed effects model was run in R to use analyze the data. In this analysis, the independent variables were time and risk group, as well as their interactions. The covariates were age at baseline, sex, and education. The dependent variable was the feature, which was calculated using this formula: feature ~ risk group + time + age at baseline + sex + education + risk group*time + (1|participant ID). The (1|participant ID) term allowed each unique individual to have their own intercept, rather than assuming a single group intercept.

Chapter 4: Results

4.1 Demographics

Table 1. Demographics for healthy, MCI, and AD participants from the ADNI database (n=721)

	Normal			MCI			AD		
	Low Risk	High Risk	P value	Low Risk	High Risk	P value	Low Risk	High Risk	P value
Male	81	30	1.294E-06	107	121	0.3538	25	61	0.0001036
Female	72	27	6.106E-06	58	67	0.4208	28	44	0.05935
Total	153	57	3.481E-11	165	188	0.2209	53	105	0.00003520
Age	78.15 (5.54)	77.38 (5.21)	3.704E-01	77.36 (8.00)	75.46 (6.80)	0.01689	76.22 (8.75)	74.54 (6.79)	0.1883
Years of Education	16.25 (2.75)	15.98 (2.78)	5.373E-01	15.75 (2.94)	15.65 (2.91)	0.7562	15.28 (3.18)	14.61 (2.96)	0.1933
Hispanic or Latino	1	1		6	5		0	1	
Not Hispanic or Latino	151	56		158	182		53	103	
Unknown	1	0		1	1		0	1	
American Indian or Alaskan Native	0	0		0	0		0	0	
Asian	2	1		6	2		2	0	
Native Hawaiian or Other Pacific Islander	0	0		0	0		0	0	
Black or African American	10	4		4	7		1	4	
White	141	52		155	198		50	100	
More than one race	0	0		0	0		0	1	
Unknown	0	0		0	0		0	0	
Systolic Blood Pressure	131.80 (14.99)	134.70 (16.41)	0.227454143	134.24 (17.43)	132.09 (16.41)	0.234504778	130.30 (17.52)	136.45 (17.51)	0.040166532
Diastolic Blood Pressure	73.70 (9.78)	73.12 (9.18)	0.701228467	73.46 (9.82)	74.15 (8.80)	0.491740378	72.49 (10.72)	74.58 (8.16)	0.177604268
Mean Arterial Pressure	93.07 (9.59)	93.65 (9.61)	0.696705051	93.72 (10.55)	93.46 (9.85)	0.810699927	91.76 (11.43)	95.20 (9.31)	0.045572025

Note: For categories: Male, Female and Total, the P value was determined through a chi square test. For categories: Age, Years of Education, Systolic Blood Pressure, Diastolic Blood Pressure and Mean Arterial Pressure, the P value was determined through a homoscedastic independent t test.

Table 1 shows the demographic breakdown for healthy participants, participants with MCI, and participants with AD. The low risk group represents e2 or e3 carriers, and the high risk group represents e4 carriers.

4.2. Linear Mixed Effects Model

4.2.1 Cognitive testing

4.2.1.1 Executive function

Figure 1. LME results for Executive Function in healthy, MCI, and AD participants from the ADNI database

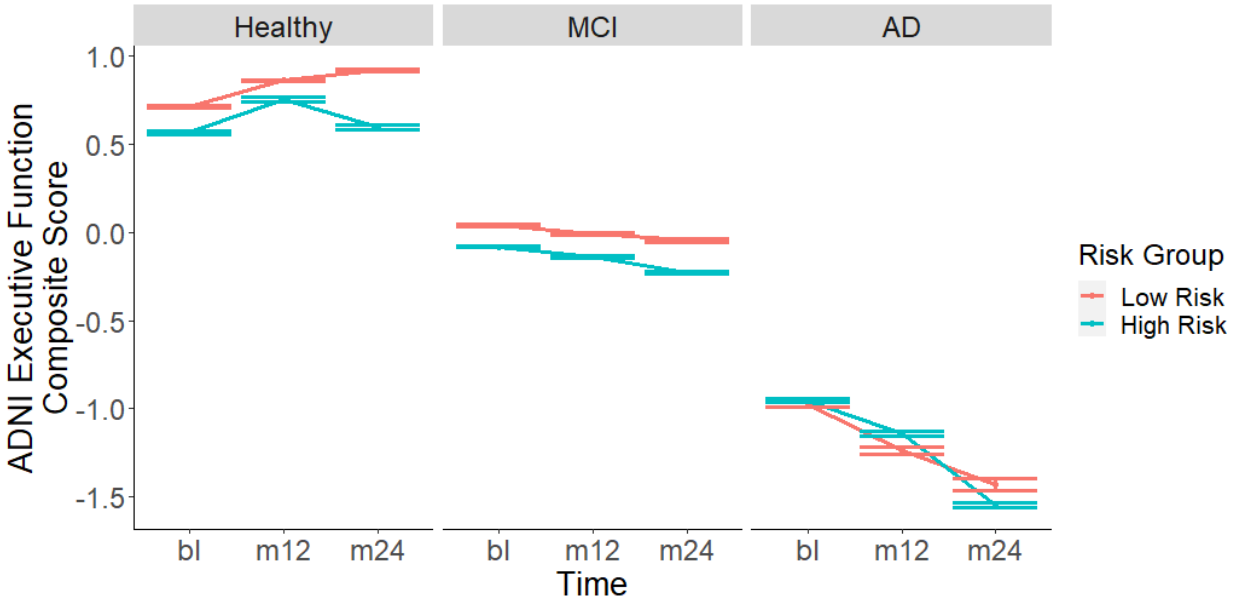

Figure 1 illustrates the LME results for executive function scores over time for healthy participants, participants with MCI, and participants with AD. The low risk group in red represents e2 or e3 carriers, and the high risk group in blue represents e4 carriers.

Table 2. LME results for Executive Function in healthy, MCI, and AD participants from the ADNI database

	Variable	Estimate	Standard Error	T Value	P value
Healthy (n=567)	Intercept	2.42094	0.651608	3.715	0.000258
	Month 12	0.139081	0.049002	2.838	0.004786
	Month 24	0.184449	0.051475	3.583	0.000384
	Age Baseline	-0.04067	0.007675	-5.298	2.87E-07
	Education	0.087029	0.015771	5.518	9.94E-08
	Risk Group e4 x Month 24	-0.17102	0.094825	-1.803	0.072131
MCI (n=925)	Age Baseline	-0.0143	0.00635	-2.246	0.025317
	Education	0.061	0.016	3.81	0.000164
	Risk Group e4 x Month 24	-0.143	0.0727	-1.964	0.050012
AD (n=394)	Intercept	-1.68633	0.851016	-1.982	0.049194
	Month 12	-0.28509	0.08237	-3.461	0.000637
	Month 24	-0.44733	0.097264	-4.599	6.82E-06
	Age Baseline	0.017481	0.009386	1.862	0.064335

Table 2 demonstrates the LME results for executive function scores for healthy participants, participants with MCI, and participants with AD. The low risk group represents e2 or e3 carriers, and the high risk group represents e4 carriers.

For healthy participants there was a significant effect of time, such that there was a significant increase of executive function scores at month 12 and 24. There was also a significant negative effect of age and a significant positive effect in education. For participants with MCI, there was a significant decrease in executive function scores due to age, but a significant increase due to education. For participants with AD, there was a significant effect of time, such that there was a significant decrease at month 12 and 24. For details of these results see Figure 1 and Table 2.

4.2.1.2 Language

Figure 2. LME results for Language in healthy, MCI, and AD participants from the ADNI database

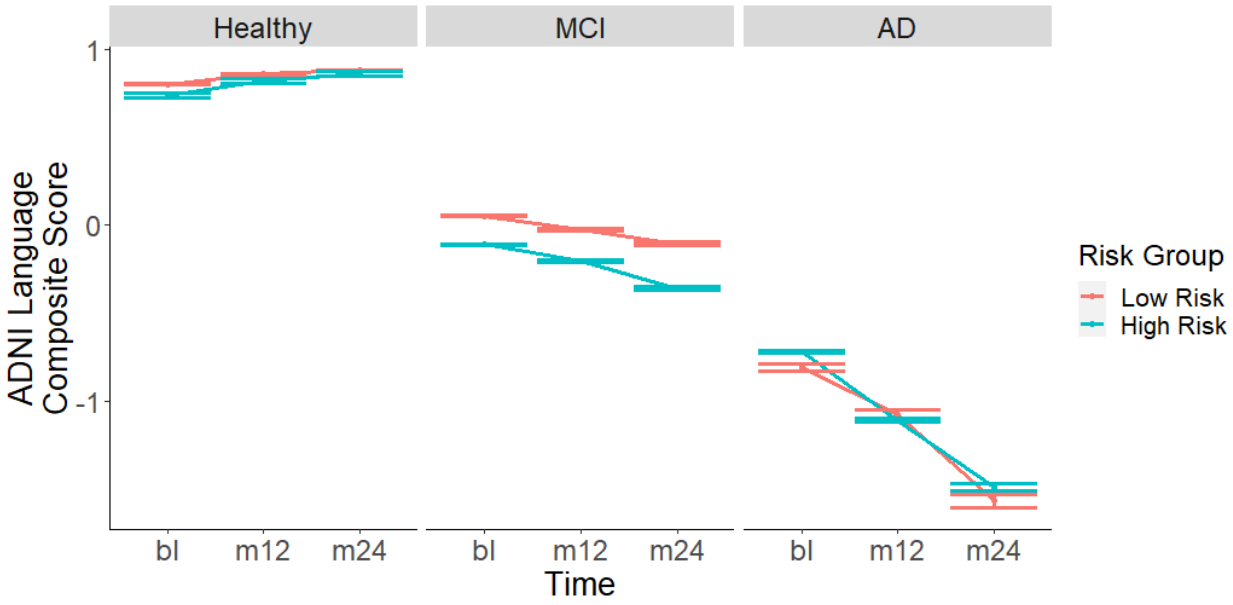

Figure 2 illustrates the LME results for language scores over time for healthy participants, participants with MCI, and participants with AD. The low risk group in red represents e2 or e3 carriers, and the high risk group in blue represents e4 carriers.

Table 3. LME results for Language in healthy, MCI, and AD participants from the ADNI database

	Variable	Estimate	Standard Error	T Value	P value
Healthy (n=567)	Intercept	1.065511	0.642698	1.658	0.0988
	Age Baseline	-0.01878	0.007571	-2.48	0.0139
	Sex (Female)	0.218454	0.086553	2.524	0.0123
	Education	0.068539	0.015573	4.401	1.71E-05
MCI (n=925)	Risk Group e4	-0.18932	0.092891	-2.038	0.0421
	Month 12	-0.07874	0.047113	-1.671	0.0952
	Month 24	-0.14533	0.052629	-2.761	0.00594
	Age Baseline	-0.01504	0.005792	-2.596	0.00982
	Education	0.057867	0.014607	3.962	9.02E-05
	Risk Group e4 x Month 24	-0.16136	0.071949	-2.243	0.02529
AD (n=394)	Month 12	-0.28291	0.103189	-2.742	0.00658
	Month 24	-0.69037	0.121653	-5.675	3.90E-08

Table 3 demonstrates the LME results for language scores for healthy participants, participants with MCI, and participants with AD. The low risk group represents e2 or e3 carriers, and the high risk group represents e4 carriers.

For healthy participants, there was a significant negative effect of age. There was also a significant increase in language scores for sex and education. For participants with MCI, there was a significant decrease in language scores for risk, such that participants with e4 scored lower than participants with e2 and e3. There was also a significant effect of time, such that there was a significant decrease at month 24. Furthermore, there was a significant negative interaction between risk and time at month 24. There was also a significant negative effect of age, and a significant positive effect of education. For participants with AD, there was a significant effect of time, such that there was a significant decrease at month 12 and 24. For details of these results see Figure 2 and Table 3.

4.2.1.3 Memory

Figure 3. LME results for Memory in healthy, MCI, and AD participants from the ADNI database

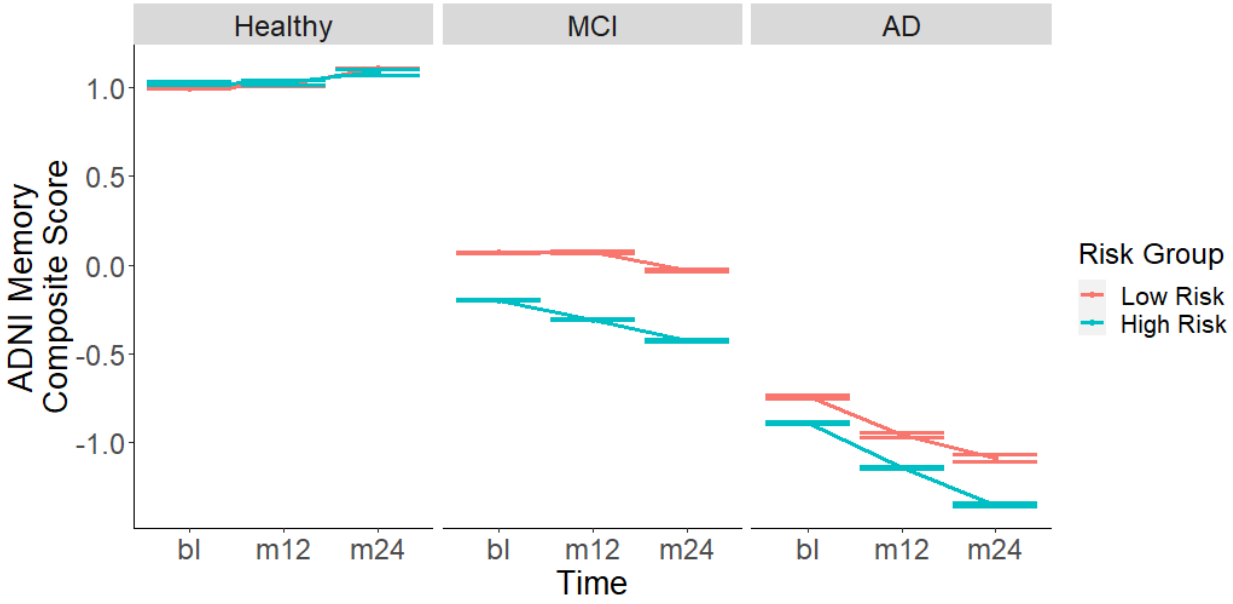

Figure 3 illustrates the LME results for memory scores over time for healthy participants, participants with MCI, and participants with AD. The low risk group in red represents e2 or e3 carriers, and the high risk group in blue represents e4 carriers.

Table 4. LME results for Memory in healthy, MCI, and AD participants from the ADNI database

	Variable	Estimate	Standard Error	T Value	P value
Healthy (n=567)	Month 24	0.102837	0.034364	2.993	0.00295
	Sex (Female)	0.419001	0.072898	5.748	3.14E-08
	Education	0.053977	0.013127	4.112	5.65E-05
MCI (n=925)	Risk Group e4	-0.27836	0.072646	-3.832	0.000146
	Month 24	-0.083	0.034899	-2.378	0.017722
	Education	0.03576	0.011684	3.061	0.002378
	Risk Group e4 x Month 12	-0.10038	0.042942	-2.338	0.019752
	Risk Group e4 x Month 24	-0.18075	0.047715	-3.788	0.000168
AD (n=394)	Month 12	-0.22463	0.054555	-4.118	5.29E-05
	Month 24	-0.33708	0.064409	-5.233	3.61E-07
	Intercept	-1.38331	0.555964	-2.488	0.0138

Table 4 demonstrates the LME results for memory scores for healthy participants, participants with MCI, and participants with AD. The low risk group represents e2 or e3 carriers, and the high risk group represents e4 carriers.

For healthy participants, there was a significant effect of time, such that there was a significant increase at month 24. There was also a significant increase in memory scores for sex and education. For participants with MCI, there was a significant negative effect of risk, such that participants with e4 scored lower than participants with e2 and e3. There was also a significant effect of time, such that there was a significant decrease at month 24. Furthermore, there was also a significant negative interaction between risk and time at month 12 and 24. There was also a significant positive effect of education. For participants with AD, there was a significant effect of time, such that there was a significant decrease at month 12 and 24. For details of these results see Figure 3 and Table 4.

4.2.1.4 Visuospatial ability

Figure 4. LME results for Visuospatial Ability in healthy, MCI, and AD participants from the ADNI database

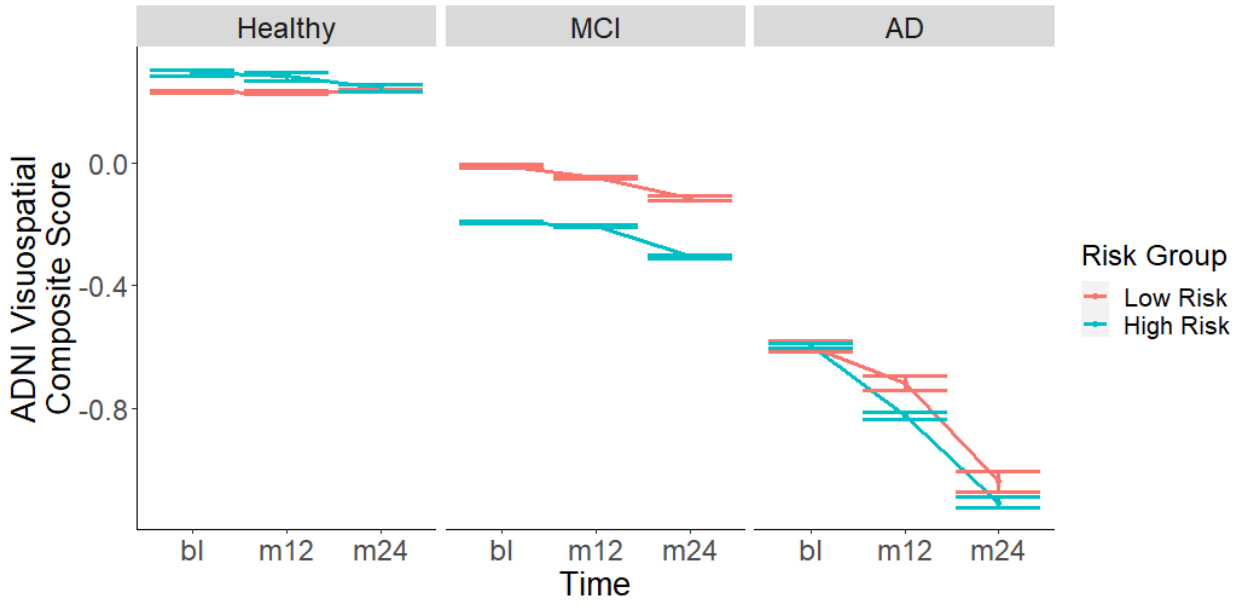

Figure 4 illustrates the LME results for visuospatial ability scores over time for healthy participants, participants with MCI, and participants with AD. The low risk group in red represents e2 or e3 carriers, and the high risk group in blue represents e4 carriers.

Table 5. LME results for Visuospatial Ability in healthy, MCI, and AD participants from the ADNI database

	Variable	Estimate	Standard Error	T Value	P value
Healthy (n=567)	Education	4.48E-02	1.23E-02	3.629	0.000361
MCI (n=925)	Intercept	-1.06448	0.431218	-2.469	0.014
	Risk Group e4	-0.17114	0.085363	-2.005	0.0454
	Education	0.049089	0.011859	4.14	4.36E-05
AD (n=394)	Intercept	-2.38748	0.854774	-2.793	0.00584
	Month 24	-0.48595	0.147489	-3.295	0.00113
	Age Baseline	0.024223	0.009409	2.575	0.01093

Table 5 demonstrates the LME results for visuospatial ability scores for healthy participants, participants with MCI, and participants with AD. The low risk group represents e2 or e3 carriers, and the high risk group represents e4 carriers.

For healthy participants, there was a significant negative effect of education on the visuospatial composite score. For participants with MCI, there was a significant negative effect of risk, shown through the decreased scores in e4 compared to e2 and e3. There was also a positive effect of education. For participants with AD, there was a significant effect of time such

that there was a significant decrease at month 24. There was also a significant positive influence of age. For details of these figures see Figure 4 and Table 5.

4.2.2 Imaging

4.2.2.1 Left Hippocampal Volume

Figure 5. LME results for Left Hippocampal Volume in healthy, MCI, and AD participants from the ADNI database

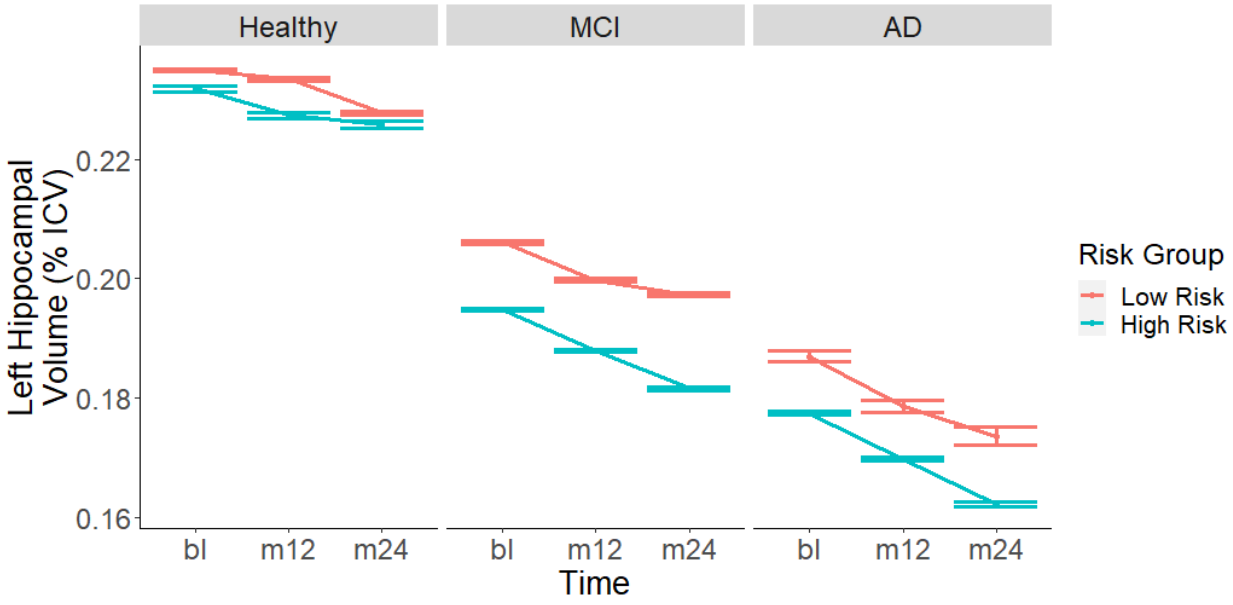

Figure 5 illustrates the LME results for left hippocampal volume over time for healthy participants, participants with MCI, and participants with AD. The low risk group in red represents e2 or e3 carriers, and the high risk group in blue represents e4 carriers.

Table 6. LME results for left hippocampal volume in healthy, MCI, and AD participants from the ADNI database

	Variable	Estimate	Standard Error	T Value	P value
Healthy (n=567)	Intercept	3.94E-01	3.17E-02	12.435	4.95E-27
	Month 24	-0.00459	9.80E-04	-4.684	4.00E-06
	Age	-0.00174	3.73E-04	-4.659	5.60E-06
	Sex (Female)	1.03E-02	4.27E-03	2.419	0.0164
	Education	-1.75E-03	7.71E-04	-2.271	0.0241
	Risk Group e4 x Month 24	-3.27E-03	1.80E-03	-1.819	0.0697
MCI (n=925)	Intercept	3.19E-01	2.23E-02	14.318	5.66E-37
	Risk Group e4	-1.39E-02	3.70E-03	-3.763	0.000195
	Month 12	-5.27E-03	9.49E-04	-5.551	4.36E-08
	Month 24	-9.12E-03	1.06E-03	-8.577	9.02E-17
	Age	-1.21E-03	2.44E-04	-4.978	1.01E-06
	Education	-1.33E-03	6.15E-04	-2.158	0.031642
	Risk Group e4 x Month 24	-2.82E-03	1.45E-03	-1.937	0.053231
AD (n=394)	Intercept	2.93E-01	2.91E-02	10.071	7.89E-19
	Risk Group e4	-1.02E-02	5.25E-03	-1.944	0.0535
	Month 12	-6.88E-03	1.32E-03	-5.226	3.84E-07
	Month 24	-1.32E-02	1.56E-03	-8.472	2.63E-15
	Age	-1.57E-03	3.21E-04	-4.899	2.31E-06

Table 6 demonstrates the LME results for left hippocampal volume for healthy participants, participants with MCI, and participants with AD. The low risk group represents e2 or e3 carriers, and the high risk group represents e4 carriers.

For healthy participants, there was a significant effect of time, such that there was a significant decrease at month 12. There was a significant decrease in left hippocampal volume for age and education. However, there was a significant positive effect of sex, such that women had a greater volume than men. For participants with MCI, there was a significant decrease in the left hippocampal volume scores for risk, such that e4 carriers had a lesser volume than e2 and e3 carriers. There was also a significant effect of time, such that there was a significant decrease at month 12 and month 24. Furthermore, there was a significant negative effect of age and education. For participants with AD, there was a significant effect of time, such that there was a

significant decrease at month 12 and month 24. Furthermore, there was a significant negative effect of age. For details of these results, see Figure 5 and Table 6.

4.2.2.2 Right Hippocampal Volume

Figure 6. LME results for Right Hippocampal Volume in healthy, MCI, and AD participants from the ADNI database

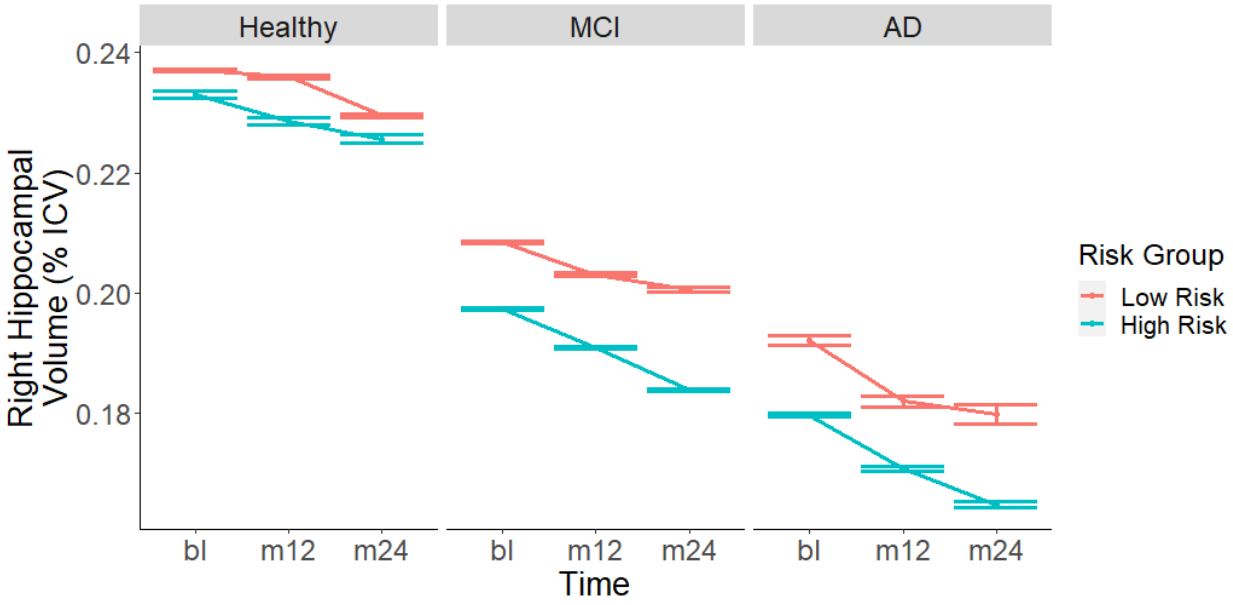

Figure 6 illustrates the LME results for right hippocampal volume over time for healthy participants, participants with MCI, and participants with AD. The low risk group in red represents e2 or e3 carriers, and the high risk group in blue represents e4 carriers.

Table 7. LME results for right hippocampal volume in healthy, MCI, and AD participants from the ADNI database

	Variable	Estimate	Standard Error	T Value	P value
Healthy (n=567)	Intercept	4.39E-01	3.16E-02	13.873	1.37E-31
	Month 24	-5.15E-03	9.64E-04	-5.339	1.66E-07
	Age	-1.99E-03	3.73E-04	-5.33	2.49E-07
	Sex (Female)	7.16E-03	4.27E-03	1.678	0.0949
	Education	-3.09E-03	7.70E-04	-4.012	8.35E-05
	Risk Group e4 x Month 24	-3.41E-03	1.77E-03	-1.927	0.0548
MCI (n=925)	Intercept	3.18E-01	2.30E-02	13.83	4.73E-35
	Risk Group e4	-1.34E-02	3.80E-03	-3.533	0.000463
	Month 12	-4.55E-03	8.98E-04	-5.067	5.47E-07
	Month 24	-9.15E-03	1.01E-03	-9.092	1.57E-18
	Age Baseline	-1.27E-03	2.52E-04	-5.029	7.87E-07
	Sex (Female)	6.74E-03	3.92E-03	1.719	0.086436
	Risk Group e4 x Month 24	-3.03E-03	1.38E-03	-2.203	0.028001
AD (n=394)	Intercept	3.01E-01	3.20E-02	9.407	4.98E-17
	Risk Group e4	-1.42E-02	5.77E-03	-2.454	0.0152
	Month 12	-7.73E-03	1.25E-03	-6.207	2.44E-09
	Month 24	-1.30E-02	1.48E-03	-8.804	2.95E-16
	Age Baseline	-1.60E-03	3.54E-04	-4.514	1.22E-05

Table 7 demonstrates the LME results for right hippocampal volume for healthy participants, participants with MCI, and participants with AD. The low risk group represents e2 or e3 carriers, and the high risk group represents e4 carriers.

For healthy participants, there was a significant effect of time in the right hippocampal volume, such that there was a significant decrease at month 24. There was also a significant negative effect of age and education. For participants with MCI, there was a significant negative effect of risk, such that e4 carriers had a lesser volume than e2 and e3 carriers. There was a significant effect of time in the right hippocampal volume, such that there was a significant decrease at month 12 and month 24. Furthermore, there was a significant negative interaction for the risk group at month 24. There was also a significant negative effect of age. For participants with AD, there was a significant negative effect of risk on right hippocampal volume. In addition there was a significant effect of time, such that there was a significant decrease at months 12 and

24. There was also a significant negative effect of age. For more details of these results, see Figure 6 and Table 7.

4.2.2.3 Total Hippocampal Volume

Figure 7. LME results for Total Hippocampal Volume in healthy, MCI, and AD participants from the ADNI database

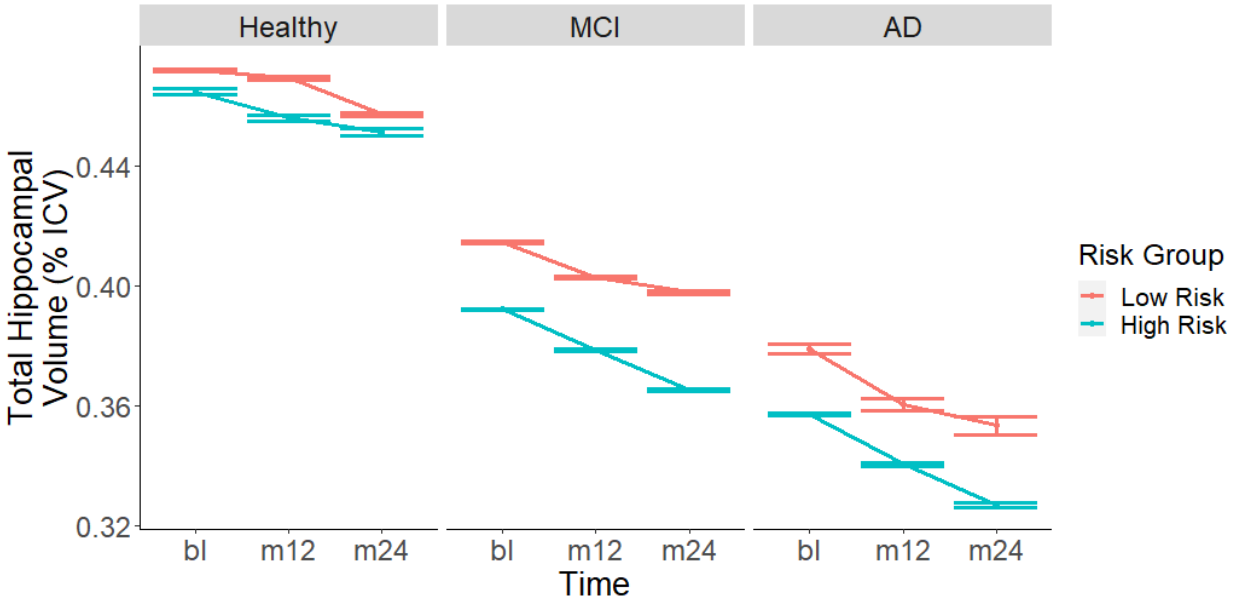

Figure 7 illustrates the LME results for total hippocampal volume over time for healthy participants, participants with MCI, and participants with AD. The low risk group in red represents e2 or e3 carriers, and the high risk group in blue represents e4 carriers.

Table 8. LME results for total hippocampal volume in healthy, MCI, and AD participants from the ADNI database

	Variable	Estimate	Standard Error	T Value	P value
Healthy (n=567)	Intercept	8.33E-01	6.08E-02	13.688	5.33E-31
	Month 24	-9.72E-03	1.78E-03	-5.457	9.09E-08
	Age	-3.73E-03	7.17E-04	-5.197	4.74E-07
	Sex	1.75E-02	8.20E-03	2.132	0.03417
	Education	-4.84E-03	1.48E-03	-3.269	0.00126
	Risk Group e4 x Month 24	-6.70E-03	3.27E-03	-2.051	0.04099
MCI (n=925)	Intercept	0.636645	0.043651	14.585	5.00E-38
	Risk Group e4	-0.02734	0.007213	-3.79	0.000175
	Month 12	-0.0098	0.001623	-6.039	2.80E-09
	Month 24	-0.01828	0.00182	-10.043	5.60E-22
	Age	-0.00248	0.000478	-5.187	3.61E-07
	Education	-0.00223	0.001206	-1.848	0.065425
	Risk Group e4 x Month 24	-0.00583	0.002489	-2.343	0.019482
AD (n=394)	Intercept	5.94E-01	5.79E-02	10.252	2.60E-19
	Risk Group e4	-2.44E-02	1.04E-02	-2.335	0.0207
	Month 12	-1.46E-02	2.19E-03	-6.668	1.85E-10
	Month 24	-2.62E-02	2.60E-03	-10.113	3.44E-20
	Age	-3.17E-03	6.40E-04	-4.952	1.84E-06

Table 8 demonstrates the LME results for left hippocampal volume for healthy participants, participants with MCI, and participants with AD. The low risk group represents e2 or e3 carriers, and the high risk group represents e4 carriers.

For healthy participants, there was a significant effect of time, such that there was a significant decrease at month 24. Furthermore, there was a significant negative interaction of the risk group at month 24. There was a significant negative effect of age and education, and a significant positive effect of sex. For participants with MCI, there was a significant negative effect of risk, such that e4 carriers had a lesser volume than e2 and e3 carriers. There was a significant effect of time, such that there was a significant decrease at month 12 and month 24. Furthermore, there was a significant negative interaction of the risk group at month 24. There was also a significant negative effect of age and education. For participants with AD, there was a significant negative effect of risk, such that e4 carriers had a lesser volume than e2 and e3

carriers. There was a significant effect of time, such that there was a significant decrease at month 12 and month 24. There was also a significant negative effect of age. For details of these results see Figure 7 and Table 8.

4.2.3 Blood biomarkers

4.2.3.1 Aβ42

Figure 8. LME results for Aβ42 in healthy, MCI, and AD participants from the ADNI database

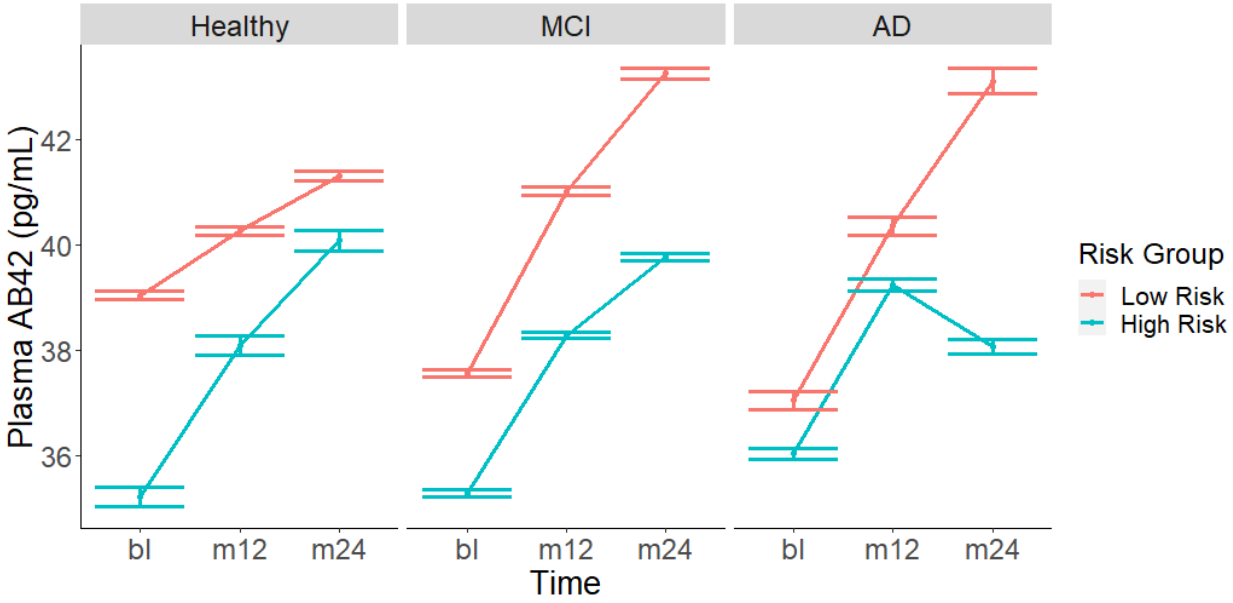

Figure 8 illustrates the LME results for Aβ42 over time for healthy participants, participants with MCI, and participants with AD. The low risk group in red represents e2 or e3 carriers, and the high risk group in blue represents e4 carriers.

Table 9. LME results for AB42 in healthy, MCI, and AD participants from the ADNI database

	Variable	Estimate	Standard Error	T Value	P value
Healthy (n=567)	Interept	21.7972	10.4741	2.081	0.038606
	Risk Group e4	-3.4134	1.6894	-2.02	0.044179
	Month 12	1.6237	0.7328	2.216	0.027312
	Month 24	2.6868	0.77	3.489	0.000543
MCI (n=925)	Intercept	15.80179	6.51216	2.427	0.0157
	Month 12	3.79067	0.64287	5.896	6.31E-09
	Month 24	5.53881	0.71743	7.72	4.97E-14
	Age Baseline	0.29162	0.07121	4.095	5.24E-05
	Sex (Female)	-2.86757	1.10993	-2.584	0.0102
AD (n=394)	Intercept	24.20145	8.3106	2.912	0.004109
	Month 12	3.47365	1.19615	2.904	0.004037
	Month 24	5.17112	1.40249	3.687	0.000279

Table 9 demonstrates the LME results for Aβ42 levels for healthy participants, participants with MCI, and participants with AD. The low risk group represents e2 or e3 carriers, and the high risk group represents e4 carriers.

For healthy participants, there was a significant negative effect of risk, such that e4 carriers had lower Aβ42 levels than e2 and e3 carriers. There was also a significant effect of time, such that there was a significant increase in levels at month 12 and 24. For participants with MCI, there was a significant effect of time, such that there was a significant increase at month 12 and 24. There was a significant positive effect of age, and a significant negative effect of sex. For participants with AD, there was a significant effect of time, such that there was a significant increase at month 12 and 24. For details of these results see Figure 8 and Table 9.

4.2.3.2 Aβ40

Figure 9. LME results for Aβ40 in healthy, MCI, and AD participants from the ADNI database

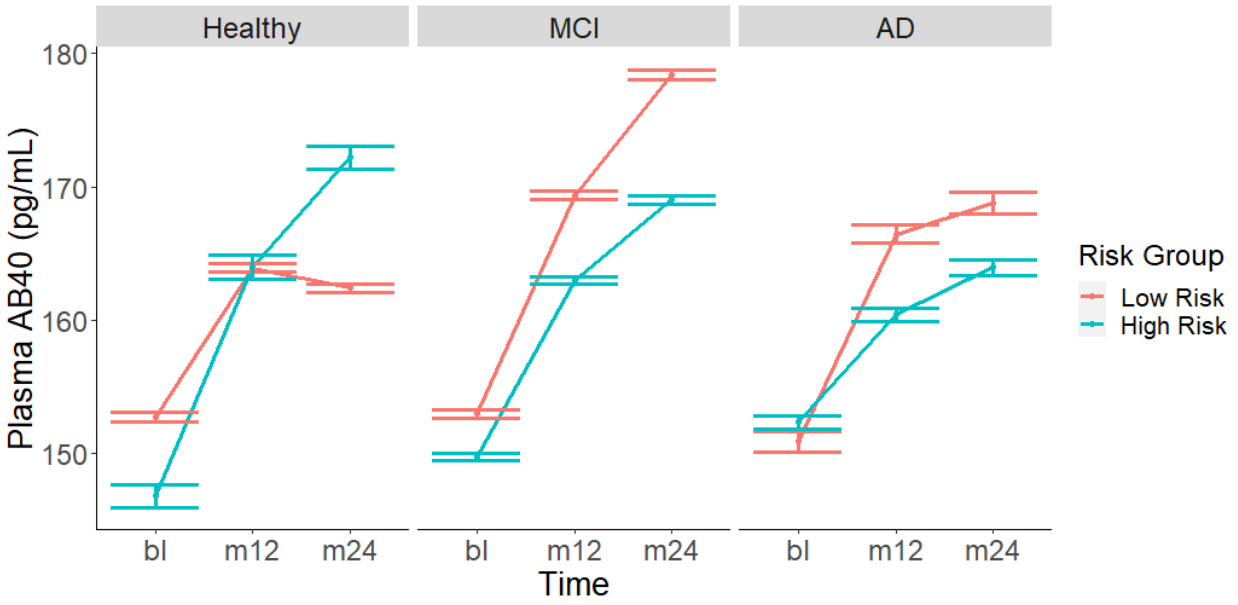

Figure 9 illustrates the LME results for Aβ40 over time for healthy participants, participants with MCI, and participants with AD. The low risk group in red represents e2 or e3 carriers, and the high risk group in blue represents e4 carriers.

Table 10. LME results for AB40 in healthy, MCI, and AD participants from the ADNI database

	Variable	Estimate	Standard Error	T Value	P value
Healthy (n=567)	Intercept	121.2001	41.499	2.921	0.00387
	Month 12	11.9336	3.7017	3.224	0.00138
	Month 24	11.5008	3.884	2.961	0.00326
	Sex (Female)	-9.5864	5.5824	-1.717	0.08742
	Risk Group e4 x	13.8357	7.1673	1.93	0.05433
MCI (n=925)	Intercept	43.9146	26.2623	1.672	0.0954
	Month 12	16.6731	3.5022	4.761	2.44E-06
	Month 24	23.3688	3.8916	6.005	3.31E-09
	Age Baseline	1.4283	0.2868	4.98	1.00E-06
	Sex (Female)	-11.1407	4.4692	-2.493	0.0131
AD (n=394)	Intercept	117.2939	35.0378	3.348	0.00102
	Month 12	15.78506	6.02739	2.619	0.0094
	Month 24	16.48535	7.0295	2.345	0.0198

Table 10 demonstrates the LME results for Aβ40 levels for healthy participants, participants with MCI, and participants with AD. The low risk group represents e2 or e3 carriers, and the high risk group represents e4 carriers.

For healthy participants, there was a significant effect of time, such that there was a significant increase at month 12 and 24. There was also a significant negative effect effect of sex. For participants with MCI, there was a significant effect of time, such that there was a significant increase at month 12 and 24. There was also a significant positive effect of age, and a significant negative effect of sex. For participants with AD, there was a significant effect of time, such that there was a significant increase at month 12 and 24. For details of these results see Figure 9 and Table 10.

4.2.3.3 Aβ42/40 ratio

Figure 10. LME results for Aβ42/40 in healthy, MCI, and AD participants from the ADNI database

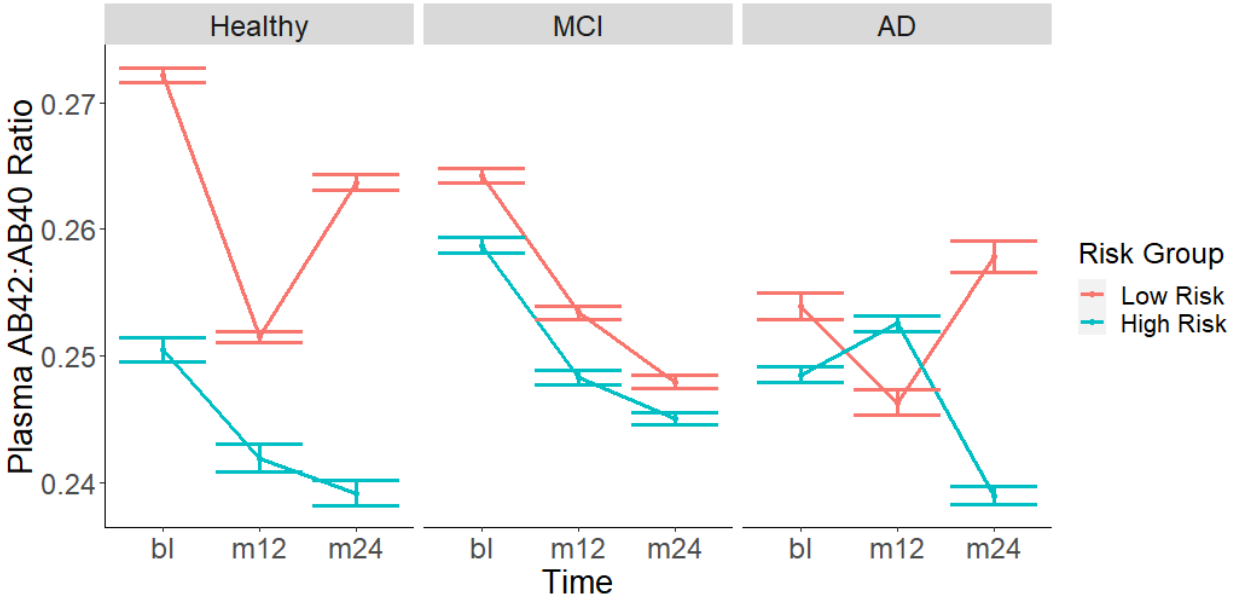

Figure 10 illustrates the LME results for the Aβ42/40 ratio over time for healthy participants, participants with MCI, and participants with AD. The low risk group in red represents e2 or e3 carriers, and the high risk group in blue represents e4 carriers.

Table 11. LME results for AB42/40 ratio in healthy, MCI, and AD participants from the ADNI database

	Variable	Estimate	Standard Error	T Value	P value
Healthy (n=567)	Intercept	2.20E-01	6.54E-02	3.357	0.000936
	Risk Group e4	-2.26E-02	1.08E-02	-2.103	0.036218
	Month 12	-1.99E-02	5.01E-03	-3.979	8.37E-05
	Month 24	-1.06E-02	5.26E-03	-2.017	0.044441
MCI (n=925)	Intercept	2.68E-01	5.47E-02	4.904	1.44E-06
	Month 12	-9.71E-03	5.59E-03	-1.736	0.083
	Month 24	-1.39E-02	6.24E-03	-2.229	0.0262
AD (n=394)	Intercept	2.47E-01	4.36E-02	5.663	6.53E-08

Table 11 demonstrates the LME results for the Aβ42/40 ratio for healthy participants, participants with MCI, and participants with AD. The low risk group represents e2 or e3 carriers, and the high risk group represents e4 carriers.

For healthy participants, there was a significant negative effect of risk, such that e4 carriers had a smaller Aβ42/40 ratio than e2 and e3 carriers. There was also a significant effect of time, such that there was a significant decrease at month 12 and month 24. For participants with MCI, there was a significant effect of time, such that there was a significant decrease at month 12 and month 24. For details of these results see Figure 10 and Table 11.

Chapter 5: Discussion

5.1 Findings

5.1.1 Cognitive testing

5.1.1.1 Executive function

Data collected on executive function for those with mild cognitive impairment and Alzheimer's disease align with findings from previous studies. Fairly recently was executive function considered when it came to Alzheimer's over the last years (Guarino et al., 2019). However, within the past twenty years, this view has changed, and more recent studies have confirmed the presence in Alzheimer's Disease of early impairment in many tasks aimed at

investigating executive functions (Guarino et al., 2019). Along with this, impairment in executive cognition is now being recognized as being relatively common among older persons with mild cognitive impairment and may be able to predict the development of dementia (Brandt et al., 2009). Through research conducted in other studies, it is observed that specific aspects of executive function have been impacted, while others appear to remain intact (Brandt et al., 2009). This goes beyond the scope of our research; however, this could be another avenue to investigate.

Though we expected executive function to be affected for participants with Alzheimer's Disease along with healthy participants and those with mild cognitive impairment, our research shows that those with Alzheimer's Disease did not show a decrease in executive function like participants in other categories. There is a possibility that other factors we did not research impacted this outcome. For example, results could have differed if we conducted further research into types of tests for executive function. Some tests could have included planning, problem solving, working memory, and judgment that had proven less reliable (Brandt et al., 2009). Selection bias could also be a contributing factor to differences we have recognized between our research and the research conducted by others. Generally speaking, the rest of our data aligned with what we expected, this being regression in executive function that comes with development of MCI and AD.

5.1.1.2 Language

A 2009 study by Snitz et al. aimed to determine the standardized effects of age, gender, race and education on language ability in older adults. Participants aged 65 years and older did two tasks assessing language ability: an animal fluency task (AFT) and the Indiana University

Token Test (IUTT). For both language tests, improved language results significantly were associated with lower age, higher education and white race. On the IUTT only, sex was also a significant factor with women performing better than men. Furthermore, the study concluded that age and education were more potent variables than race and gender (Snitz et al., 2009). Our results supported the positive effect of education and negative effect of age on language ability in healthy participants, and found that women performed better on language tasks than men. However, the impact of sex on language ability was larger than the impact of age and education. We were not able to draw conclusions about the effect of race on language ability in healthy participants due to our limited participant sample.

To test the effect of genetic risk on language ability in participants with MCI, Biundo et al. (2011) did a category fluency task with 30 participants with amnestic MCI and 22 healthy age matched cognitively healthy participants. In the task, participants were asked to produce words, and researchers compared them based on lexical characteristics such as age of acquisition, typicality and familiarity. In general, e4 carriers with MCI produced words that were earlier acquired, and that the MCI subgroup overall produced more typical words than controls, which suggests that e4 carriers with MCI and participants with MCI overall had worse language ability than the cognitively healthy and e2 or e3 carrier control group (Biundo et al., 2011). Our results supported the negative effect of the risk group on language ability in participants with MCI.

5.1.1.3 Memory

The memory scores that resulted in our data showed that APOE-e4 allele had a significant negative effect on memory for participants with MCI as well as increased decline over time for MCI individuals with the e4 risk allele. The findings in our research correlates to a

2015 study by Carrasquillo et al. which studied the association of nine risk variants from genome-wide association studies (GWAS) to memory. The study analyzed longitudinal data with n=2262 Caucasian elderly subjects for association of memory decline and incident MCI/AD with the nine GWAS variants over a five-year period and found that APOE-e4 had the strongest association with low baseline memory as well as increased decline of memory. Each subject underwent more than two clinical evaluations which included the 30-minute delayed recall scores (LMDR) and the logical memory subtests. Results showed that the APOE-inclusive risk scores were significantly associated with low memory and increased incident MCI/AD. An interesting finding for memory scores for participants with MCI was that education had a positive effect and therefore those with higher education showed less decline in memory. Although previous studies also show that higher education may be associated with higher memory scores (Carrasquillio et al., 2015), participants with higher education were younger than other participants and therefore, further analysis must be done to isolate education as a factor when studying association to memory decline in MCI participants in order to draw a significant conclusion. Our analysis also showed that for participants already diagnosed with AD, the APOE-e4 risk allele did not have a significant effect in memory. This data was supported by results from the 2015 study by Carrasquillio et al. in which the results also found that there was no APOE-e4 risk allele significance in memory decline for AD participants and therefore could not be used for prediction of AD progression. They also found that age and sex variable for prediction of progression of AD was not significant and provided no further improvement for prediction (Carrasquillo et al., 2015) which also correlates with our findings.

5.1.1.4 Visuospatial ability

The analysis of the visuospatial composite scores gave a unique insight into the cognitive function decline of different participants. Past research has indicated that changes in the brain, including size and structure, due to aging can contribute to MCI and eventually Alzheimer's. Based on the data analysis of the visuospatial composite score, multiple trends were observed. Arguably, the most important trend would be the one seen in individuals with AD. For this group, time was seen to have a negative effect on score. This correlates to past research indicating that as individuals age, their brain structure alters. Specifically, brain volume and white matter decline which leads to less brain mass which strongly influences cognitive impairment and Alzheimer's Disease (Weintraub et al., 2018). This idea of time having a strong impact on AD progression has been established, however, looking at the scores of healthy individuals also provided interesting information. Education appears to have influenced the scores negatively in healthy individuals. Knowing the information from the analysis aids in future research and directions. Focusing on time and education as possible accelerants of cognitive impairment can lead to possible solutions to slow the process and assist in limiting the chances of AD and even MCI.

5.1.2 Imaging

It is known that a decrease in hippocampal volume, assessed using MRI, is one of the most commonly used biomarkers of AD (de Flores et al., 2015; Shi et al., 2009). Diffusion tensor imaging (DTI) is often used to identify the association of microstructural changes in the hippocampus and the decline of verbal memory (Müller et al., 2005). In a study with 18 memory clinic MCI patients, as well as 18 age and gender cross-correlated healthy controls, high-

resolution MRI scans, neuropsychological physical testing, and DTI were used to identify the significance of both hippocampal volumes. The left hippocampal volume was noted to be significantly lower in MCI patients compared to the healthy control patients (Müller et al., 2005). Quantitatively, the difference was noted to value -11% with a p-value of 0.02 (Müller et al., 2005).

However, studies have shown that the extent of hippocampal atrophy tends to have more variation among individuals with MCI. Our results suggest that hippocampal atrophy may be a valid biomarker to assess both MCI and AD. Although total hippocampal atrophy was observed to be associated with the normal process of aging in all participants, the decrease in total hippocampal volume over time for participants with MCI and AD was much greater than the decrease in total hippocampal volume over time for healthy participants. This indicates that hippocampal atrophy, as an effect of normal aging, can be differentiated from the hippocampal atrophy associated with MCI and AD based on the rate of decrease.

Other studies show that the e4 allele, the risk-associated allele of the APOE gene, may be associated with increased hippocampal atrophy in healthy adults (O'Dwyer et al., 2012). This APOE gene is an important component of a signaling pathway that contributes to normal neuronal development, synaptic plasticity, and lipid transport. However, our findings do not suggest a decrease in hippocampal volume in healthy individuals who are carriers of this risk allele. It was found that left hippocampal volume showed a significant decrease in e4 carriers with MCI and right hippocampal volume showed a significant decrease in e4 carriers with AD. No significant risk group effects were observed for total hippocampal volume of any e4 carries.

These inconsistencies leave us unable to draw conclusions from the effect of carrying this risk allele.

These results do not support the “left-less-than-right” trend that has been found in other research regarding hippocampal volume for Alzheimer’s Disease (Shi et al., 2009). It appears that there is no trend as to which side of the hippocampus experiences atrophy for any of the groups, and thus it cannot be concluded that there is an asymmetrical effect causing more atrophy in the right hippocampus than the left.

5.1.3 Blood biomarkers

Amyloid-β (Aβ) in the brain is the most representative sign of AD pathology. On the other side of the blood-brain barrier, levels of Aβ have been shown to predict status in the CSF.

5.1.3.1 Aβ40

Aβ40 oligomers showed an increase in presence across multiple covariates. By being able to initially detect the presence of Aβ40, this will allow for the detection of and early diagnosis of Alzheimer’s disease (Gao, et. al, 2010). As the results indicate, over time, the presence of the Aβ40 protein did increase, however, in the healthy control low risk group, there was a drop in levels at month 24. From these results, many questions arise of how Aβ40 progresses over time, and at what point does detection of Aβ42 become more helpful in tracing cognitive decline. Furthermore, it is important to note how these levels are impacted factors, such as sex, across patients with MCI and AD.

5.1.3.2 Aβ42

The Aβ42 is typically found in senile plaques (Gato, et. al, 2010), so while it is not the best indicator for early detection of Alzheimer’s it can play a role in the disease later on. In most

healthy, MCI, and AD groups, there was an increase in levels over time. However, as seen in the high risk AD group, the Aβ42 levels do decrease at month 24. This decrease can be attributed to multiple factors, such as: protease degradation, microglial uptake, or clearance from the brain interstitial fluid to the blood (Patel, et. al, 2009). Aβ42 decrease is a common characteristic of cognitive decline, so the results, particularly for the AD high risk group, support this fact.

5.1.3.3 Aβ42/40 ratio

Given the trends seen in the detection of Aβ40 and 42, the ratio accordingly shows similar trends in behaviors of these oligomers. While in MCI low and high risk groups as well as the health high risk groups, the presence decreased over the 24 month period, the healthy and AD low risk groups saw a decrease by month 12, and an increase by month 24. For the AD high risk group, the ratio increased by month 12 and decreased by month 24. As Aβ40 increases, the ratio will decrease, which is reflected in the healthy high risk group, MCI groups and AD high risk groups for the most part. The ratio provides a tool for further analysis, by allowing one to understand which oligomer is at high levels at the moment, and what its impact is. If the ratio decreases over time, we can assume that the Aβ40 levels are higher than the Aβ42 levels, and if it increases over time, we can assume that the Aβ42 levels are greater in levels than Aβ40 and are increasing over time as well.

5.2 Equity Impact Report

It is known that people of color are disproportionately affected by Alzheimer's Disease, with Black Americans being 1.5 to 2 times more likely to develop the disease (*Data Shows Racial Disparities in Alzheimer's Disease Diagnosis between Black and White Research Study Participants*, 2021.) . However, these groups are severely underrepresented in most research

studies, including the research collected in the ADNI database. Although Alzheiemer's Disease can affect anyone, African Americans specifically have the highest prevalence of the disease of any race in the United States. These racial inequalities are perpetuated by a number of causes. Racial minorities often do not have the same level of healthcare accessibility as white Americans due to lack of health insurance or lack of transportation for example, decreasing the possibility of early detection and treatment. Currently, diagnosing Alzheimer's Disease requires an invasive CSF lumbar puncture procedure and cognitive testing, both of which are incredibly expensive without insurance. Drugs used to treat the condition, such as acetylcholinesterase inhibitors, are also incredibly expensive.

In addition to the financial burden placed on individuals diagnosed with the disease, there is an immense financial and emotional burden placed upon the caretakers of these individuals who often have to take time away from work to ensure the safety and health of their family member or friend. If family and friends are unable to assume the responsibility of being a caretaker due to their own financial obligations, it becomes necessary to hire a professional caretaker, adding to the financial burden of these families. The racial divide based on socio-economic status renders this burden more serious for families of racial minorities.

Psychological and cultural factors are also known to contribute to the perpetuation racial inequalities in healthcare and regarding the care of AD patients. White coat syndrome, or a fear to pursue medical consultation is especially prevalent among racial and cultural minorities. Additionally, cultural beliefs that loss of memory is part of the normal aging process can lead people to shy away from seeking medical consultations. Although some types of memory, such as time-based prospective memory, are known to deteriorate in the normal aging process, the

memory loss and personality changes associated with Alzheimer's Disease are distinct (Transparent Meta-Analysis of Prospective Memory and Aging - PMC, n.d.). Awareness of the features that differentiate normal aging from dementia among immigrant communities and communities with low education resources must be increased. This is something that our team worked to do through sharing the knowledge of Alzheimer's Disease and our research with members of the community through virtual presentations.

It is imperative that this paradox, the fact that the most under researched populations are affected most prominently by AD, be resolved with future research. It was our goal to acquire a sample of participants that would be as diverse as possible, both racially and in terms of socio-economic status in order to ensure that the results can be generalizable to everyone. However, given the circumstances of the COVID-19 pandemic, the team was unable to proceed with plans of in-person testing and recruitment of a diverse pool of participants. We tried to maximize the diversity of our sample from the ADNI database, but our sample was still overwhelmingly composed of white majority population given the limitation of relying upon a pre-existing database. Unfortunately, this means that our results are not necessarily an accurate depiction of the cognitive, memory, language, imaging, and biomarker changes that may result in minority populations.

Since control of the diversity of the sample was limited by the diversity of the database's sample, the team decided to reach out to these underrepresented populations in other ways in an effort to increase educational awareness of Alzheimer's Disease. In November of 2021, the team virtually presented "Alzheimer's Disease In a Nutshell" at the weekly "Kickstart Your Health" program organized by the African American Health Program of Montgomery County, Maryland

via Zoom. Accompanied by the program director, nurses, physicians and about 20 geratric community members, mainly of African American background, members of the team presented an informational session introducing Alzheimer's disease, its importance, causative risks, signs and symptoms, preventative tactics including nutritional diets and healthy lifestyle options, disproportionate racial impact, as well as a synopsis of the current research collected at that time. Additionally, the team held a discussion portion questioning experiences with clinical research. The team was met with an overwhelming response from the audience regarding personalized experiences about friends with the disease, care-taking concerns, hesitancy in participating in clinical studies and further questions about how to better prepare themselves. Due to the lack of medical credentials amongst the team, a few questions and concerns were also answered by the physicians who offered clinical advice to the grateful participants. Despite the pandemic, this opportunity allowed us to interact with community members and apply our research for greater use to educate and positively impact our local community. The team was well thanked and requested to return and present our final results at a future program event.

5.3 Limitations

The main limitation in this research project arises from the reliance on ADNI for data. One limitation, as discussed in the Equity Impact Report, is the lack of diversity in the sample of participants collected by the ADNI initiative. We were unable to draw conclusions about cognitive, imaging and biomarker changes that may result in minority populations.

The cross sectional design of the study also resulted in limitations. The ADNI database consists of several individual studies which look at different biomarkers in different participants. In conducting our analysis, our aim was to see the big picture of cognitive function, brain

imaging and blood biomarkers. In order to achieve this, we only included participants who had been included in the analysis of each of these markers, which limited our participant population. There were many participants who had only a few of the markers we were interested in who we elected not to include in our analysis. Because of the lack of participant overlap with other variables, we also were unable to include some markers we hoped to analyze.

In particular, there was limited access to further biomarkers, such as insulin and Tau protein. These biomarkers are key in Alzheimer's pathology, and would allow us to form a more complete model in our data analysis. Furthermore, there was limited access to other imaging, such as PET, fMRI, dMRI, sMRI and CT. This imaging, available in different datasets in ADNI, looked at brain areas other than the hippocampus, in collected different data other than volume.

Lastly, the ADNI database did not include key lifestyle factors in the analysis. There was a physical function category that did not overlap with our participants, but we didn't have access to information about physical activity, diet, sleep, socialization, etc.

5.4 Future Directions

The research that the team has performed over the past three years was conducted with the ultimate goal of finding possible trends that may increase accessibility to Alzheimer's Disease diagnosis and care. It is our hope that with more research on risk factors and biomarkers of Alzheimer's Disease, the medical community will get closer to finding a method of predicting and/or diagnosing the condition that is far less invasive and more affordable than the methods that exist today.

We hope that in the future with more time and resources, we are able to identify important lifestyle factors that can help in eliminating or decreasing the development of

Alzheimer's Disease and MCI. With less limitations, we would be able to collect original research data, and diversify our research population. We also hope to expand the research on blood biomarkers, as some biomarkers contain additional factors that may affect how they present MCI and ADI to us during analysis. With more time we could also initiate a study on lifestyle factors, such as physical exercise, occupation or geographical location, to correlate with AD development. Another future direction is to apply machine learning to the data found in the ADNI database and original research data.

When it comes to impacting the community, we hope to be able to implement further outreach initiatives and raise awareness to MCI and AD to those populations that are at higher risk. This can be done through social media, and in-person visits to facilities of the middle-aged and elderly. In the future we can determine more affordable, accessible, and less invasive predictive/diagnostic methods. Hopefully through this, we will be able to also encourage clinical research participation, and obtain results that are representative of a more diverse population.

Bibliography

An, Y., Varma, V. R., Varma, S., Casanova, R., Dammer, E., Pletnikova, O., … Thambisetty, M. (2018). Evidence for Brain Glucose Dysregulation in Alzheimer's Disease. *Alzheimer's & Dementia*, *14*(3), 318–329. https://doi.org/10.1016/j.jalz.2017.09.011

Awada, A. A. (2015). Early and Late-Onset Alzheimer's Disease: What are the Differences? *Journal of Neurosciences in Rural Practice*, *6*(3), 455–456. https://doi.org/10.4103/0976-3147.154581

Apostolova, L. G., Dinov, I. D., Dutton, R. A., Hayashi, K. M., Toga, A. W., Cummings, J. L., & Thompson, P. M. (2006). 3D comparison of hippocampal atrophy in amnestic mild cognitive impairment and Alzheimer's disease. *Brain*, *129*(11), 2867–2873. https://doi.org/10.1093/brain/awl274

Bastos-Leite António J., van der Flier Wiesje M., van Straaten Elisabeth C.W., Staekenborg Salka S., Scheltens Philip, & Barkhof Frederik. (2007). The Contribution of Medial Temporal Lobe Atrophy and Vascular Pathology to Cognitive Impairment in Vascular Dementia. *Stroke*, *38*(12), 3182–3185. https://doi.org/10.1161/STROKEAHA.107.490102

Bateman, R. J., Xiong, C., Benzinger, T. L. S., Fagan, A. M., Goate, A., Fox, N. C., … Morris, J. C. (2012). Clinical and Biomarker Changes in Dominantly Inherited Alzheimer's Disease. *New England Journal of Medicine*, *367*(9), 795–804. https://doi.org/10.1056/NEJMoa1202753

Beltrán, J. F., Wahba, B. M., Hose, N., Shasha, D., Kline, R. P., & Alzheimer's Disease Neuroimaging Initiative. (2020). Inexpensive, non-invasive biomarkers predict Alzheimer transition using machine learning analysis of the Alzheimer's Disease Neuroimaging (ADNI) database. *PloS One*, *15*(7), e0235663. {Updating}

Beta-amyloid and the amyloid hypothesis. (2017). Alzheimer's Association.

https://www.alz.org/national/documents/topicsheet_betaamyloid.pdf

Bi, T. M., & Daggett, V. (2018). The Role of α-sheet in Amyloid Oligomer Aggregation and Toxicity. *The Yale Journal of Biology and Medicine, 91*(3), 247–255.*Biomarkers Consortium Project: Use of Targeted Multiplex Proteomic Strategies to Identify Plasma-Based Biomarkers in Alzheimer's Disease*. (2010). http://adni.loni.usc.edu/wp-content/uploads/2010/11/BC_Plasma_Proteomics_Data_Primer.pdf

Biundo, R., Gardini, S., Caffarra, P., Concari, L., Martorana, D., Neri, T. M., Shanks, M. F., & Venneri, A. (2011). Influence of APOE Status on Lexical–Semantic Skills in Mild Cognitive Impairment. *Journal of the International Neuropsychological Society*, *17*(3), 423–430. https://doi.org/10.1017/S135561771100021X

Brandt, J., Aretouli, E., Neijstrom, E., Samek, J., Manning, K., Albert, M. S., & Bandeen-Roche, K. (2009). Selectivity of executive function deficits in mild cognitive impairment. Neuropsychology, 23(5), 607–618. https://doi.org/10.1037/a0015851

Byun, M. S., Kim, H. J., Yi, D., Choi, H. J., Baek, H., Lee, J. H., … KBASE Research Group. (2017). Differential Effects of Blood Insulin and HbA1c on Cerebral Amyloid Burden and Neurodegeneration in Nondiabetic Cognitively Normal Older Adults. *Neurobiology of Aging*, *59*, 15–21. https://doi.org/10.1016/j.neurobiolaging.2017.07.004

Calero, M., Rostagno, A., Matsubara, E., Zlokovic, B., Frangione, B., & Ghiso, J. (2000, July 27). Apolipoprotein J (clusterin) and Alzheimer's disease. *Microscopy Research and Technique*, Retrieved October 5, 2019, from https://onlinelibrary.wiley.com/doi/abs/10.1002/1097-0029(20000815)50:4<305::AID-JEMT10>3.0.CO;2-L

Calsolaro, V., & Edison, P. (2016). Alterations in Glucose Metabolism in Alzheimer's Disease. *Recent*

Patents on Endocrine, Metabolic & Immune Drug Discovery, *10*(1), 31–39.

Carrasquillo, M. M., Crook, J. E., Pedraza, O., Thomas, C. S., Pankratz, V. S., Allen, M., Nguyen, T., Malphrus, K. G., Ma, L., Bisceglio, G. D., Roberts, R. O., Lucas, J. A., Smith, G. E., Ivnik, R. J., Machulda, M. M., Graff-Radford, N. R., Petersen, R. C., Younkin, S. G., & Ertekin-Taner, N. (2015). Late-onset alzheimer's risk variants in memory decline, incident mild cognitive impairment, and alzheimer's disease. *Neurobiology of Aging*, *36*(1), 60–67. https://doi.org/10.1016/j.neurobiolaging.2014.07.042

Cavedo, E., Lista, S., Khachaturian, Z., Aisen, P., Amouyel, P., Herholz, K., … Hampel, H. (2014). The Road Ahead to Cure Alzheimer's Disease: Development of Biological Markers and Neuroimaging Methods for Prevention Trials Across all Stages and Target Populations. *The Journal of Prevention of Alzheimer's Disease*, *1*(3), 181–202. https://doi.org/10.14283/jpad.2014.32

Chan, D., Fox, N. C., Scahill, R. I., Crum, W. R., Whitwell, J. L., Leschziner, G., … Rossor, M. N. (2001). Patterns of Temporal Lobe Atrophy in Semantic Dementia and Alzheimer's Disease. *Annals of Neurology*, *49*(4), 433–442. https://doi.org/10.1002/ana.92

Chiaravalloti, A., Koch, G., Toniolo, S., Belli, L., Lorenzo, F. D., Gaudenzi, S., … Martorana, A. (2016). Comparison between Early-Onset and Late-Onset Alzheimer's Disease Patients with Amnestic Presentation: CSF and 18F-FDG PET Study. *Dementia and Geriatric Cognitive Disorders Extra*, *6*(1), 108–119. https://doi.org/10.1159/000441776

Choi, S.-E., Mukherjee, S., Gibbons, L. E., Sanders, R. E., Jones, R. N., Tommet, D., Mez, J., Trittschuh, E. H., Saykin, A., Lamar, M., Rabin, L., Foldi, N. S., Sikkes, S., Jutten, R. J., Grandoit, E., Mac Donald, C., Risacher, S., Groot, C., Ossenkoppele, R., … Crane, P. K. (2020).

Development and validation of language and visuospatial composite scores in ADNI. *Alzheimer's & Dementia (New York, N. Y.)*, *6*(1), e12072. https://doi.org/10.1002/trc2.12072

Crane, P. K., Carle, A., Gibbons, L. E., Insel, P., Mackin, R. S., Gross, A., Jones, R. N., Mukherjee, S., Curtis, S. M., Harvey, D., Weiner, M., Mungas, D., & Alzheimer's Disease Neuroimaging Initiative. (2012). Development and assessment of a composite score for memory in the Alzheimer's Disease Neuroimaging Initiative (ADNI). *Brain Imaging and Behavior*, *6*(4), 502–516. https://doi.org/10.1007/s11682-012-9186-z

Crous-Bou, M., Minguillón, C., Gramunt, N., & Molinuevo, J. L. (2017). Alzheimer's Disease Prevention: From Risk Factors to Early Intervention. *Alzheimer's Research & Therapy*, *9*(1), 71. https://doi.org/10.1186/s13195-017-0297-z

Cunningham, E., McGuinness, B., Herron, B., & Passmore, A. (2015). Dementia. *The Ulster Medical Journal*, *84*(2), 79–87.

De Flores, R., La Joie, R., & Chételat, G. (2015). Structural imaging of hippocampal subfields in healthy aging and Alzheimer's disease. *Neuroscience*, *309*, 29–50. https://doi.org/10.1016/j.neuroscience.2015.08.033

De Roeck, E. E., De Deyn, P. P., Dierckx, E., & Engelborghs, S. (2019). Brief Cognitive Screening Instruments for Early Detection of Alzheimer's Disease: A Systematic Review. *Alzheimer's Research & Therapy*, *11*(1), 21. https://doi.org/10.1186/s13195-019-0474-3

Du, X., Wang, X., & Geng, M. (2018). Alzheimer's Disease Hypothesis and Related Therapies. *Translational Neurodegeneration*, *7*(1), 2. https://doi.org/10.1186/s40035-018-0107-y

Duan, Y., Dong, S., Gu, F., Hu, Y., & Zhao, Z. (2012). Advances in the Pathogenesis of Alzheimer's Disease: Focusing on Tau-Mediated Neurodegeneration. *Translational Neurodegeneration*, *1*(1),

24. https://doi.org/10.1186/2047-9158-1-24

Dubois, B., & Albert, M. L. (2004). Amnestic MCI or Prodromal Alzheimer's disease? *The Lancet Neurology*, *3*(4), 246–248. https://doi.org/10.1016/S1474-4422(04)00710-0

Eke, C. S., Jammeh, E., Li, X., Carroll, C., Pearson, S., & Ifeachor, E. (2021). Early Detection of Alzheimer's Disease with Blood Plasma Proteins Using Support Vector Machines. *IEEE Journal of Biomedical and Health Informatics*, *25*(1), 218–226. https://doi.org/10.1109/JBHI.2020.2984355

Fandos, N., Pérez-Grijalba, V., Pesini, P., Olmos, S., Bossa, M., Villemagne, V. L., … AIBL Research Group. (2017). Plasma Amyloid β 42/40 Ratios as Biomarkers for Amyloid β Cerebral Deposition in Cognitively Normal Individuals. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring*, *8*(1), 179–187. https://doi.org/10.1016/j.dadm.2017.07.004

Flicker, L. (2010, May 26). *Modifiable Lifestyle Risk Factors for Alzheimer's Disease—IOS Press*. https://content.iospress.com/articles/journal-of-alzheimers-disease/jad091624

Folstein, M. F., Folstein, S. E., & McHugh, P. R. (1975). "Mini-Mental State": A Practical Method for Grading the Cognitive State of Patients for the Clinician. *Journal of Psychiatric Research*, *12*(3), 189–198. https://doi.org/10.1016/0022-3956(75)90026-6

Frisardi, V., Panza, F., Seripa, D., Farooqui, T., & Farooqui, A. A. (2011). Glycerophospholipids and Glycerophospholipid-Derived Lipid Mediators: A Complex Meshwork in Alzheimer's Disease Pathology. *Progress in Lipid Research*, *50*(4), 313–330. https://doi.org/10.1016/j.plipres.2011.06.001

Gallaway, P. J., Miyake, H., Buchowski, M. S., Shimada, M., Yoshitake, Y., Kim, A. S., & Hongu, N. (2017). Physical Activity: A Viable Way to Reduce the Risks of Mild Cognitive Impairment,

Alzheimer's Disease, and Vascular Dementia in Older Adults. *Brain Sciences*, *7*(2). https://doi.org/10.3390/brainsci7020022

Gao, C. M., Yam, A. Y., Wang, X., Magdangal, E., Salisbury, C., Peretz, D., Zuckermann, R. N., Connolly, M. D., Hansson, O., Minthon, L., Zetterberg, H., Blennow, K., Fedynyshyn, J. P., & Allauzen, S. (2010). Ab40 Oligomers Identified as a Potential Biomarker for the Diagnosis of Alzheimer's Disease. *PLoS ONE*, *5*(12), 7.

Gao, Y., Tan, L., Yu, J.-T., & Tan, L. (2018). Tau in Alzheimer's Disease: Mechanisms and Therapeutic Strategies. *Current Alzheimer Research*, *15*(3), 283–300. https://doi.org/10.2174/1567205014666170417111859

Gauthier, S., Reisberg, B., Zaudig, M., Petersen, R. C., Ritchie, K., Broich, K., Belleville, S., Brodaty, H., Bennett, D., Chertkow, H., Cummings, J. L., de Leon, M., Feldman, H., Ganguli, M., Hampel, H., Scheltens, P., Tierney, M. C., Whitehouse, P., Winblad, B., & International Psychogeriatric Association Expert Conference on mild cognitive impairment. (2006). Mild cognitive impairment. *Lancet (London, England)*, *367*(9518), 1262–1270. https://doi.org/10.1016/S0140-6736(06)68542-5

Guarino, A., Favieri, F., Boncompagni, I., Agostini, F., Cantone, M., & Casagrande, M. (2019). Executive Functions in Alzheimer Disease: A Systematic Review. Frontiers in aging neuroscience, 10, 437. https://doi.org/10.3389/fnagi.2018.00437

Gibbons, L. E., Carle, A. C., Mackin, R. S., Harvey, D., Mukherjee, S., Insel, P., Curtis, S. M., Mungas, D., Crane, P. K., & Alzheimer's Disease Neuroimaging Initiative. (2012). A composite score for executive functioning, validated in Alzheimer's Disease Neuroimaging Initiative (ADNI) participants with baseline mild cognitive impairment. *Brain Imaging and Behavior*, *6*(4), 517–

527. https://doi.org/10.1007/s11682-012-9176-1

Guo, T., Noble, W., & Hanger, D. P. (2017). Roles of Tau Protein in Health and Disease. *Acta Neuropathologica*, *133*(5), 665–704. https://doi.org/10.1007/s00401-017-1707-9

Gupta, V. B., Doecke, J. D., Hone, E., Pedrini, S., Laws, S. M., Thambisetty, M., … Martins, R. N. (2016). Plasma Apolipoprotein J as a Potential Biomarker for Alzheimer's Disease: Australian Imaging, Biomarkers and Lifestyle Study of Aging. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring*, *3*, 18–26. https://doi.org/10.1016/j.dadm.2015.12.001

Han, S.-H., Park, J.-C., Byun, M. S., Yi, D., Lee, J. H., Lee, D. Y., & Mook-Jung, I. (2019). Blood Acetylcholinesterase Level is a Potential Biomarker for the Early Detection of Cerebral Amyloid Deposition in Cognitively Normal Individuals. *Neurobiology of Aging*, *73*, 21–29. https://doi.org/10.1016/j.neurobiolaging.2018.09.001

Hang, J. (2019). Alzheimer Disease. In *Neurologic Disorders*. *Merck Manual*. Retrieved from https://www.merckmanuals.com/professional/neurologic-disorders/delirium-and-dementia/alzheimer-disease

Himmelstein, D. S., Ward, S. M., Lancia, J. K., Patterson, K. R., & Binder, L. I. (2012). Tau as a therapeutic target in neurodegenerative disease. *Pharmacology & Therapeutics*, *136*(1), 8–22. https://doi.org/10.1016/j.pharmthera.2012.07.001

Hishikawa, D., Hashidate, T., Shimizu, T., & Shindou, H. (2014). Diversity and Function of Membrane Glycerophospholipids Generated by the Remodeling Pathway in Mammalian Cells. *Journal of Lipid Research*, *55*(5), 799–807. https://doi.org/10.1194/jlr.R046094

Hoveida, R., Alaei, H., Oryan, S., Parivar, K., & Reisi, P. (2011). Treadmill running improves spatial memory in an animal model of Alzheimer's disease. Behavioural Brain Research, 216(1), 270–

274. https://doi.org/10.1016/j.bbr.2010.08.003

Hsu, J.-L., Lee, W.-J., Liao, Y.-C., Wang, S.-J., & Fuh, J.-L. (2017). The Clinical Significance of Plasma Clusterin and Aβ in the Longitudinal Follow-Up of Patients with Alzheimer's Disease. *Alzheimer's Research & Therapy*, *9*(1), 91. https://doi.org/10.1186/s13195-017-0319-x

Hu, H., Chen, K.-L., Ou, Y.-N., Cao, X.-P., Chen, S.-D., Cui, M., Dong, Q., Tan, L., Yu, J.-T., & Alzheimer's Disease Neuroimaging Initiative. (2019). Neurofilament light chain plasma concentration predicts neurodegeneration and clinical progression in nondemented elderly adults. *Aging*, *11*(17), 6904–6914. https://doi.org/10.18632/aging.102220

James, O. G., Doraiswamy, P. M., & Borges-Neto, S. (2015). PET Imaging of Tau Pathology in Alzheimer's Disease and Tauopathies. *Frontiers in Neurology*, *6*. https://doi.org/10.3389/fneur.2015.00038

Johansson, J. (2005). Amyloid fibrils. *The FEBS Journal*, *272*(23), 5941–5941. https://doi.org/10.1111/j.1742-4658.2005.05026.x

Jouanne, M., Rault, S., & Voisin-Chiret, A.-S. (2017). Tau Protein Aggregation in Alzheimer's Disease: An Attractive Target for the Development of Novel Therapeutic Agents. *European Journal of Medicinal Chemistry*, *139*, 153–167. https://doi.org/10.1016/j.ejmech.2017.07.070

Karantzoulis, S., & Galvin, J. E. (2011). Distinguishing Alzheimer's Disease from Other Major Forms of Dementia. *Expert Review of Neurotherapeutics*, *11*(11), 1579–1591. https://doi.org/10.1586/ern.11.155

Koch, M., DeKosky, S. T., Fitzpatrick, A. L., Furtado, J. D., Lopez, O. L., Kuller, L. H., … Jensen, M. K. (2018). Apolipoproteins and Alzheimer's Pathophysiology. *Alzheimer's & Dementia : Diagnosis, Assessment & Disease Monitoring*, *10*, 545–553.

https://doi.org/10.1016/j.dadm.2018.07.001

Kosicek, M., & Hecimovic, S. (2013). Phospholipids and Alzheimer's Disease: Alterations, Mechanisms and Potential Biomarkers. *International Journal of Molecular Sciences*, *14*(1), 1310–1322. https://doi.org/10.3390/ijms14011310

Laakso, M. P., Partanen, K., Riekkinen, P., Lehtovirta, M., Helkala, E. L., Hallikainen, M., Hanninen, T., Vainio, P., & Soininen, H. (1996). Hippocampal volumes in Alzheimer's disease, Parkinson's disease with and without dementia, and in vascular dementia: An MRI study. *Neurology*, *46*(3), 678–681. https://doi.org/10.1212/wnl.46.3.678

Lane, C. A., Hardy, J., & Schott, J. M. (2018a). Alzheimer's Disease. *European Journal of Neurology*, *25*(1), 59–70. https://doi.org/10.1111/ene.13439

Lanoiselée, H.-M., Nicolas, G., Wallon, D., Rovelet-Lecrux, A., Lacour, M., Rousseau, S., … Campion, D. (2017). APP, PSEN1, and PSEN2 Mutations in Early-Onset Alzheimer Disease: A Genetic Screening Study of Familial and Sporadic Cases. *PLoS Medicine*, *14*(3). https://doi.org/10.1371/journal.pmed.1002270

Laske, C., Leyhe, T., Stransky, E., Hoffmann, N., Fallgatter, A. J., & Dietzsch, J. (2011). Identification of a blood-based biomarker panel for classification of Alzheimer's disease. *International Journal of Neuropsychopharmacology*, *14*(9), 1147–1155. https://doi.org/10.1017/S1461145711000459

Lehmann, S., Delaby, C., Boursier, G., Catteau, C., Ginestet, N., Tiers, L., … Gabelle, A. (2018). Relevance of Aβ42/40 Ratio for Detection of Alzheimer Disease Pathology in Clinical Routine: The PLMR Scale. *Frontiers in Aging Neuroscience*, *10*, 138. https://doi.org/10.3389/fnagi.2018.00138

Lewczuk, P., Ermann, N., Andreasson, U., Schultheis, C., Podhorna, J., Spitzer, P., Maler, J. M.,

Kornhuber, J., Blennow, K., & Zetterberg, H. (2018). Plasma Neurofilament Light as a Potential Biomarker of Neurodegeneration in Alzheimer's Disease. *Alzheimer's Research & Therapy*, *10*(1), 71. https://doi.org/10.1186/s13195-018-0404-9

Liang, K. Y., Mintun, M. A., Fagan, A. M., Goate, A. M., Bugg, J. M., Holtzman, D. M., … Head, D. (2010). Exercise and Alzheimer's Disease Biomarkers in Cognitively Normal Older Adults. *Annals of Neurology*, *68*(3), 311–318. https://doi.org/10.1002/ana.22096

Lin, J. S., O'Connor, E., Rossom, R. C., Perdue, L. A., & Eckstrom, E. (2013). Screening for Cognitive Impairment in Older Adults: A Systematic Review for the U.S. Preventive Services Task Force. *Annals of Internal Medicine*, *159*(9), 601–612. https://doi.org/10.7326/0003-4819-159-9-201311050-00730

Liu, C.-C., Kanekiyo, T., Xu, H., & Bu, G. (2013, January 8). *Apolipoprotein E and Alzheimer disease: Risk, mechanisms and therapy | Nature Reviews Neurology*. https://www.nature.com/articles/nrneurol.2012.263

Marden, J. R., Mayeda, E. R., Tchetgen Tchetgen, E. J., Kawachi, I., & Glymour, M. M. (2017). High Hemoglobin A1c and Diabetes Predict Memory Decline in the Health and Retirement Study. *Alzheimer Disease & Associated Disorders*, *31*(1), 48–54. https://doi.org/10.1097/WAD.0000000000000182

Mattsson, N., Cullen, N. C., Andreasson, U., Zetterberg, H., & Blennow, K. (2019). Association Between Longitudinal Plasma Neurofilament Light and Neurodegeneration in Patients With Alzheimer Disease. *JAMA Neurology*, *76*(7), 791–799. https://doi.org/10.1001/jamaneurol.2019.0765

Metcalfe, M. J., & Figueiredo-Pereira, M. E. (2010). Relationship Between Tau Pathology and

Neuroinflammation in Alzheimer's Disease. *Mount Sinai Journal of Medicine: A Journal of Translational and Personalized Medicine*, *77*(1), 50–58. https://doi.org/10.1002/msj.20163

Mendez, M. F. (2017). Early-Onset Alzheimer Disease. *Neurologic Clinics*, *35*(2), 263–281. https://doi.org/10.1016/j.ncl.2017.01.005

Mielke, M. M., & Lyketsos, C. G. (2010). Alterations of the Sphingolipid Pathway in Alzheimer's Disease: New Biomarkers and Treatment Targets? *Neuromolecular Medicine*, *12*(4), 331–340. https://doi.org/10.1007/s12017-010-8121-y

Mielke, M. M., Wiste, H. J., Weigand, S. D., Knopman, D. S., Lowe, V. J., Roberts, R. O., Geda, Y. E., Swenson-Dravis, D. M., Boeve, B. F., Senjem, M. L., Vemuri, P., Petersen, R. C., & Jack, C. R. (2012). Indicators of amyloid burden in a population-based study of cognitively normal elderly. *Neurology*, *79*(15), 1570–1577. https://doi.org/10.1212/WNL.0b013e31826e2696

Milojevic, J., & Melacini, G. (2011). Stoichiometry and Affinity of the Human Serum Albumin-Alzheimer's Aβ Peptide Interactions. *Biophysical Journal*, *100*(1), 183–192. https://doi.org/10.1016/j.bpj.2010.11.037

Morris, M. C., Tangney, C. C., Wang, Y., Sacks, F. M., Bennett, D. A., & Aggarwal, N. T. (2015). MIND diet associated with reduced incidence of Alzheimer's disease. *Alzheimer's & Dementia*, *11*(9), 1007–1014. https://doi.org/10.1016/j.jalz.2014.11.009

Muir, S. W., Speechley, M., Wells, J., Borrie, M., Gopaul, K., & Montero-Odasso, M. (2012). Gait Assessment in Mild Cognitive Impairment and Alzheimer's Disease: The Effect of Dual-Task Challenges Across the Cognitive Spectrum. *Gait & Posture*, *35*(1), 96–100. https://doi.org/10.1016/j.gaitpost.2011.08.014

Mulder, C., Verwey, N. A., van der Flier, W. M., Bouwman, F. H., Kok, A., van Elk, E. J., Scheltens, P.,

& Blankenstein, M. A. (2010). Amyloid-β(1–42), Total Tau, and Phosphorylated Tau as Cerebrospinal Fluid Biomarkers for the Diagnosis of Alzheimer Disease. *Clinical Chemistry*, *56*(2), 248–253. https://doi.org/10.1373/clinchem.2009.130518

Müller, M. J., Greverus, D., Dellani, P. R., Weibrich, C., Wille, P. R., Scheurich, A., Stoeter, P., & Fellgiebel, A. (2005). Functional implications of hippocampal volume and diffusivity in mild cognitive impairment. NeuroImage, 28(4), 1033–1042. https://doi.org/10.1016/j.neuroimage.2005.06.029

Murphy, M. P., & LeVine III, H. (2010). Alzheimer's Disease and the Amyloid-β Peptide. *Journal of Alzheimer's Disease*, *19*(1), 311–323. https://doi.org/10.3233/JAD-2010-1221

Nasreddine, Z. S., Phillips, N. A., Bédirian, V., Charbonneau, S., Whitehead, V., Collin, I., … Chertkow, H. (2005). The Montreal Cognitive Assessment, MoCA: A Brief Screening Tool For Mild Cognitive Impairment. *Journal of the American Geriatrics Society*, *53*(4), 695–699. https://doi.org/10.1111/j.1532-5415.2005.53221.x

O'Dwyer, L., Lamberton, F., Matura, S., Tanner, C., Scheibe, M., Miller, J., Rujescu, D., Prvulovic, D., & Hampel, H. (2012). Reduced Hippocampal Volume in Healthy Young ApoE4 Carriers: An MRI Study. PLoS ONE, 7(11), 1–10. https://doi.org/10.1371/journal.pone.0048895

O'Caoimh, R., Gao, Y., Svendovski, A., Gallagher, P., Eustace, J., & Molloy, D. W. (2017). Comparing Approaches to Optimize Cut-off Scores for Short Cognitive Screening Instruments in Mild Cognitive Impairment and Dementia. *Journal of Alzheimer's Disease*, *57*(1), 123–133. https://doi.org/10.3233/JAD-161204

Paulsen, J. S. (2009). Biomarkers to Predict and Track Diseases. *The Lancet Neurology*, *8*(9), 776–777. https://doi.org/10.1016/S1474-4422(09)70203-0

Perl, D. P. (2010). Neuropathology of Alzheimer's Disease. *Mount Sinai Journal of Medicine: A Journal of Translational and Personalized Medicine*, *77*(1), 32–42. https://doi.org/10.1002/msj.20157

Petersen, R. C., Aisen, P. S., Beckett, L. A., Donohue, M. C., Gamst, A. C., Harvey, D. J., Jack, C. R., Jagust, W. J., Shaw, L. M., Toga, A. W., Trojanowski, J. Q., & Weiner, M. W. (2010). Alzheimer's Disease Neuroimaging Initiative (ADNI): Clinical characterization. *Neurology*, *74*(3), 201–209. https://doi.org/10.1212/WNL.0b013e3181cb3e25

Pillai, J. A., Appleby, B. S., Safar, J., & Leverenz, J. B. (2018). Rapidly Progressive Alzheimer's Disease in Two Distinct Autopsy Cohorts. *Journal of Alzheimer's Disease : JAD*, *64*(3), 973–980. https://doi.org/10.3233/JAD-180155

Pini, L., Pievani, M., Bocchetta, M., Altomare, D., Bosco, P., Cavedo, E., … Frisoni, G. B. (2016). Brain Atrophy in Alzheimer's Disease and Aging. *Ageing Research Reviews*, *30*, 25–48. https://doi.org/10.1016/j.arr.2016.01.002

Portugal, E. M. M., Vasconcelos, P. G. T., Souza, R., Lattari, E., Monteiro-Junior, R. S., Machado, S., & Deslandes, A. C. (2015). Aging Process, Cognitive Decline and Alzheimer's Disease: Can Strength Training Modulate these Responses? *CNS & Neurological Disorders Drug Targets*, *14*(9), 1209–1213. https://doi.org/10.2174/1871527315666151111121749

Proitsi, P., Kim, M., Whiley, L., Simmons, A., Sattlecker, M., Velayudhan, L., … Legido-Quigley, C. (2017). Association of Blood Lipids with Alzheimer's Disease: A Comprehensive Lipidomics Analysis. *Alzheimer's & Dementia*, *13*(2), 140–151. https://doi.org/10.1016/j.jalz.2016.08.003

Qi, Y., Klyubin, I., Cuello, A. C., & Rowan, M. J. (2018). NLRP3-dependent synaptic plasticity deficit in an Alzheimer's disease amyloidosis model in vivo. *Neurobiology of Disease*, *114*, 24–30.

https://doi.org/10.1016/j.nbd.2018.02.016

Raji C, Wang MB, Moe E, Palacios EM, Mukherjee P. Tract based spatial statistics in persons who will develop Alzheimer's dementia: A study from the Alzheimer's Disease Neuroimaging Initiative. Radiological Society of North America meeting. Nov. 25, 2018.

Reitz, C. (2015). Novel Susceptibility Loci for Alzheimer's Disease. *Future Neurology*, *10*(6), 547–558. https://doi.org/10.2217/fnl.15.42

Roberts, R. O., Knopman, D. S., Cha, R. H., Mielke, M. M., Pankratz, V. S., Boeve, B. F., … Lowe, V. J. (2014). Diabetes and Elevated Hemoglobin A1c Levels Are Associated with Brain Hypometabolism but Not Amyloid Accumulation. *Journal of Nuclear Medicine*, *55*(5), 759–764. https://doi.org/10.2967/jnumed.113.132647

Santhanam, L. (2019, March 5). *Why the number of Americans with Alzheimer's could more than double by 2050*. PBS NewsHour. https://www.pbs.org/newshour/health/why-the-number-of-americans-with-alzheimers-could-more-than-double-by-2050

Schindler, S. E., Bollinger, J. G., Ovod, V., Mawuenyega, K. G., Li, Y., Gordon, B. A., Holtzman, D. M., Morris, J. C., Benzinger, T. L. S., Xiong, C., Fagan, A. M., & Bateman, R. J. (2019). High-precision plasma β-amyloid 42/40 predicts current and future brain amyloidosis. *Neurology*, 10.1212/WNL.0000000000008081. https://doi.org/10.1212/WNL.0000000000008081

Seshadri, S., Beiser, A., Selhub, J., Jacques, P. F., Rosenberg, I. H., D'Agostino, R. B., … Wolf, P. A. (2002). Plasma Homocysteine as a Risk Factor for Dementia and Alzheimer's Disease. *New England Journal of Medicine*, *346*(7), 476–483. https://doi.org/10.1056/NEJMoa011613

Sherwani, S. I., Khan, H. A., Ekhzaimy, A., Masood, A., & Sakharkar, M. K. (2016). Significance of HbA1c Test in Diagnosis and Prognosis of Diabetic Patients. *Biomarker Insights*, *11*, 95–104.

https://doi.org/10.4137/BMI.S38440

Shi, F., Liu, B., Zhou, Y., Yu, C., & Jiang, T. (2009). Hippocampal Volume and Asymmetry in Mild Cognitive Impairment and Alzheimer's Disease: Meta-Analyses of MRI Studies. *Hippocampus*, *19*(11), 1055–1064. https://doi.org/10.1002/hipo.20573

Small, G. W., Kepe, V., Ercoli, L. M., Siddarth, P., Bookheimer, S. Y., Miller, K. J., Lavretsky, H., Burggren, A. C., Cole, G. M., Vinters, H. V., Thompson, P. M., Huang, S.-C., Satyamurthy, N., Phelps, M. E., & Barrio, J. R. (2006). PET of Brain Amyloid and Tau in Mild Cognitive Impairment. *New England Journal of Medicine*, *355*(25), 2652–2663. https://doi.org/10.1056/NEJMoa054625

Snitz, B. E., Unverzagt, F. W., Chang, C.-C. H., Bilt, J. V., Gao, S., Saxton, J., Hall, K. S., & Ganguli, M. (2009). Effects of age, gender, education and race on two tests of language ability in community-based older adults. *International Psychogeriatrics*, *21*(6), 1051–1062. https://doi.org/10.1017/S1041610209990214

Stanyon, H. F., & Viles, J. H. (2012). Human Serum Albumin Can Regulate Amyloid-β Peptide Fiber Growth in the Brain Interstitium IMPLICATIONS FOR ALZHEIMER DISEASE. *Journal of Biological Chemistry*, *287*(33), 28163–28168. https://doi.org/10.1074/jbc.C112.360800

Strimbu, K., & Tavel, J. A. (2010). What are Biomarkers? *Current Opinion in HIV and AIDS*, *5*(6), 463. https://doi.org/10.1097/COH.0b013e32833ed177

Vijayakumar, A., & Vijayakumar, A. (2012). Comparison of Hippocampal Volume in Dementia Subtypes. *ISRN Radiology*, *2013*, 174524. https://doi.org/10.5402/2013/174524

Wang, H.-X., Karp, A., Winblad, B., & Fratiglioni, L. (2002). Late-Life Engagement in Social and Leisure Activities Is Associated with a Decreased Risk of Dementia: A Longitudinal Study from

the Kungsholmen Project. *American Journal of Epidemiology*, *155*(12), 1081–1087. https://doi.org/10.1093/aje/155.12.1081

Wang, L., Zang, Y., He, Y., Liang, M., Zhang, X., Tian, L., Wu, T., Jiang, T., & Li, K. (2006). Changes in Hippocampal Connectivity in the Early Stages of Alzheimer's Disease: Evidence from Resting State fMRI. *NeuroImage*, *31*(2), 496–504. https://doi.org/10.1016/j.neuroimage.2005.12.033

Wattmo, C., & Wallin, Å. K. (2017). Early- Versus Late-Onset Alzheimer's Disease in Clinical Practice: cognitive and Global Outcomes Over 3 Years. *Alzheimer's Research & Therapy*, *9*(1), 70. https://doi.org/10.1186/s13195-017-0294-2

Weintraub, S., Carrillo, M. C., Farias, S. T., Goldberg, T. E., Hendrix, J. A., Jaeger, J., … Randolph, C. (2018). Measuring Cognition and Function in the Preclinical Stage of Alzheimer's Disease. *Alzheimer's & Dementia : Translational Research & Clinical Interventions*, *4*, 64–75. https://doi.org/10.1016/j.trci.2018.01.003

Wu, X.-L., Piña-Crespo, J., Zhang, Y.-W., Chen, X.-C., & Xu, H.-X. (2017). Tau-mediated Neurodegeneration and Potential Implications in Diagnosis and Treatment of Alzheimer's Disease. *Chinese Medical Journal*, *130*(24), 2978–2990. https://doi.org/10.4103/0366-6999.220313

Wu, C.-W., Chen, Y.-C., Yu, L., Chen, H., Jen, C. J., Huang, A.-M., … Kuo, Y.-M. (2007). Treadmill Exercise Counteracts the Suppressive Effects of Peripheral Lipopolysaccharide on Hippocampal Neurogenesis and Learning and Memory. *Journal of Neurochemistry*, *103*(6), 2471–2481. https://doi.org/10.1111/j.1471-4159.2007.04987.x

Xiang, C.-P., Han, J.-X., Li, X.-C., Li, Y.-H., Zhang, Y., Chen, L., Qu, Y., Hao, C.-Y., Li, H.-Z., Yang, C.-R., Zhao, S.-J., & Xu, M. (2017). Chemical Composition and Acetylcholinesterase Inhibitory

Activity of Essential Oils from Piper Species. *Journal of Agricultural and Food Chemistry*, *65*(18), 3702–3710. https://doi.org/10.1021/acs.jafc.7b01350

Youn, Y. C., Kang, S., Suh, J., Park, Y. H., Kang, M. J., Pyun, J.-M., … Kim, S. (2019). Blood Amyloid-β Oligomerization Associated with Neurodegeneration of Alzheimer's Disease. *Alzheimer's Research & Therapy*, *11*(1), 40. https://doi.org/10.1186/s13195-019-0499-7

Zetterberg, H. (2019). Blood-Based Biomarkers for Alzheimer's Disease—An Update. *Journal of Neuroscience Methods*, *319*, 2–6. https://doi.org/10.1016/j.jneumeth.2018.10.025

Zhang, Y., Lu, L., Jia, J., Jia, L., Geula, C., Pei, J., … Pan, N. (2014). A Lifespan Observation of a Novel Mouse Model: In Vivo Evidence Supports Aβ Oligomer Hypothesis. *PLOS ONE*, *9*(1), e85885. https://doi.org/10.1371/journal.pone.0085885

Zhong, Y., Huang, L., Cai, S., Zhang, Y., von Deneen, K. M., Ren, A., & Ren, J. (2014). Altered effective connectivity patterns of the default mode network in Alzheimer's disease: An fMRI study. *Neuroscience Letters*, *578*, 171–175. https://doi.org/10.1016/j.neulet.2014.06.043

Zunzunegui, M.-V., Alvarado, B. E., Del Ser, T., & Otero, A. (2003). Social networks, social integration, and social engagement determine cognitive decline in community-dwelling Spanish older adults. *The Journals of Gerontology. Series B, Psychological Sciences and Social Sciences*, *58*(2), S93–S100. https://doi.org/10.1093/geronb/58.2.s93