Team BRAIN Prospectus

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, characterized by significant loss of memory and cognitive dysfunction, that has a significant impact on the health, social and financial burden on society. From past AD research, it can be seen that there is no clear understanding of the mechanism behind tau-mediated degeneration, that there can be other factors than genetics that influence the risk of developing AD, and that there is no single test best suited for the detection of AD in all patients. The purpose of the research is to observe, over the course of six months, the way that early-stage AD patients change in terms of biomarker presence, lifestyle, relationships and more. Team BRAIN plans on using 3T MRI imaging to look at tissue volume, as well as doing analysis of blood samples to analyze the presence of the Plasma/serum Nfl, Aβ42/40 ratio, Sphingolipids, Glyerophospholipids, Aβ, Tau, Plasma clusterin Insulin, and Apolipoprotein J (Apo J) biomarkers. We will be investigating the different biomarkers that AD presents and comparing them to determine what their relationships are to each other, and to different lifestyle factors from self-report data. Over the course of 6 months we will be analyzing 20 individuals without cognitive impairments, as well as an additional 20 individuals showing signs of cognitive impairments. At the beginning of the study, all of these individuals will have blood drawn in order to observe and analyze several blood biomarkers. Additionally, all participants will provide self-report data on their lifestyle and will receive brain scans. After 6 months, blood will be redrawn and brain scans readministered, in order to compare these results with the preliminary data collected at the beginning of the study. The blood will then be analyzed using the ELISA technique. To evaluate our data, we will use within subject T tests to determine the difference between the baseline and 6-month period and the correlation

coefficients between biomarkers. For a normal distribution we will be using Pearson coefficients, otherwise we plan to use Spearman coefficients. We hypothesize that there will be a complex relationship between the self-reported lifestyle factors and biomarker data, as some factors can lower risk while others increase risk. Furthermore, we expect to see a decreased volume of the hypothalamus in the structural MRI scans, as well as changing levels of blood biomarkers.

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. 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 global health concern because of its significant impact on the health, social and financial burden on society. An estimated 5.8 million Americans are currently living with AD and is projected to rise to about 13.8 million by the year 2050 (Santhanam, 2019). In the United States, AD is the 6th leading cause of death with a significant financial burden that exceeds $200 billion annually on direct patient care of these individuals (Santhanam, 2019). There is no way to definitively diagnose AD is a brain autopsy. Currently, there are no curative method of treatment (Santhanam, 2019), however, there is active research in the development for early detection and treatment.

There are two types of Alzheimer's Disease: familial AD, which occurs in <0.5% of the population and mutations seen in three genes: amyloid precursor protein, presenilin 1, and presenilin 2 and sporadic AD. With respect to age of onset, there are two types of AD: early-onset AD (EOAD) and late-onset AD (LOAD). Early-onset AD occurs in individuals before the

age of 65, typically occurring between 30 and 50 years of age, and LAD which presents typically in individuals >65 years of age. It is believed that the epidemiology of AD is multifactorial including genetics, lifestyle and environment.

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 the exclusion of nondegenerative causes of the symptoms including depression, nutritional deficiencies, substance abuse, medications and metabolic and endocrine disorders. However, a definitive diagnosis of AD can only be made on autopsy. 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 leading to build up inside the nerve cell. These features are the gold standard for the diagnosis and can be seen in autopsy pathological evaluations.

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: cholinergic hypothesis, amyloid cascade hypothesis and tau hypothesis. In the cholinergic hypothesis, there is reduced synthesis of acetylcholine and destruction of these neurons causes disruptions in distant neuronal networks. 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. 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. Although these are competing hypotheses, some studies have shown some interplay between them. Research by Zhagn and Li (2014) looked at the relationship between amyloid-beta and tau. This work shows that although tau protein may be dependent on amyloid-beta aggregation, 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 and monitor for efficacy. 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%. CSF biomarkers presents 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 condition in which a person develops memory problems not severe enough to significantly impact daily activities in their lives. They do not experience the hallmark symptoms of AD such as personality changes, but they tend to lose things, forget previously scheduled appointments or events, and have more trouble producing words than other people of the same age group ("What Is Mild Cognitive Impairment?," n.d.).

MCI puts older people at a greater risk for developing AD, but does not guarantee a person will develop any form of later cognitive impairment, as some afflicted with MCI return to normal cognitive ability ("Alzheimer's Disease Fact Sheet," n.d.). About 8 out of 10 people diagnosed with MCI develop AD within 7 years, but adults over 65 years old with normal cognitive abilities are far less likely to develop AD within 1 year; only about 1-3% do ("What Is Mild Cognitive Impairment?," n.d.). Since MCI is a significant predictor for AD, Team BRAIN will be recruiting participants with MCI to determine how these early forms of cognitive impairment may progress throughout a six month duration. 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 refer to any molecule within the body that is able to be measured to assess health levels. These bodily molecules can be taken from body fluids, tissue, or blood. Relevant to this study being conducted, blood is being used ("Biomarker Testing," 2019). 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 (Elliott, 2013). 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, 2018). 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, 2018). 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 ("Tau Protein and Beta Amyloid," 2019). 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 start to 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). White matter connects the different regions of the brain so they can work together and function (Woodruff, 2018). The decrease in white matter and brain mass results in slower processing speed, working memory, and reasoning. However, vocabulary and world knowledge tend to increase with age, indicating that cognition is not dependent on a single factor.

Cognition decreases as AD patients' progress. Cognition is evaluated to determine the stage of AD. AD is characterized by declining cognitive abilities, such as solving problems, making decisions, and exercising judgement. Symptoms of declining cognition include losing track of time, disorientation, and forgetfulness (Harvard Health, 2019). 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 tracked 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. Certain indicators of later stages of AD include decreased brain size in the temporal and parietal lobes and declining brain activity (Radiology, 2019). 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 (Woodruff, 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 (Harvard Health, 2019). 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. Ideally, MRI scans will be taken of the 20 to 40 participants whose blood is taken for analysis. However, due to funding this may not be possible.

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 it's onset. Team BRAIN is looking to correlate biomarker levels and lifestyle choices with the progression of Alzheimer's Disease. Each participant's physical health and cognitive function will be assessed using an Alzheimer's Cognitive Battery. Enzyme Linked Immunosorbent Assays and structural MRI data will be used to analyze the levels of biomarkers and observe their change in concentration over a six month period, which will be compared with data from the self report survey. We expect to find that the blood biomarkers we chose to test will become more prominent after the six month period. By analyzing changes in a set of biomarkers

characteristic of the progression of Alzheimer's Disease, we hope to propose a novel, quantitative way to diagnose 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 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, 2017).

Furthermore, there is another protein, called the tau protein that is also known for the manifestation of AD, through its excess accumulation in a phosphorylated form in the cerebral cortex. The tau protein is helpful for its regulation of and the stability and assembly of microtubules, as well as its role in axonal transport. The phosphorylation of tau proteins is a part

of the post-translational tau modification, and leads to the decreased affinity for microtubules and microtubule stability (Guo et. al. 2019).

In healthy individuals, tau manifests in a non-phosphorylated form, and is very soluble. 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. 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). EOAD is characterized as the development of AD before 65 years of age, while LOAD occurs after 65 years of age. 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, in comparison to LOAD tends to have a faster rate of progression and a shorter duration of the disease. 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. 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).

Late Onset of AD, or LOAD, is a 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, executive functions, ideomotor praxis, and visuospatial skills, 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

2.3.1 Blood

A recent study demonstrated a new way of assessing risk 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 ("Assessing Risk for Alzheimer's Disease," n.d.). The most common gene associated with late onset AD is apolipoprotein E, or APOE for short. 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. Lastly, APOE e4 is less common than APOE e3 and increases the risk of developing AD. Individuals with only one copy of the APOE e3 gene have a lower risk than those who have two copies of the variant. Interestingly, not everyone who has one or both copies of APOE e3 develop AD, and therefore the gene is said to increase risk and not predict future development. Other gene variants that increase risk for late onset AD include ABCA7, CLU, CR1, PICALM, PLD3, TREM2 and SORL1. 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, so genetics is not the only variable influencing development ("The role of genes in your Alzheimer's risk," n.d.).

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 ("Alzheimer's Disease Fact Sheet," n.d.) and increase risk. 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 Mayo Clinic highlights the MIND diet (Mediterranean-DASH Intervention for Neurodegenerative Delay) as a framework for organizing eating patterns with the potential to slow mental degradation. 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. This 15-point diet is a more flexible version of the Mediterranean diet, and it does not have to be followed exactly. If each rule is counted as a point, those who scored 7.5 points cut their risk for AD by more than a third ("15 diet tweaks that could cut your Alzheimer's risk," n.d.). 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, 2007). Studies 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, 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, 2011). 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, 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, Karp, Winblad, & Fratiglioni, 2002). 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, Alvarado, Del Ser, & Otero, 2003).

2.5 Methods of Assessing AD Progression

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 ("Alzheimer's Disease Fact Sheet," n.d.). These amyloid plaques are tough deposits of fibrils that impedes normal brain function and leads to dementia when enough has accumulated. 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. 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 "[changing] shape and [aggregating] into long fibrils… which form dense plaques on nerve cells" (Goodsell, 2006).

Amyloid-β starts to accumulate in the brain 10-15 years before the first clinical symptoms start to appear (Bateman et al., 2012). 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 (Han et al., 2019). 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, Piña-Crespo, Zhang, Chen, & Xu, 2017). Additionally, tau protein serves an important role in axonal transport (Wu, Piña-Crespo, Zhang, Chen, & Xu, 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, Rault, & Voisin-Chiret, 2017). It is likely a result of a combination of both processes being deregulated, resulting in an imbalance that favors hyperphosphorylated tau (Duan, Dong, Gu, Hu, & Zhao, 2012).

In its non phosphorylated form, tau is actually a highly soluble protein and is unlikely to aggregate (Jouanne, Rault, & Voisin-Chiret, 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, Rault, & Voisin-Chiret, 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, Dong, Gu, Hu, & Zhao, 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 ester at the postsynaptic membrane. Because it also exists on the cell membrane of red blood cells, it may serve as a promising biomarker than can be taken from the blood, as opposed to the CSF (Han et al., 2019).

A study performed by Han et al. (2019) shows that blood AChE is associated to the deposits of Aβ in the brain. The study was done on CN, MCI, and AD patients and data was collected using the Pittsburg 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 by Mattsson et al. (2019), 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, Cullen, Andreasson, Zetterberg, & Blennow, 2019). The AD dementia condition had the highest concentration at 45.9ng/L (Mattsson, Cullen, Andreasson, Zetterberg, & Blennow, 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 ("Plasma Neurofilament Light Predicts Alzheimer Neurodegeneration | Psychiatry & Behavioral Health Learning Network," n.d.). Not only are NfL levels important, the change in NfL levels can also be a good predictor of dementia progression (Mattsson, Cullen, Andreasson, Zetterberg, & Blennow, 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. 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 ("The Hemoglobin A1c Test & Chart," n.d.). 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 remain 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). 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). 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). The risk of developing AD was 1.8 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). 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 et. al, 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 et. al, 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 (Hishikawa et. al, 2014), 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 (Hishikawa et. al, 2014), 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.

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 modelling 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 Dagget (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 (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 (Gao et al., 2010), therefore encouraging

further study on the utility of soluble oligomers as biomarkers. 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 (Mielke, 2012). 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, 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, 2012). There is insufficient development of high-throughput sphingolipid assays that can be used in this study, so sphingolipids will not be used as a biomarker (Mielke, 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 (L. 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 (L. 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 is 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 is 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, Doraiswamy & Borges-Neto, 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 AD Battery

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 (M. Folstein, S. Folstein & Hugh, 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, De Deyn, Dierckx & Engelborghs, 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, De Deyn, Dierckx & Engelborghs, 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 & Molloy, 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 able

to 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

Team BRAIN will be recruiting participants with MCI and early onset AD and evaluating the progression of their impairments throughout a six month period through cognitive and physical assessments. In addition, this research aims to search for correlations between lifestyle factors and blood biomarkers that may be predictive of AD. The methods of this research will include gathering self-report data through a Qualtrics survey on lifestyle factors, drawing the blood of participants two times with six months in between, performing cognitive and physical assessments, and taking fMRI brain scans of select participants.

3.0 Population

Participants of this study will consist of individuals with AD, more specifically, those who have mild cognitive impairments and/or early AD symptoms in their later stages of life. Individuals with more severe impairments and symptoms pose a risk for our study due to the inability to effectively communicate among other obstacles that potentially could arise, specifically with the use of the delicate brain scans. Individuals who have entered more serious stages of cognitive decline may be unable to calmly undergo blood testing, memory testing, and brain scanning. Certain exclusions may include other affecting conditions: such as, neurological

disorders, blood disorder, stroke patients, as well as individuals who are claustrophobic and/or have any type of metal implants or devices in their body.

3.1 Self Report

Participants will self-report their own lifestyle choices by completing a Qualtrics survey. This survey will ask for information such as their dietary habits, activity/exercise habits, whether or not the individual smokes or drinks, and about their perception of their own cognitive decline. This survey will allow us to assess if any specific habits may be associated with the risk of developing AD.

3.2 MRI Scans

Given enough funding, structural MRI will be performed, magnetic resonance imaging, scans to determine hippocampal volume of a set of study participants. This set will be composed of two subsets: individuals with mild cognitive impairment and individuals who are neurotypical healthy controls. Ideally, we would like to conduct the scans 20 participants from each group, but realistically we may only scan 10 individuals from each subset. The scans will then be compared to determine differences in hippocampal volume progression over time. Those who have metal in their bodies or claustrophobia will be excluded from the MRI scanning.

3.3 Blood Draw and Assessment

Team members will be trained and certified in phlebotomy by the University Health Center before taking blood samples from study participants. Samples will be taken from each participant two times, separated by a six month interval. These blood samples will be tested for the following blood biomarkers: Plasma/serum Nfl, Aβ42/40 ratio, Sphingolipids, Glyercophospholipids, Aβ, Tau, Plasma clusterin Insulin, and Apolipoprotein J (Apo J). The

quantification of these biomarkers in the blood at the beginning of the six month period will be compared to the quantification of these biomarkers in the blood at the end of the six month period. Then, this information will be analyzed to determine which biomarkers exhibit the most noticeable changes and correlate most closely to the progression of cognitive deficits associated with AD.

3.4 Cognitive Assessment

To assess patients' cognitive functioning, an Alzheimer's Cognitive Battery will be conducted, which will be modeled after Dr. Smith's. Dr. Smith's cognitive battery will be modified so that it best matches our research questions. This battery will allow correlation of blood biomarker levels with levels of dementia progression, which will act as a control for different stages of the disease progression in the participants.

3.5 Physical Assessment

An assessment of each participant's physical health will be conducted prior to drawing their blood. This physical assessment will occur when blood is drawn for the first time, and again when blood is drawn again after six months. Any abnormalities in the participant's physical health will be recorded, as well as any changes in their physical appearance of health after the sixth month period. This information will give insight on how the progression of their dementia has impacted their physical health, in addition to their cognitive functioning.

The gait of each participant will also be accounted. Participants will walk along a pre-made track, and how fast they walk and the manner of their walk will be observed. Any disturbances in gait will be used as a measure of cognitive decline.

Chapter 4: Appendix

4.1 Timeline

Our future plans for this project include IRB approval, fundraising, recruitment, data collection and data analysis. We will send our project for IRB approval late this semester (Fall 2019). We estimate that the approval will take two to three months, and in this time we plan to practice our data collection methods such as obtaining self report data, administering the informed consent form and the AD Battery. Before this time, we will also get Phlebotomy Certification from the Health Center which will require practice drawing blood as well.

Fundraising will start in November 2019 for Alzheimer's Disease Awareness month, and in doing so we will reach out to potential donors through email and a social media campaign. This money will be sent as a donation to the Gemstone Program. We will have all of our materials ready in February 2020 in order to start our Launch UMD Campaign in March 2020, just in time to align with Giving Day. We will continue fundraising through our social media campaign, particularly if we require more funds than originally predicted.

Recruitment will start next semester, during Spring 2020. We plan on recruiting participants with MCI from Senior Living Facilities and Memory Care Facilities in the area. However, we will accept participants from all stages of AD into our study. For our control condition, we will recruit healthy participants in the same age range as our participants with MCI and AD. Hopefully, many of the participants in the control condition will be spouses or family members of those with MCI or AD. In order to recruit, we will be visiting these facilities in smaller groups and giving presentations about our project, its importance and predicted impact on the field.

We predict that data collection will start during Fall 2020. However, our methods allow us to have data collection on a rolling basis. We will be starting the 6 month period for each participant at a different time in order to stagger the end of the 6 month period and the data collection that follows. This way, we can collect and analyze data more efficiently. Lastly, data analysis is estimated to start in Fall 2021. This is not a finite deadline because the rolling basis for the start of data collection may allow us to start before this date.

4.2 Budget

It is difficult to determine a budget because we have not yet determined which biomarkers we will be using, and thus the cost of blood assays is unknown. We estimate a structural MRI will cost $400 and will be done twice on each participant within a select group, so in only structural MRI costs we expect to spend $800 per participant. For an estimated number of 20 participants each with two MRI scans, we can expect to spend at least $16,000. We predict our budget will be around $18,000 including the blood assays.

Presenting your budget in a table is more helpful. Estimate costs.

4.3 Mentor Feedback

Dr. Smith helped us break down the Thesis Prospectus in a logical way. This allows the document to be understandable to those who do not have a background in Neuroscience, AD, or blood. He also told us about a good resource called ADNI (Alzheimer's Disease Neuroimaging Initiative) to use for our research.

He also clarified what set of study participants we will be conducting MRI scans on. We originally wrote that this set would include only individuals with MCI, but we have now broadened it to include neurotypical individuals who will be used as healthy controls.

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