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How Alzheimer's Disease is Taking Over (Your Brain)

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Abstract

As the population continues to age and the burden on our care system grows, it is urgent to understand, treat, and cure Alzheimer's Disease (AD). Despite decades of research, there is currently no known cause for the development of dementia or AD. There are two prominent explanations currently dominant in neuroscience: amyloid plaques and neurofibrillary tangles. I will delve into the hypotheses and definitions of each of these pathologies and specifically address how they affect overall neural activity that is proposed to result in the neurodegenerative symptoms of AD. I will also discuss the recent research surrounding biomarkers, age-related neurodegeneration, and genetics and their correlations to an AD diagnosis. Finally, I will reflect on the findings of the new Seattle Alzheimer's Disease Brain Cell Atlas. The intention is to present a comprehensive overview and holistic understanding of this geriatric pandemic in an effort to identify specific areas that warrant further exploration to advance prevention strategies and find a cure.

Keywords: Alzheimer's Disease (AD), amyloid plaques, neurofibrillary tangles, Aducanumab, Lecanemab, neurodegeneration, ApoE

Introduction

Alzheimer's Disease (AD) is a neurodegenerative disorder that can lead to short-term memory loss, paranoia, and delusional ideas (Kavitha et al., 2022). One out of every ten people over the age of 65 will develop symptoms, and this number increases to one in three after the age of 85 (Alzheimer's Disease Research Center). About 60% of people over the age of 60 are now living with AD and with no known cure, early detection is crucial to slow the neural deterioration that occurs with AD (An et al., 2008). With higher life expectancy and the population of the elderly increasing at an exponential rate, the percentage of those diagnosed with AD will also rise drastically, thereby having an effect on social and economic stability of societies (Kavitha et al., 2022). While there is no known cause of AD, there are two hypotheses that have risen to prominence in neuroscientific explanations: amyloid plaques and neurofibrillary tangles. Amyloid plaques form intercellularly in the brain as a result of clumping of amyloid proteins. Neurofibrillary tangles form intracellularly as a result of an abnormal form of the tau protein. Examining these two accounts, along with relative biomarkers, age-related neurodegeneration, and genetic factors, may lead to information that will help us better understand, treat, and detect AD at its earlier stages.

The brain is composed of a hundred billion neurons with over a trillion different synapses connecting these neurons and aiding in communication between the cells. In AD, communication amongst these synapses is disrupted. According to the National Institute of Health (NIH), when these connections are disrupted, it leads to the loss of neuron functioning and, eventually, neuronal death (NIH). In order for a neuron to maintain its function, it must maintain its connection with other neurons, but because AD causes a major disruption in this process, neurons are unable to maintain a connection with other neurons and ultimately die.

History

Alzheimer's Disease is named after Dr. Alois Alzheimer, a German psychiatrist, who discovered significant neural changes in a woman named Auguste Deter who died of a mysterious medical condition in 1906 (Hippius & Neundörfer, 2003). Auguste Deter was 50 years old and a patient of Alzheimer's for 5 years; he followed her case of severe paranoia, memory loss, aggression, sleep disturbance, and confusion until her death. Following her death, Alzheimer noticed various distinctive changes in her neural tissue - changes that we now know were amyloid plaques and neurofibrillary tangles. Over the course of these 5 years, Alzheimer noted the progression of the illness seen in his patient. Alzheimer shared his findings at the Tubingen meeting with fellow scientists in 1906, telling them that this pathology is something that has never been seen before. However, the response from the scientific community following Alzheimer's lecture was disappointing, as very few people had questions or comments. No further discussions occurred about the topic after the meeting, but Alzheimer continued to investigate the cause of this "mysterious" disease. Auguste Deter's diagnosis was rolled out as a rare case of "presenile dementia with some unusual histological signs [plaques and neurofibrillary tangles]" (Hippius & Neundörfer, 2003).

The case of Josef F in 1911 also had a significant impact on the development of what we now know as AD. Alzheimer examined patient Josef F and diagnosed him with AD due to the very similar behavioral symptoms experienced by Auguste Deter. However, after Josef F's death, there was a very important distinction between his neural tissue and that of Auguste Deter's. Josef only had plaques, with no evidence showing that he had neurofibrillary tangles. Alzheimer ruled Josef's case as "plaques-only Alzheimer's Disease." Due to the lack of interest of the scientific community, both of these cases went unnoticed for more than 50 years, but this has

changed over recent decades. Neural tissue of both Auguste Deter and Josef F were investigated in 1995 using modern neurohistological techniques which led to the discovery that Josef F's "plaques-only" case was likely due to the disorder being at an earlier stage.

Josef F and Auguste Deter were the first documented cases of AD in history, but it is highly likely this disease has been around for much longer than a century. In spite of that, there is still no known cause or treatment for the disease over 100 years after its first diagnosis. AD went un-researched for over 50 years after the death of Dr. Alois Alzheimer in 1915, but this has changed over recent decades. In the late 1960s, doctors and scientists began to see the importance of amyloid plaques in the elderly, and by the early 1970s, doctors began to distinguish AD from other forms of dementia (Ellison, 2021). However, it was not until 2011 that AD was officially redefined and was understood as a unique pathological process that develops into a clinical disease over the span of several decades (Ellison, 2021). The attitude of the scientific community has shifted, and many have been eager to fund and conduct research related to the disease. To date, AD is one of the largest funded research projects around the world, with $3.1 billion of annual federal funding in the US and additional significant funds through various private foundations being dedicated to its research (Alzheimer's Association, 2020).

Over recent years, scientists have developed new and better ways of testing for and detecting AD. Machine learning (ML) algorithms are currently the major predictors of AD, using different techniques involved in analyzing brain images (Kavitha et al., 2022). ML algorithms use brain imaging techniques to get a better visual look at the amyloid plaques and neurofibrillary tangles forming in different areas of the brain. ML algorithms are significant improvements to existing methods because they provide results with better accuracy and validity and provide more information regarding the stage of AD that the patient is in. Based on the Open

Access Series of Imaging Studies (OASIS) that test for precision, recall, and accuracy, tests provided to examine for AD have gotten significantly better over the last few years, having an 83% accuracy rate compared to earlier diagnostic methods (Kavitha et al., 2022). In addition to ML algorithms, new techniques are being used to diagnose AD at earlier stages including magnetic resonance imaging (MRI) scans and biomarkers (Kavitha et al., 2022). Biomarkers are measurable indicators of a biological process or disease in the body that can be used to more accurately diagnose, monitor, and track disease progression. Using ML algorithms to detect biomarker patterns in AD may be helpful in providing a better understanding of AD on a more cellular level, since it can provide critical information such as protein and chemical levels.

Diagnostic Criteria

According to the NIH, the current diagnostic criteria for AD is divided into 3 stages: preclinical, mild cognitive impairment (MCI), and Alzheimer's dementia (NIH). The preclinical stage notes brain changes, including amyloid buildup and other nerve cell changes, with very few to no clinical symptoms evident. Amyloid plaques may be present, but there are no behavioral symptoms experienced by the patient in this stage. The MCI stage is marked by symptoms of memory and/or cognitive deficits that are greater than normal for a person's age and education, but these changes do not interfere with the person's independence and functionality. The final stage, Alzheimer's dementia, is marked by evident symptoms of memory loss, word-finding difficulties, and visuospatial problems; these symptoms impair a person's ability to function independently (NIH). These three stages, and the duration of each as experienced by different patients, are directly correlated with the severity of the progression of the illness.

According to the Alzheimer's Association, the final stage of Alzheimer's dementia is further broken down into three separate sub-stages of mild, moderate, and severe (Alzheimer's

Association, 2022). Mild Alzheimer's dementia is characterized when the behavioral symptoms have a mild intervention on the person's day-to-day life, and some assistance is required with some activities such as handling money and paying bills. Common daily tasks also take more time to complete, but things like driving and going to work are not severely affected. Moderate Alzheimer's dementia is often the longest stage, where individuals experience more issues with memory and language. Confusion is also often expressed by the individual, and they may start having personality and behavioral changes. Severe Alzheimer's dementia is characterized when the individual's ability to verbally communicate and physically move is greatly affected, and the individual needs around-the-clock care. Individuals in this stage may become bed-bound due to damage to brain areas involved in movement. Being bed-bound opens the individual to other health issues such as blood clots, skin infections, and trouble eating and drinking. In fact, one of the leading causes of death among AD patients is the result of a lung infection called aspiration pneumonia, which occurs when supine or reclining individuals swallow food into the windpipe rather than the esophagus and food particles are deposited in the lungs (Alzheimer's Association, 2022).

Another important factor when looking at the diagnosis of AD is the age of the patient. Early-onset AD is present in patients under the age of 65 whereas late-onset AD is present in patients over the age of 65 (Awada, 2015). Early-onset AD is quite rare and only occurs in about 5% of patients; this form of the disease likely occurs due to a genetic predisposition (Awada, 2015). Late-onset AD is much more common and is likely a result of other neurodegenerative factors in addition to genetic predisposition, making it more complex than early-onset AD from a pathological standpoint. While genetics may not be the sole cause of developing the disease, they may tell a small part of the story with regards to how the disease progresses in the brain.

Understanding these less complex forms could lead to additional understanding of the disease etiology and progression. The role that genetics play in the development of AD will be discussed further below.

Despite well-developed diagnostic criteria for AD, the underlying changes in the brain are not easily testable and are not fully understood. To improve this, scientists have sought to identify the underlying biomarkers of AD progression. Some of these biomarkers include amyloid plaques and neurofibrillary tangles. How these might be detected at earlier stages rather than in post-mortem examination will be discussed in more detail in the next sections.

Another concern with AD current diagnostic criteria is that, although behavioral symptoms may begin to manifest in stage 2 of MCI, AD cannot currently reliably be detected in its earlier stage. Furthermore, once the physiological and behavioral symptoms are experienced by the patient, there is no current method of reversing the damage. In other words, once a patient is diagnosed with stage 2 of the AD diagnostic criteria, there is no known medical intervention able to reverse the neural damage and improve the clinical symptoms. This is why it is crucial to develop methods of detection and prevention *before* neural damage becomes progressive and irreversible.

The research surrounding AD has remained largely unchanged for the past five decades. The majority of previous approaches were aimed at trying to improve the symptomatology of AD primarily by trying to remove amyloid plaques or through pharmaceutical agents at arresting symptoms. It is crucial to change our approach to trying to find the actual biological root cause of the illness rather than treating its symptoms at a much later stage. The current diagnostic criteria for AD calls for medical intervention at a stage where it is too late to treat the illness. This must change if a cure is ever to be found. Efforts made to define AD's pathogenesis will

arguably have a larger impact than treating the person once they have already developed the disease.

Amyloid Plaques

Amyloid beta, or Aβ, is a protein that is normally produced in the brain in a soluble state but can adopt different oligomers (different states) that ultimately form amyloid plaques (Santin et al., 2016). Brains of AD patients have shown extracellular plaques made up of a peptide called Aβ (Sanes & Holtzman, 2021). These amyloid plaques are plaques formed by the clumping of amyloid precursor proteins that form in the synapses, ultimately resulting in the loss of connections between neurons. Amyloid plaques are thought to cause several issues in the brain: (1) neuritic dystrophy occurs where the plaques are surrounded by swollen axons and dendrites, (2) inflammatory cells surround these neural processes, and (3) amyloid deposits may form in the arterioles in the brain, producing cerebral amyloid angiopathy and causing issues with blood flow to the brain (Sanes & Holtzman, 2021).

Amyloid plaques result in neuroinflammation that holds toxic soluble forms of Aβ, which is why limiting the amyloid produced by the brain may be a key therapeutic strategy for preventing the formation of amyloid plaques (Santin et al., 2016). Microglia appear to rapidly migrate toward the plaques and contribute to their formation by facilitating the conversion of existing Aβ to oligomeric Aβ (Nagele et al., 2004). The unusual aspect of this phenomenon is that microglia typically act as the brain's "immune system," so rather than internalizing and removing these plaques, they are contributing to their formation. A study conducted in 2016 used *in vivo* imaging performed with positron emission tomography (PET) to evaluate current anti-amyloid therapies at both the clinical and preclinical levels. However, one issue with this method is that PET scans do not provide a very visible and reliable image that can visualize

individual plaques, which is also why the current modern approach has shifted to using MRIs and fMRIs as a replacement to PET scans (Santin et al., 2016).

Amyloid plaques are currently being used to detect preclinical AD because they presumably appear during the early stages of AD (Nagele et al., 2004). The issue is that their presence is not in a specific location in the brain, as they appear in areas of the cerebral cortex, entorhinal cortex, hippocampus, ventral striatum, and basal forebrain (Nagele et al., 2004). Two things that researchers are trying to figure out are how to prevent these plaques from forming and how to get rid of them when they do - both of which require pre-clinical research to achieve.

A study conducted in 2012 found that vitamin D3 may activate specific genes to stimulate the immune system to clear the amyloid plaques (Mizwicki et al., 2012). In this study, scientists discovered that specific types of immune cells in AD patients responded to vitamin D3 and curcumin, a chemical found in turmeric spice (Mizwicki et al., 2012). By stimulating the immune cells, scientists hope that the immune system will clear the amyloid plaques on its own. The study performed by researchers at UCLA used blood samples from AD patients and healthy control patients where specific immune cells called macrophages were isolated. Macrophages play a critical role in getting rid of amyloid beta and other toxins and waste products from the brain (Mizwicki et al., 2012). The results found that when a specific active form of vitamin D3 was mixed with the blood samples, it had a significant effect on macrophages by opening chloride channel 3 (CLC3). CLC3 is important in phagocytosis, the process in which the body gets rid of bacteria, by supporting the uptake of amyloid beta (Mizwicki et al., 2012). Curcumin plays a role in activating the CLC3 in Type I macrophages, enhancing the phagocytosis process.

Researchers have also found there to be a correlation between vitamin D deficiency and AD (Engelen, 2009). The explanation for this correlation is that vitamin D plays a role in

neuroprotection and reducing inflammation (Engelen, 2009). However, there is also a correlation between vitamin D deficiency and old age, so it is unknown whether or not vitamin D deficiency results in AD or is simply just a correlation. A more recent review published in 2021 stated that vitamin D aids in synaptic plasticity and neurotransmission of dopaminergic neural circuits while working to reduce inflammation (Bivona et al., 2021). Another study also found that vitamin D deficiency was associated with a significantly increased risk of developing dementia and AD, supporting the hypothesis that vitamin D may play a neuroprotective role (Littlejohns, 2014). Given all of the studies listed above, some of the next stages of research include testing the effects of vitamin D in a clinical trial to further examine its role on AD patients. Considering the results of vitamin D's role in the immune system, more research needs to be done to further assess this as a potential treatment option for the removal or prevention of amyloid plaques.

Neurofibrillary Tangles

Along with amyloid plaques, neurofibrillary tangles are another factor identified in AD progression. Tau is a protein naturally produced by the body that plays a crucial role in maintaining the structural integrity of a neuron and intracellular transport, particularly in axons, by binding to and stabilizing microtubules (Sanes & Holtzman, 2021). Typically, this tau protein binds to microtubules inside the cell, but in an AD patient, this protein detaches itself from the microtubule, becomes hyperphosphorylated, and sticks to other tau molecules in the cell, forming tangles (Sanes & Holtzman, 2021). Neurofibrillary tangles form intracellularly in the cell bodies and dendrites of neurons and are made up of paired helical filaments, an abnormal form of the tau protein (Furcila et al., 2019). Additionally, tau proteins are able to pass through synaptically-connected neurons, resulting in the formation of neurofibrillary tangles, synaptic loss, axonal retraction, and cell death (Robbins et al., 2021). The fact that tau deposition is

transmitted synaptically means that the number of neurons with neurofibrillary tangles drastically increases over time. While both amyloid plaques and neurofibrillary tangles have been found when classifying AD, there appears to be a stronger correlation between the accumulation of tau pathology and cognitive decline (DeVos et al., 2018). A possibility for this may be because the regional patterns of brain atrophy - reduced brain volume or cortical thinning - in AD patients have been found to coincide with the distribution of neurofibrillary tangles (Emrani et al., 2020). In other words, because neurofibrillary tangles cause neuronal dysfunction and eventually lead to neuronal death, this causes a loss of brain volume over time. This loss of brain volume may translate to cognitive decline symptoms in patients.

There seems to be evidence suggesting that amyloid plaque formation leads to the formation of neurofibrillary tangles by driving tau aggregation (Sanes & Holtzman, 2021). This theory has come to be known as the "amyloid cascade hypothesis." This hypothesis states that the formation of amyloid plaques is the causative component of AD, which further leads to the formation of neurofibrillary tangles and cognitive impairment. Both neurofibrillary tangles and amyloid plaques, while sparsely located in different regions of the brain, have been found in abundance in medial temporal structures, such as the entorhinal cortex, amygdala, and hippocampus, with both their number and the proportion of the cortex affected increasing as the disease progresses over time (Furcila et al., 2019). These areas of the brain are also implicated in functions associated with cognitive impairments in AD (Furcila et al., 2019).

In the initial stages of AD, neuronal loss and several other neurodegenerative changes affect primarily the hippocampal regions (Furcila et al., 2019). The hippocampus plays a central role in the formation of memory, so the location of the plaques and tangles disrupts that process. A study conducted in 2017 found that the regional deposition of tau aggregates in AD patients

affects higher-order cognitive networks predominantly more than primary sensory-motor networks, but is not specific to any single large-scale functional brain network as a whole (Hansson et al., 2017). This suggests that tau deposition within a given region would primarily spread within the interconnected networks before spreading to other brain regions. These higher-level cognitive processes may indicate that they have an inherent susceptibility to tau aggregation, likely due to its specific functional role and network of interconnected neurons (Hansson et al., 2017). This could mean that these tangles directly affect the actions of the higher-level cognitive processes, which in turn affect the lower-level processes of the brain.

Although there is some evidence linking plaques and tangles, it remains inconclusive whether there is a definitive connection between the two. Many researchers hypothesize that the number of neurofibrillary tangles, not amyloid plaques, correlates best with the presence and degree of dementia in patients with AD (Iqbal & Grundke-Iqbal, 2008). A reason for this could be because amyloid plaques can appear long before AD symptoms are present. This is beneficial to scientists and doctors treating AD and other types of dementia because it gives them the ability to see the intensity and duration of these tangles and how the abundance or presence of the tangles affects the symptoms of the illness. This could be because the primary role of the tau protein in the brain is to maintain cellular structure and transport within the cell, and when this process gets disrupted by abnormal tau aggregation, neurons are unable to do their jobs. Therefore, cognitive decline and cell death are bound to happen eventually due to a loss of communication between cells.

Unfortunately, to date, there is no available treatment for the prevention or removal of neurofibrillary tangles. A study published in 2020 found that vitamin E may play a role in a therapeutic effect in the reduction of both neurofibrillary tangles and amyloid plaques in the

hippocampus (Jahanshahi et al., 2020). This study is based on research done on scopolamine given to rats. Scopolamine is a drug that mimics AD pathophysiology by significantly increasing the density of amyloid plaques and neurofibrillary tangles in the hippocampus (Jahanshahi et al., 2020). The results found that vitamin E injections reversed the scopolamine-induced increases in the densities of the plaques and tangles in rats. However, one limitation of this study is that the results are based on artificially increasing the densities of the plaques and tangles, so it is unknown if vitamin E will have the same effects on a human subject with AD. Furthermore, the actual role of vitamin E and its effects on neurofibrillary tangles and amyloid plaques is poorly understood, so more research needs to be done in order to learn more about this being a potential treatment option for AD.

Because the appearance and location of neurofibrillary tangles seems to be highly correlative with brain atrophy and overall cognitive decline, preventing or trying to reduce these tangles may be a key therapeutic approach to slow down AD progression and overall cognitive decline. However, targeting the removal of neurofibrillary tangles is more difficult than targeting the removal of amyloid plaques because neurofibrillary tangles form intracellularly whereas amyloid plaques form extracellularly. It is much more difficult to develop a treatment that would improve the cell's internal environment than it is to target the cell's external environment. Currently, there are several tau-targeting drugs that are being tested and are undergoing clinical trials: leuco-methylthioninium (LMTX), AADvac1, and Zagotenemab.

LMTX, or better known by its brand name Lucidity, is a tau aggregation inhibitor. Its purpose is to decrease the levels of aggregated tau proteins in the brain in order to further alleviate tau-related neuronal damage (Huang et al., 2020). In fact, it is the only tau-specific agent that has reached phase III clinical trials. The phase III clinical trial was concluded and the

results were negative (Gauthier et al., 2016). The findings indicated that LMTX did not have any benefit for patients with mild to moderate AD (Gauthier et al., 2016). According to the National Registry of Clinical Trials, a study was posted in May 2018, but has not yet been completed, to further evaluate LMTX as a treatment for early and mild-moderate AD. Given that LMTX is the only tau-specific drug to reach stage III clinical trials, it is yet to be approved by the FDA as a treatment option for AD.

The development of AADvac1 is an entirely new and different therapeutic approach of treating AD. AADvac1 is an active vaccine that is designed to induce the body's natural immune response by targeting various epitopes in pathological tau forms, resulting in the inhibition of aggregating and a reduction in the formation of neurofibrillary tangles (Huang et al., 2020). A phase I 12-week clinical trial was conducted in 2013 to test the safety and efficacy of AADvac1 on 30 patients, ages 50-85, with mild to moderate AD (Novak et al., 2017). Of the 30 patients who received the vaccine, 29 developed an IgG immune response (Novak et al., 2017). Following the results of this study, a phase II randomized study was performed to see if these results could be replicated and to further evaluate the immunogenicity and efficacy of the vaccine. The results of this study found that the patients who received the vaccine had higher levels of IgG antibodies, but there were no significant effects on cognitive or functional tests (Novak et al., 2021).

Zagotenemab is an anti-tau antibody that acts to capture and neutralize tau aggregation (Huang et al., 2020). Two phase I clinical trials were performed to test the safety and efficacy of the drug, one in 2016 and one in 2017, but the results are not yet published (Abyadeh et al., 2021). Specifically, these trials were conducted on patients with MCI or mild to moderate AD, so it seems that this drug is only targeting tau removal in patients with early stage AD. A phase II

clinical trial was started in 2018 to further examine the safety, tolerability, and efficacy of Zagotenemab, but the results are not yet published (Abyadeh et al., 2021).

It is important to note that none of these medications targeting neurofibrillary tangles have been approved by the FDA as a treatment option for AD. Targeting neurofibrillary tangles as a potential therapeutic approach against AD is still being discussed by the scientific community. We can see a strong correlation between the presence of neurofibrillary tangles, brain atrophy, and cognitive decline, but there is not enough research available to indicate how to reverse this process or whether doing so would improve cognitive decline that may or may not be caused as a result tau accumulation and the formation of neurofibrillary tangles.

Biomarkers

The early detection of neurofibrillary tangles and amyloid plaques is crucial for effective intervention and management of AD. Identifying these pathologies before the onset of symptoms could provide a significant breakthrough in the development of effective treatments and preventative measures. MRI scans allow for structural images of the brain while biomarkers allow for a more detailed report of chemicals and blood flow in the brain. Research surrounding biomarkers and AD are relatively new and there is much that is not yet known. Cerebrospinal fluid (CSF) analysis is a valuable diagnostic tool as it provides a direct measure of biomarkers since it is in direct contact with the extracellular space of the brain and can be examined to evaluate changes that occur inside the brain (Anoop et al., 2010). CSF is a translucent bodily fluid that flows in and around the brain and spinal cord; it essentially acts as the waste management of the brain (Anoop et al., 2010). However, the process of obtaining CSF from patients via lumbar puncture is invasive and can potentially be painful for the individual (Anoop

et al., 2010). Lumbar puncture, also known as a spinal tap, is a procedure in which a doctor inserts a needle into the spinal cord in the lower back and withdraws CSF (Mayo Clinic, 2022).

One study conducted in 2021 revealed that there were differences in CSF in patients diagnosed with AD compared to controls. Specifically, a significantly higher number of synaptophysin-bearing microvesicles were found in the CSF of AD patients compared to the control group (Utz et al., 2021). Microvesicles are small fragments of the membrane that are released as exosomes (Camussi et al., 2010). Exosomes are extracellular vesicles that carry components, such as proteins, lipids, DNA, or RNA (Zhang et al., 2021). Specifically, the synaptophysin-bearing microvesicles that are found in abundance in the CSF of AD patients play an important role in blocking the release of some neurotransmitters, such as acetylcholine (ACh) - a neurotransmitter primarily functioning at the neuromuscular junction, aiding in muscle movements, learning, and memory (Utz et al., 2021).

Scientists found that patients diagnosed with AD have deteriorating levels of ACh-producing neurons in the brain, which may play a significant role in the movement difficulties associated with AD. This may be due to the correlation found between acetylcholinesterase (AChE) and the progression of AD (Auti & Kulkarni, 2019). AChE breaks down ACh leading to decreased levels in the brain. Exposure to heavy metals may also increase the risk for AD because aluminum is linked to the activation of AChE activity (Hussien et al., 2018). Several medications, such as donepezil and galantamine, are drugs that are currently approved to help inhibit AChE and improve the movement difficulty symptoms associated with AD (Hansen et al., 2008). However, the main issue with AChE inhibitory drugs that are currently available is the fact that they have many adverse side effects, including nausea, vomiting, and

diarrhea, so the need for developing a better treatment option is pressing (Auti & Kulkarni, 2019).

More research is currently being conducted on other chemical and neurotransmitter abnormalities in the CSF of AD patients compared to controls. A systematic review published in 2013 stated that there are 5 AD biomarkers that have been incorporated into the diagnostic criteria and can be seen below (Jack Jr & Holtzman, 2013).

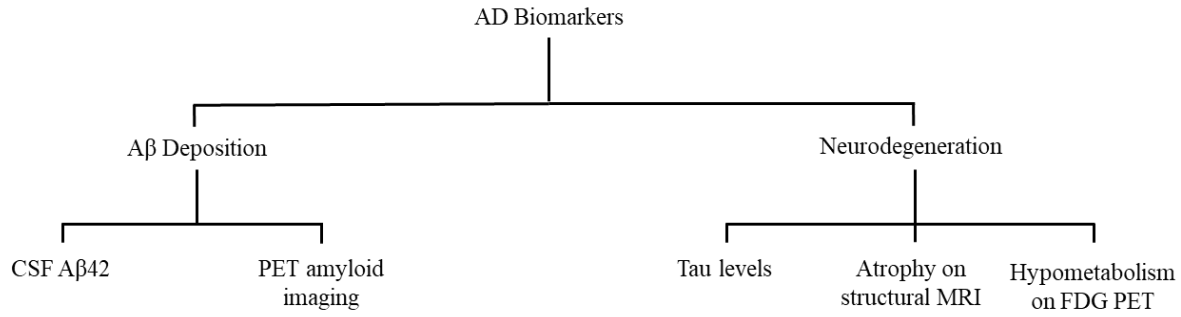

Figure 1. Five AD biomarkers that have been incorporated into diagnostic criteria

These biomarkers are divided into two categories: biomarkers of A□ deposition and tau-related neurodegeneration. Of these 5 biomarkers, 3 are imaging measures and 2 are CSF analytes. Biomarkers of A□ deposition are the first known biomarkers to become abnormal in AD patients, as amyloid plaques typically develop early-on in AD (Jack Jr & Holtzman, 2013). Biomarkers of A□ deposition are CSF A□42 and PET amyloid imaging. CSF A□42 simply refers to the accumulation of A□ that is measured in the CSF. By measuring the levels of A□, this will give scientists and medical professionals a better idea of how much A□ is present in the brain, with increased levels indicating larger and more abundant amyloid plaques. PET amyloid imaging refers to structural amyloid plaques that can be seen via PET scan. PET and MRI scans allow scientists and medical professionals to see exactly where and how large amyloid plaques

are formed in the brain, which may also aid in explaining specific behavioral symptoms and diagnosing the specific stage of AD in a patient.

Biomarkers of tau-related neurodegeneration are the measurements of tau levels, atrophy on structural MRI, and hypometabolism on fluorodeoxyglucose (FDG) PET. Tau levels can be measured via CSF and used to diagnose and characterize the stage of AD, with higher tau levels indicating greater AD pathology, neurodegeneration, and cognitive decline. Because brain atrophy is known to topographically follow the formation of neurofibrillary tangles, scientists can use structural MRI to see what parts of the brain have experienced these tangles and ultimately endured neuronal cell loss that resulted in atrophy. On structural MRI, neurofibrillary tangles are characterized macroscopically as brain atrophy and microscopically as the loss of neurons and neuronal processes (Jack Jr & Holtzman, 2013). The last biomarker of tau-related neurodegeneration is hypometabolism that could be seen via FDG PET. Hypometabolism is characterized by a reduction in cerebral glucose utilization (Zilberter & Zilberter, 2017). FDG PET is used to visualize the distribution of neural injury or synaptic dysfunction and to identify specific phenotypes of dementia due to AD and other forms of dementia (Kato et al., 2016). Specifically, AD shows hypometabolism in specific parts of the brain, such as the parietotemporal association area, which is a predictor for the progression from MCI to AD dementia (Kato et al., 2016). Age-related hypometabolism is mainly observed in the anterior cingulate cortex and anterior temporal lobe (Kato et al., 2016). FDG hypometabolism is also correlated with neurofibrillary tangles and is used to visualize further neuronal and synaptic loss and dysfunction.

These biomarkers can aid in seeing changes in amyloid buildup and tau accumulation over time in AD patients. They can also give a better understanding of the temporal evolution of

AD biomarkers as we can see the time it took to progress from one stage of AD to another using neuroimaging and CSF analysis. It is also important to note that AD pathology may look different and manifest differently for early-onset versus late-onset AD, and specific models for each type are still under investigation. New research emerging about certain biomarkers and their relationships to an AD diagnosis may be useful in the treatment and management of AD. The use of neuroimaging and continued testing on CSF biomarkers may lead to new insights into better understanding AD on a more cellular and chemical level. I am very curious to see if there are more significant differences in the CSF between AD and control patients. Understanding AD on a more chemical level may lead to further insights into how AD manifests in the brain.

Age-Related Neurodegeneration

As we age, our brain also ages, causing changes in how our neurons behave and interact with each other. As part of the aging process, neurons become increasingly vulnerable to damage. Specifically for cognitive decline, these changes include narrowed gyri, widened sulci, reduced brain weight, and enlarged ventricles (Sanes & Holtzman, 2021). It is important to address the importance of typical age-related neurodegeneration in order to better understand the cellular and molecular basis of pathological neurodegeneration in AD. By learning more about how the brain changes as we age, we will be better able to understand, target, and treat AD. The hallmarks of brain aging can be characterized by several categories: primary, antagonistic, and integrative hallmarks (Azam et al., 2021). The hallmarks of brain aging can be seen in the figure below.

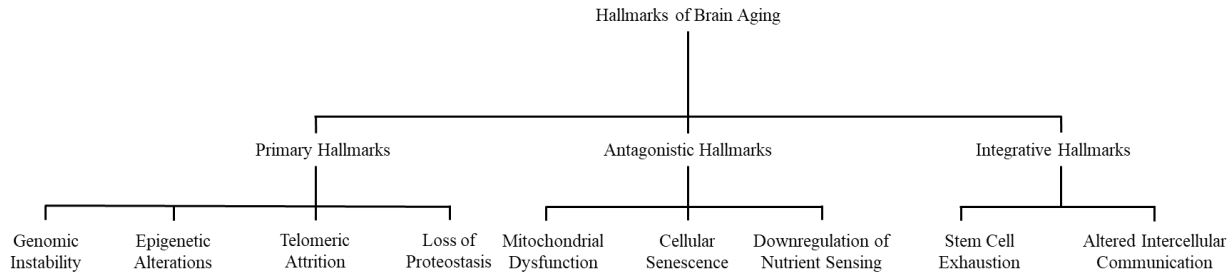

Figure 2. Nine biological hallmarks of brain aging

Primary hallmarks include genomic instability, epigenetic alterations, telomeric attrition, and loss of proteostasis, which is the process by which protein homeostasis is maintained over time. Antagonistic hallmarks describe the compensatory responses that occur in response to primary damage associated with aging and include mitochondrial dysfunction, cellular senescence, and downregulation of nutrient sensing (Azam et al., 2021). Integrative hallmarks are the result of the damage caused by both the primary and antagonistic hallmarks and include stem cell exhaustion and altered intercellular communications. These hallmarks are not specific to one disease but are rather an overall result of the brain aging.

Several of these hallmarks have been associated with the progression of AD, including genetic mutations, epigenetic modifications, cellular senescence, and altered intercellular communications. Key factors in AD include dysregulation of DNA repair machinery and an increase in DNA mutations, which are significant contributors to disease pathogenesis (Azam et al., 2021). Epigenetic modifications include DNA methylation, PARylation, and acetylation and can strongly influence chromatin functions. These modifications cause physical changes in the structure of DNA, thereby changing the activity of the gene. Accelerated DNA damage leads to an elevation in cellular energy demands, prompting mitochondrial fusion, which in turn can contribute to stress-induced neurodegeneration (Azam et al., 2021). Mitochondrial dysfunction is

categorized when mitochondria do not work as well as they should. Mitochondrial dysfunction is very dangerous for multiple reasons: (1) the mitochondria release ATP (energy), so when this process is disrupted, there is a loss of energy in the cell, and (2) when it occurs in the brain, it can impair brain development and cause a decrease in adult neural stem cells and loss of adult neurogenesis, thereby causing a decline in brain function and cognitive impairment (Khacho et al., 2017). Additionally, damaged DNA causes an increase in energy demands, which can lead to more issues when mitochondrial dysfunction is also present.

Cellular senescence is a process by which a cell ages and stops dividing but does not die (Di Micco et al., 2020). The cellular senescence process can accelerate aging and contribute to the development of aging-related diseases (Azam et al., 2021). In the aging brain, cellular senescence occurs typically as a result of prolonged DNA damage. Previous research suggests that aging not only diminishes the ability of cells to repair DNA damage but also introduces complex DNA repair mechanisms that may cause additional mutations (Azam et al., 2021).

Altered intercellular communications is a key component of AD (Azam et al., 2021). As discussed earlier, AD disrupts neuronal connections, ultimately leading to neuronal death. Amyloid plaques form intercellularly between synapses, causing the connections between the neurons to weaken. Changes in synapses often cause changes in behavior. Too many synapses and connections being severed causes significant neural damage as neurons are no longer able to communicate with each other, resulting in neuronal and synaptic loss and cognitive deficits. It is important to note that altered intercellular communications are not only a result of amyloid plaques as they can also be caused by cellular senescence and epigenetic modifications (Azam et al., 2021).

Shortened telomere length (TL) has also been associated with AD. A meta-analysis of 13 studies demonstrated a significant difference in TL between 860 AD patients and 2,022 controls, suggesting a consistent shortening of telomeres in AD patients (Azam et al., 2021). Telomeres are located at the end regions of chromosomes and are composed of proteins and DNA that can become very vulnerable to degradation over time. Telomeres gradually become shorter over time with each cell division. The primary role of telomeres is to protect the terminal regions of chromosomes from degradation and fusion with other chromosomes, while also serving critical functions in genomic replication, repair, and maintenance machinery (Cai et al., 2012). TL has long been seen as a biomarker of aging, and research has shown that it can play a causal role in age-related neurodegenerative diseases, including AD (Cai et al., 2012). In fact, many aging-related illnesses are associated with shortened TL including AD, diabetes, Huntington's disease, Parkinson's disease, and osteoporosis. Specifically with AD, telomere shortening has been associated with cognitive impairment, amyloid plaque pathology, and tau hyperphosphorylation (Cai et al., 2012). Telomere shortening also plays a role in the pathogenesis of AD via the mechanisms of oxidative stress and inflammation (Cai et al., 2012). Recent findings suggest that shorter telomeres in the brain may lead specifically to the aging of neural stem cells, impacting neurogenesis and the maintenance of neurons and synapses in the brain (Cai et al., 2012).

The presence of Aβ may also accelerate telomere shortening in microglia, suggesting that telomere shortening plays a role in the deterioration of microglia, which may in turn alter the brain's natural immune response (Cai et al., 2012). Telomere shortening in T cells - white blood cells which are part of the immune system - correlate with AD status, so it seems that peripheral as well as central telomere shortening impair immune function (Cai et al., 2012). Impairment of

immune function aids in the progression of AD because it prevents the body from naturally getting rid of pathologies associated with AD, such as amyloid plaques and neurofibrillary tangles. Therefore, targeting TL and maintaining TL may be a key therapeutic approach to the treatment of AD.

Short TL has been shown to accelerate aging (Azam et al., 2021). Telomeres naturally shorten over time with every cell division unless parent cells express telomerase to prevent this process (Azam et al., 2021). TA-65 is a dietary supplement that reportedly increases TL by increasing telomerase activity. TA-65 reportedly adds length to critically short telomere segments, which may slow down or possibly reverse aging (Cai et al., 2012). This finding is based on a study that was published in 2011 that reported that TA-65 elongates short telomeres in adult mice, which ultimately resulted in an enhanced health span (de Jesus et al., 2011). TA-65 was first discovered in the year 2000 from an "empirical screen of natural product extracts from traditional Chinese medicines" (Salvador et al., 2016). TA-65 is currently designated as GRAS (generally recognized as safe) for use in medical foods and has been available to the public as a dietary supplement for over a decade. A randomized, double-blind, placebo-controlled study of TA-65 was conducted using 117 subjects aged 53-87 over a one-year period to test its effects on overall TL (Salvador et al., 2016). The results of this study reported that subjects who received the low dose of TA-65 (250 U) significantly increased TL over the 12-month period, whereas subjects in the placebo group had a significant decrease in TL (Salvador et al., 2016). Interestingly, subjects who received the high dose of TA-65 (1000 U) demonstrated improvement in TL compared to the placebo group, but these results were not statistically significant (Salvador et al., 2016). TL has also been positively linked with increased regenerative capacity of cells, reduced mortality and disease risk in humans, and increased resistance to infection, indicating

that maintaining TL may yield great clinical significance (Salvador et al., 2016). The results indicated in this study show a significant difference as it increased TL in the low dose TA-65 group. More work needs to be done to see the relationship between TL, TA-65, and cognitive impairment. Assuming that telomere shortening is directly correlated with cognitive impairment, as shortened telomeres are also associated with other neurodegenerative diseases, increasing TL may be a key therapeutic approach to prevent AD symptoms and pathology as well as other aging-related diseases.

Based on these findings, it seems that the neural aging process is a domino effect. It is not just the amyloid plaques and the neurofibrillary tangles that result in AD. Rather, it is a cascade of neural changes due to the natural aging process that causes the formation of these plaques and tangles. Targeting the removal of amyloid plaques and neurofibrillary tangles as a potential treatment option for AD is still being investigated, but it is important to also to address these other changes that occur as a result of the aging process in order to gain a greater understanding of why these plaques and tangles are forming in the first place.

AD seems to be caused by various different components of aging in addition to a weakened immune system. While there are cellular and neuronal changes in the brain that occur as a result of the natural aging process, there is also a decline in immune system function as one ages, and this affects the brain's ability to get rid of and remove amyloid plaque pathology and tau accumulation. Microglia and astrocytes, which normally act as the brain's natural immune system, do not target or remove amyloid plaques or neurofibrillary tangles, which may imply that they are not functioning at the level in which they typically do.

Genetics & The ApoE Cascade Hypothesis

Is Alzheimer's Disease genetic? In some cases yes, but not always. Early-onset AD has a higher genetic factor than does late-onset AD. About 3% of AD cases are caused directly by single mutations inherited from a parent (Alzheimer's Disease Research Center). These cases usually develop before the age of 65, characterizing them as early-onset AD. Recent studies have confirmed that there are 20 gene variants that slightly raise a person's risk of developing the disease after the age of 65 (Alzheimer's Disease Research Center). Some of the genes that are associated with early-onset AD include amyloid precursor protein (APP) on chromosome 21, presenilin-1 (PS1) on chromosome 14, and presenilin-2 (PS2) on chromosome 1. However, the gene that mostly increases an individual's risk of developing AD is apolipoprotein E (ApoE) on chromosome 19, and recent research has developed "The ApoE Cascade Hypothesis" (Martens et al., 2022). The ApoE Cascade Hypothesis states that ApoE's biochemical and biophysical properties trigger a cascade of events at the cellular and systems levels, ultimately leading to age-related pathological conditions such as AD (Martens et al., 2022). Specifically, it states that ApoE's biochemical and biophysical properties of structure, lipidation, protein levels, receptor binding, and oligomerization cause cellular homeostasis perturbation in the forms of intracellular trafficking dysregulation, increased cellular stress, and impaired lipid metabolism. This disruption in cellular homeostasis causes neuroinflammation, vascular dysfunction, and neuropathology, ultimately resulting in synaptic dysfunction and neurodegeneration.

It was first discovered in 1993 that individuals who carry the ε4 allele of ApoE will have an increased risk of developing AD, and this hypothesis has continued to develop and has become the primary genetic explanation for AD (Roses, 2006). ApoE is a 299-amino acid glycoprotein composed of an N-terminal domain, a hinge region, and the C-terminal domain

(Martens et al., 2022). ApoE is an important apolipoprotein that was initially studied for its role in transporting cholesterol and other lipids through the periphery and within the brain (Emrani et al., 2020). There are different variations of the ApoE gene (Alzheimer's Disease Research Center):

- ApoE ε2: this variation is relatively rare and may actually provide some protection against the disease. People with this variation who develop AD likely develop the disease at a much later age than if they had the ε4 variation of the gene. About 8% of the population has this variation of the gene.
- ApoE ε3: this is the most common variation of the gene and has been shown to have no effect on the development of AD. This variation of the gene neither increases nor decreases one's risk of developing the disease. About 78% of the population has this variation of the gene.
- ApoE ε4: this variation not only increases the risk for developing late-onset AD, but it is also associated with an earlier age of disease onset. A person can have multiple ApoE ε4 alleles, with each additional allele increasing the risk of developing the disease. About 14% of the population has this variation of the gene, and 37% of late-onset AD cases carry this variation, leaving room for a much greater explanation.

The link between the ε4 allele of ApoE (ApoE4) and AD susceptibility has been measured extensively, and the findings have remained relatively consistent over recent decades. The ApoE4-associated risk of AD is stratified by traits such as age, gender, and ancestry. For example, in terms of gender, studies have found that the effects of ApoE4 on AD susceptibility is more pronounced in women than in men, although these gender differences seem to lessen after

age 75 (Emrani et al., 2020). However, this still does not explain why people who have ApoE allele 4 are at an increased risk of developing AD later in life.

ApoE is primarily produced by various neuronal cell types including astrocytes, reactive microglia, vascular mural cells, and choroid plexus in the brain. ApoE plays an important role in the transportation of lipids throughout the brain, but under stress conditions, ApoE production increases, which is a possible response to repair damaged membranes caused by the stress-related event (Martens et al., 2022). ApoE and related lipoprotein particles bind to ApoE receptors, and this is an essential mechanism for cell-to-cell lipid distribution in the brain. Therefore, the ApoE Cascade Hypothesis suggests that the disruption in cellular lipid homeostasis, mediated by ApoE, triggers a pathogenic cascade that ultimately leads to AD-related cellular dysfunction (Martens et al., 2022).

As mentioned in the section above, age-related neurodegeneration is caused as a result of a cascade of issues related to cell function dysregulation. ApoE4 plays a role in some of the mechanisms related to this cellular disruption. One of the central pathways in the cellular phase of AD pathogenesis is endosomal-lysosomal dysregulation (Martens et al., 2022). A transcriptomics study conducted in 2017 found that genes involved in endosomal-lysosomal pathways are enhanced in the brains of ApoE4-TR (transgenic) mice compared to ApoE3-TR mice (Nuriel et al., 2017). ApoE4 also has several other age-related effects that overall impair the trafficking of cell surface receptors: (1) it reduces cell surface levels of ApoER2, a neuronal signaling receptor for Reelin and ApoE, as well as glutamate receptors, by sequestering them in endocytic compartments, leading to decreased synaptic activity, and (2) it suppresses cell surface insulin receptors (IRs) and impairs IR trafficking, causing significant suppression of the effects of insulin-induced glycolysis and mitochondrial respiration (Martens et al., 2022).

ApoE also causes dysregulation in the endoplasmic reticulum (ER)-mitochondria axis, which is essential in maintaining cellular homeostasis. As discussed earlier, mitochondrial dysfunction is one of the key components of age-related neurodegeneration. Mitochondrial dynamics, such as fission and fusion, are altered in the presence of ApoE4, which is also accompanied by impaired mitophagy in mouse brains (Martens et al., 2022). ApoE4 enables the physical interaction between mitochondria and ER through mitochondria-associated ER membranes [MAMs] (Martens et al., 2022). MAMs have critical roles in regulating proper cellular functions such as calcium signaling and energy homeostasis, and disruption in these membranes results in significant cell function-related issues.

ER-stress and mitochondria dysregulation are also associated with the formation of lipid droplets, which are known to accumulate in various brain cell types including neurons, astrocytes, and microglia during aging and AD (Martens et al., 2022). Naturally, lipid droplets aid and regulate cellular metabolism and buffer lipotoxicity, but can be pathogenic when dysregulated, possibly due to dysfunction of lipid metabolism (Farmer et al., 2020; Martens et al., 2022). Interestingly, ApoE4 is associated with higher lipid droplet formation in astrocytes, but is suppressed by ApoE4 in neurons. ApoE4 is also associated with decreased fatty acid degradation and lipid droplet accumulation, resulting in lipid dysregulation and accumulation of lipid droplets in astrocytes, as well as increased mitochondrial stress (Martens et al., 2022). This is an issue because impaired lipid metabolism can cause synaptic dysfunction and neurodegeneration through both neuropathology-dependent and independent mechanisms (Martens et al., 2022). A recent study found a correlation between neutral lipid accumulation and inflammatory signaling, indicating that lipid droplets may play a role in neuroinflammatory responses (Farmer et al., 2020). Astrocytes and microglia aid in maintaining the central nervous

system's homeostasis and cellular function as well as act as the brain's natural immune system - both of which seem to be most affected by ApoE. Emerging evidence indicates that the brain's immune response during aging and AD is affected by ApoE that is produced by microglia or other cells that act on microglia (Martens et al., 2022). Perhaps this explains why rather than clearing up amyloid plaques in the brain, they aid in their formation as their lipid metabolism seems to be disrupted by the effects of ApoE.

Emrani et al conducted a systematic review of studies that looked at AD patients who possess the ApoE4 allele (ApoE4+) versus AD patients who did not possess the ApoE4 allele (ApoE4-) (Emrani et al., 2020). First, they found that ApoE4+ AD patients appear to possess more tau accumulation and brain atrophy in the medial temporal lobe, resulting in greater memory impairment, whereas ApoE4- AD patients possess more tau accumulation and brain atrophy in the frontal and parietal lobes, resulting in greater impairment in executive function, visuospatial abilities, and language (Emrani et al., 2020). This suggests there may be a fundamental divergence in AD manifestation related to ApoE genotype. They also reported that different ancestral backgrounds produce unique phenotypes for those who possess the ApoE4 allele. Individuals from African ancestry populations have a higher frequency of possessing the ApoE4 allele, with a rate of about 19%, compared to Caucasian populations, but surprisingly, these individuals have a relatively lower risk of developing AD. The opposite appears to be true for East-Asian populations where people of Japanese ancestry have a relatively low frequency of possessing the ApoE4 allele, with a rate of about 9%, but are at a relatively higher ApoE4-related AD risk (Emrani et al., 2020). Another study found that the $\varepsilon 4$ allele on the ApoE gene does indeed increase the risk of developing the disease, but only in people of European ancestry (Alzheimer's Disease Research Center).

While these findings raise more questions than answers, they suggest that genes only make up a small percent of the risk factor of developing AD. The paper also found that ApoE4+ AD patients do not appear to differ in the overall rates of cognitive decline compared to ApoE4- AD patients. A meta-analysis of 3 studies that included 1,244 AD patients found that there were no ApoE4-associated differences in the rate of cognitive decline in AD, suggesting that, when analyzed in a broad fashion, AD patients who posses the ApoE4 allele do not seem to exhibit a more severe form of the disease (Emrani et al., 2020). Additionally, carriers of the ApoE4 allele may manifest symptoms of cognitive decline that are different compared to non-carriers. For example, since there seems to be more neural damage associated with the medial temporal lobe of ApoE4+ AD patients, this damage manifests as having more memory-related issues, whereas neural damage seen in ApoE4- AD patients is associated more with other areas of cortex and results in other cognitive-related issues (Emrani et al., 2020).

With regards to amyloid plaque pathology and tau accumulation, many studies show conflicting results with regards to ApoE4's role in their formation. Some studies reported that ApoE4+ AD patients do not appear to have higher A□ levels than ApoE4- AD patients, while other studies reported the exact opposite findings (Emrani et al., 2020; Leoni, 2011). However, it has been well established that carriers of the ApoE4 allele accumulate A□ in their brains at an earlier age than non-carriers, and this may occur long before the onset or diagnosis of AD (Emrani et al., 2020). Interestingly, by the age of 40, 15% of ApoE4 allele carriers will already be positive for cerebral A□, but overall A□ levels have been found to plateau before reaching a clinical diagnosis of AD, indicating that disparities in A□ levels associated with ApoE genotype are not expected to be as significant once a patient transitions to clinical AD as they are during the linear phase of A□ accumulation (Emrani et al., 2020).

ApoE has been found to contribute to amyloid pathology, tau accumulation, and overall neurodegeneration and is to-date the strongest genetic risk factor for AD, impacting 50% - 70% of all cases (Martens et al., 2022). It seems as though possessing the ApoE4 allele increases the rate at which one develops AD, increases the risk of developing early-onset AD, and provides a greater effect on memory-related areas of the brain such as the medial temporal lobe. However, it seems as though this allele works with the natural aging process in the progression of AD. AD is not caused by this gene; rather, this gene acts with the natural aging process to produce specific outcomes of the disease, such as more enhanced memory-related issues rather than other cognitive concerns, such as visuospatial issues or problems with language.

Having the ApoE4 allele, and even having multiple $\varepsilon 4$ alleles, may increase one's likelihood of developing the disease, but being a carrier does not mean that you are destined to develop the disease. Like most other diseases, genetic factors only play a small role in their development, with environmental, ancestry, nutrition, and lifestyle being the remaining risk factors. I believe that the natural aging process along with other aging-related neurodegenerative diseases comprise the main factors in developing AD. Whether AD is a genetic disorder or simply a result of the natural aging process is still unknown. Based on the evidence above, it seems as though both are significant factors.

Aducanumab, Lecanemab, & FDA Controversy

Targeting the removal of amyloid plaques may be a key therapeutic approach in aiding in the treatment of AD. Unfortunately, many drugs that were developed to treat this issue failed in clinical trials. Examples of such drugs are Verubecestat and CNP520 (Azam et al., 2021). Verubecestat is a BACE inhibitor that demonstrated ineffectiveness and toxicity in preclinical participants with AD, while CNP520, also a BACE inhibitor, resulted in increased cognitive

decline symptoms in participants with early AD (Azam et al., 2021). BACE1 is an enzyme essential for the generation and production of A□, so the purpose of these drugs was to inhibit this enzyme, resulting in the loss of production of A□, and therefore, the prevention of the formation of amyloid plaques (Das & Yan, 2019). One possible explanation for why BACE inhibitors failed as a treatment option for AD is because by blocking BACE1, other important functions of this enzyme are also being blocked, resulting in unwanted results. Additionally, these medications are given to AD patients where amyloid plaques have already formed, so preventing the production of A□ at such a late stage did not yield desired results possibly due to the previous accumulation of A□.

However, there was one drug that was developed and approved by the FDA for the treatment of AD: Aducanumab. Aducanumab, or Aduhelm, is an amyloid beta-directed monoclonal antibody that crosses the blood-brain barrier and selectively binds to A□ plaques, reducing their volume by targeting for removal by immune cells. It is this feature of selectivity that makes Aducanumab different from previously developed drugs. The FDA granted accelerated approval to Aducanumab in 2021 after it was shown to reduce A□ plaques in patients with MCI or mild dementia associated with AD (Padda & Parmar, 2021). The accelerated approval process by the FDA is granted in situations where there is uncertainty regarding the efficacy of a treatment, but there is anticipated clinical benefit, especially for a condition in which there are no currently available effective therapies (Mahase, 2021). However, its approval raised much controversy and led to three FDA advisory board members resigning: Harvard Professor of Medicine Aaron Kesselheim, Mayo Clinic neurologist David Knopman, and Washington University neurologist Joel Perlmutter.

These three FDA board members fought against Aducanumab's approval because of a lack of efficacy. Kesselheim wrote a letter to acting FDA commissioner Janet Woodcock saying that the approval of Aducanumab was "probably the worst drug approval decision in recent US history." It is important to note that Aducanumab treatment costs around $56,000 per year per patient, which means that Biogen and Eisai, the pharmaceutical companies that developed this drug, would make big profits following its approval (Sinha & Barocas, 2022). It is quite obvious that there would be a significant financial gain made off of Aducanumab's approval, but the question regarding why the FDA would approve a drug that is not effective remains unanswered. According to FDA documents, Aducanumab underwent two phase III clinical trials, both of which showed poor results. So how exactly did this drug get FDA approval?

Both of these clinical trials were stopped early due to lack of efficacy concerns. In other words, the results produced by the studies showed no statistical significance in improving cognition in AD patients. However, an analysis of one of these studies showed positive results in the group that received the highest dose of 10 mg/kg, in which there was a 23% reduction in clinical decline compared to placebo (Tagliavini et al., 2021). The FDA Nervous System Drug Advisory Committee deemed the approval followed by this outcome as questionable and premature, especially given the negative results of the study that did not meet the clinical endpoint and the significant occurrence of adverse effects with the effective dose, mostly amyloid-related imaging abnormalities (Tagliavini et al., 2021). It is important to note that health insurance companies and Medicare refused to pay for Aducanumab treatment, meaning that only people who choose to participate in trials to replicate the results and people who can afford to pay out of pocket are able to receive Aducanumab treatment. As a result of these accessibility

and equity issues, very few AD patients are currently taking Aducanumab. Other drugs are currently undergoing clinical trials as a possible treatment and/or preventative measure for AD.

Lecanemab, or Leqembi, another drug aimed at trying to reduce the volume of amyloid plaques, has just been approved in January 2023 by the FDA through their accelerated approval program. Similar to Aducanumab, Lecanemab is also a monoclonal antibody that binds with high affinity to amyloid plaques. It is important to note that Lecanemab was developed by Biogen and Eisai, the same pharmaceutical companies that developed Aducanumab. Previous evidence has indicated that Lecanemab can decrease the levels of pathogenic A□, prevent A□ deposition, and selectively lower A□ protofibrils in the brain and CSF of AD animal models (Shi et al., 2022). While there have been numerous successful clinical trials completed, several others have yielded largely negative results and failed to demonstrate significant clinical benefits for patients that clinically manifest or prodromal dementia (Shi et al., 2022). In the phase I and II trials of Lecanemab, the results showed that there was complete removal of A□ plaques in the brain, and it also alleviated cognitive decline (Shi et al., 2022). The phase III clinical trial of Lecanemab was an 18-month double-blind, placebo-controlled trial that involved 1795 participants, all between the ages of 50 and 90, and all of whom have been diagnosed with early stage AD or MCI (van Dyck et al., 2023). All participants tested positive via PET scan or CSF testing for amyloid beta. Of the 1795 participants, 898 were assigned to receive intravenous Lecanemab (10 mg per kilogram of body weight every 2 weeks) and 897 to receive placebo. During the trial, 89% of participants had adverse effects after receiving the dose, and 12.6% of participants had some sort of edema or effusion abnormalities compared to 1.7% of people taking the placebo injection (van Dyck et al., 2023). Some studies have also found that Lecanemab caused some fatal side effects in patients, such as brain swelling and hemorrhage, that resulted in three deaths

linked to the drug (Piller, 2022). Overall, it was concluded that Lecanemab reduced markers of amyloid in patients with MCI and early AD and resulted in a 27% cognitive decline improvement compared to placebo (van Dyck et al., 2023). Because the trial focused on AD patients in the MCI stage and demonstrated the most benefit to patients in this stage, this may restrict eligibility for patients seeking treatment with Lecanemab to only this subgroup of AD patients. The current price of receiving Lecanemab treatment is $26,500 per year, which is significantly cheaper than the previous Aducanumab treatment (ALZFORUM, 2023).

While the cognitive impairment delay seen with the use of Lecanemab is not clinically significant, there are other issues surrounding the validity of how these results were reached. In order to measure cognitive decline and cognitive improvement, the participants were scored on the Alzheimer's Disease Cooperative Study-Activities of Daily Living Scale for Mild Cognitive Impairment (ADCS-ADL), where lower scores indicate greater impairment. Participants who took the test before and after receiving the Lecanemab dose showed an increase in 1 to 2 points on the test. However, the people who made the ADCS-ADL questionnaire noted that differences less than 2 points are not very meaningful, and therefore, are not significant. In other words, while there was slight improvement on the scores of the ADCS-ADL questionnaire, the improvement was so minimal that it was not statistically or clinically significant. There is still a very high need for more follow-up trials to further prove the safety and effectiveness of the drug in the treatment of early AD.

Even with the recent FDA approval of Lecanemab, the current hypothesis of removing amyloid plaques is being questioned by the scientific community as a potential therapeutic approach for improving cognitive symptoms of AD, as new drugs are currently undergoing development and clinical trials targeting neurofibrillary tangles. In fact, suspicions have risen

over the entire claim of the amyloid cascade hypothesis. The first study that came out claiming that amyloid plaques in the brain were the primary cause of AD was in 2006 by neuroscientist Sylvian Lesné of the University of Minnesota, Twin Cities. However, in 2021, it was found that this paper, along with almost 70 others from Lesné, contained images that were tampered with (Piller, 2022). It was found out that the results published in the original study had been edited in order to better fit their desired hypothesis (Piller, 2022). This has resulted in numerous issues, the biggest one being the fabrication that resulted in decades of research following a fake hypothesis. This has led to major discrepancies in the scientific community that calls into question the validity of the amyloid cascade hypothesis, as well as amyloid's role in AD.

Based on the approval of Aducanumab and Lecanemab, the failed clinical trials of other drugs aimed at removing amyloid plaques, and the recent discovery of fabricated results, it is becoming more clear that the removal of amyloid plaques is not the cure. Therefore, there needs to be a shift by the scientific and medical community to broaden the current treatment approach of AD. While the approval of Lecanemab did show a slight delay in cognitive impairment in early AD patients, there is still a lot that needs to be looked at regarding Lecanemab's safety and effectiveness. Rather than focusing on the removal of amyloid plaques and neurofibrillary tangles, there should be a shift to focusing more on immunotherapy and trying to treat the body's natural immune system to overcome the pathologies that are seen in AD. The risk for developing AD increases dramatically with age, meaning that the natural aging process plays a major role in the development of the disease.

I see two primary issues that arise from the current methodology put in place for the development of drugs and treatment regimens surrounding AD. First, the development of drugs to reduce the volume of amyloid plaques are based on animal models. The issue with animal

models is that the amyloid plaques that these drugs are allegedly reducing are done on artificially manifested amyloid plaques, which means that the cellular and molecular structure of these plaques are not the same as those seen in a human model of AD. Because these drugs are working on artificially formed plaques, they will likely not have the same effects on human patients with AD. Consequently, the approach surrounding the development of these drugs needs to change to more accurately fit the true cellular and molecular basis of the disease. In humans, AD develops likely over the course of the lifetime, whereas in animal models, AD develops almost instantaneously. This may also be the reason why FDA clinical trials conducted on humans have failed to produce positive results. Second, the development of these drugs that target the removal of amyloid plaques, and even neurofibrillary tangles, are targeting a really late stage of AD where the neural damage of synaptic dysfunction and cell death have already occurred. Even if there were a drug developed that removed 100% of amyloid plaques in an AD patient, the cognitive decline symptoms would likely not improve because the neural damage is done. Rather than targeting AD pathology at a late stage, we should focus on developing therapeutic strategies that *prevent* the formation of these plaques and tangles in the first place rather than trying to remove them after they have already been formed. Research should also further investigate immunotherapy regimes that train the brain and body to maintain neural health and synaptic function.

Seattle Alzheimer's Disease Brain Cell Atlas

The scientific community has begun to slowly shift to looking at the causes of AD rather than the symptoms, with the Seattle Alzheimer's Disease Brain Cell Atlas (SEA-AD) being one of the first projects to look into this new approach. The SEA-AD is a collaborative project developed in 2020 by the Allen Institute for Brain Science, University of Washington Medicine

Alzheimer's Disease Research Center, and Adult Changes in Thought (ACT) Study from Kaiser Permanente Washington Research Institute. The SEA-AD aims to characterize the molecular and cellular changes that occur throughout the progression of AD, provide a comprehensive map of the brain cells affected by AD, and gain a deeper understanding of the disease's progression. There are three main arms of the SEA-AD: (1) classic histopathology, such as marker staining of amyloid, tau, glial cells, and neurons, (2) single nuclear transcriptomics, and (3) spatial transcriptomics. The SEA-AD is committed to open science, and all of its information, including statistics and findings, can be found on their website (https://portal.brain-map.org/explore/seattle-alzheimers-disease).

Because the data from the SEA-AD is open to the public, I was able to find information about the cohort. The Atlas has 84 brain donors all over the age of 65, half of whom have dementia and the other half are cognitively typical. In the cohort, 96% of donors identify as white/Caucasian, 3% identify as Asian, and 1% identify as American Indian/Alaska Native. Additionally, 38% are males, 62% are females. The donors are put on a pathological spectrum ranging from not affected to severely affected by AD, as seen in Figure 3. Of the 42 donors without dementia, about 27% have the ApoE4 allele. Of the 42 donors with dementia, about 62% have the ApoE4 allele. This relationship is shown in Figure 4. After running a chi-squared test, I found that $\chi^2(1, N = 84) = 2.79$, where $p = 0.095$. This means that there is a trend of diagnosis being correlated with the presence of the ApoE4 allele and a diagnosis of dementia. Donors with dementia have on average 5% lower brain weights compared to the donors without dementia, as shown in Figure 5, which is consistent with published findings of AD-associated loss of neurons and overall brain atrophy in different regions (Emrani et al., 2020). Overall brain pH of donors with dementia was lower ($M = 6.6$) compared to donors without dementia ($M = 6.75$), as seen in

Figure 6. While the difference between brain pH is not significant ($t(82) = -1.65$, $p = .104$) between donors with dementia versus donors without dementia, normal brain pH is around 7.2, which means that the pH of the brains in the dataset are a little lower than normal. This could potentially be a separate aging factor, not necessarily directly related to AD pathology. This may also be an effect of hypometabolism and increased anaerobic metabolism that could lead to an increase in brain lactate and overall acidity. Donors with dementia had on average more years of education ($M = 16.6$) compared to donors without dementia ($M = 15.8$) , as seen in Figure 7. While the difference between years of education between donors with dementia versus donors without dementia is not significant ($t(82) = 1.38$, $p = .17$), it contradicts previous hypotheses that state that individuals with lower education levels were more likely to develop AD or have quicker AD progression (Kim et al., 2020).

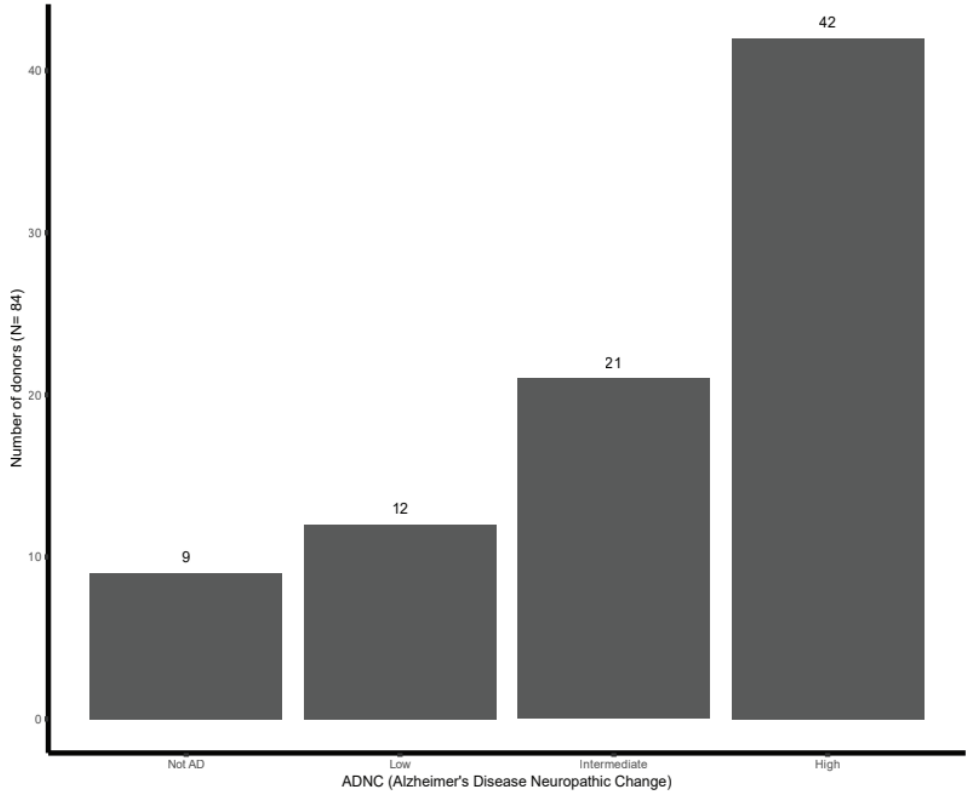

Figure 3. Graph adapted from SEA-AD. Graph depicts the number of donors on the AD pathological spectrum.

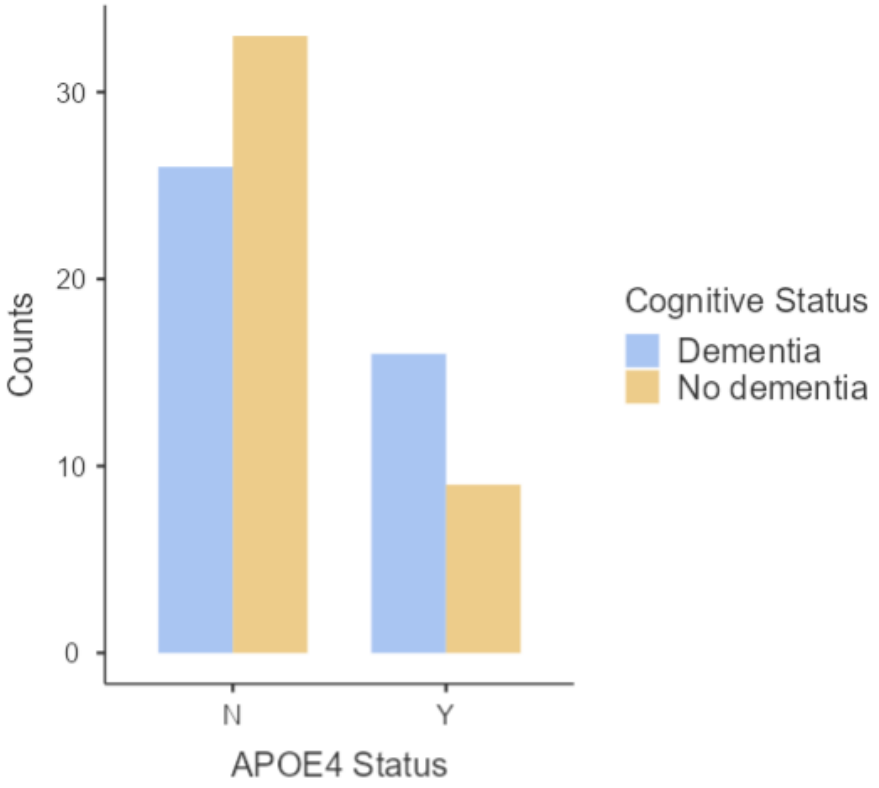

Figure 4. Data adapted from SEA-AD. Graph shows the relationship between APOE4 status and cognitive status.

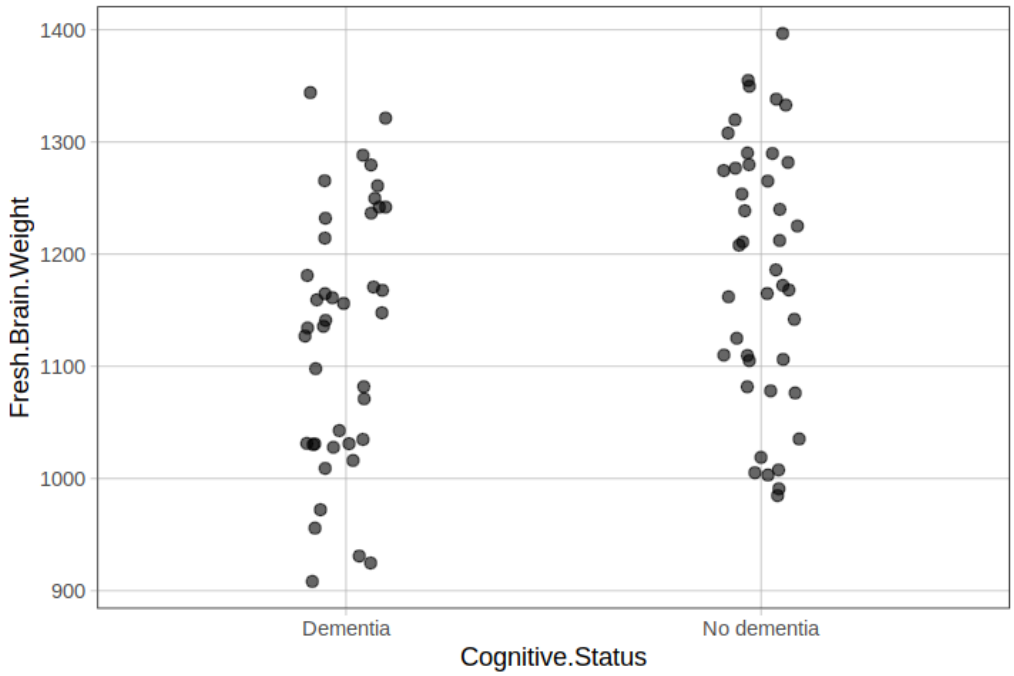

Figure 5. Data adapted from SEA-AD. Graph shows the relationship between brain weight and cognitive status.

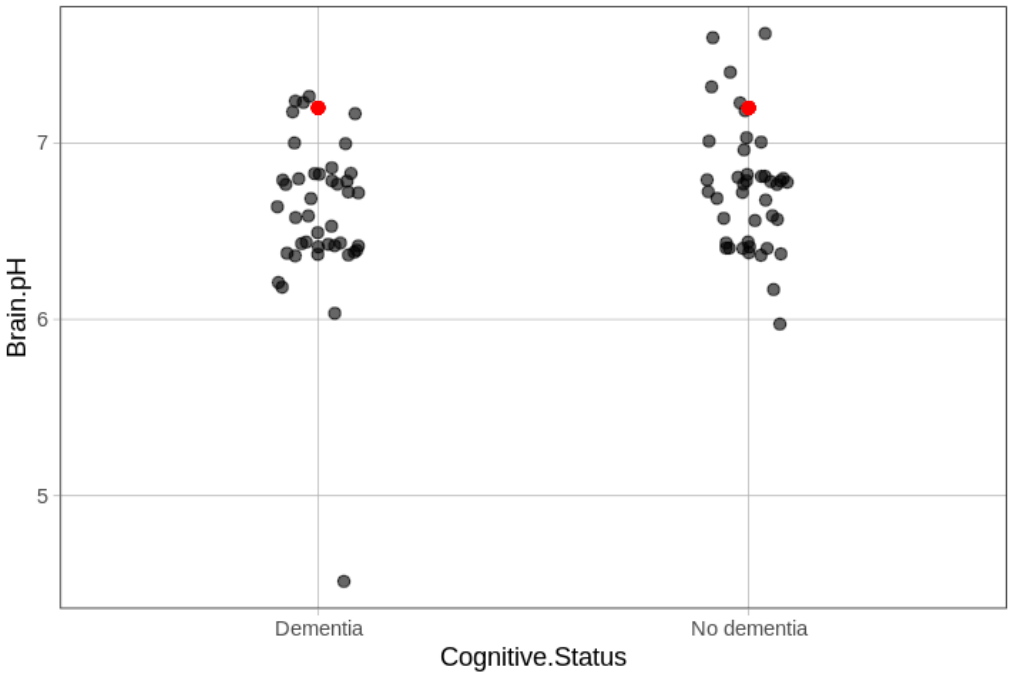

Figure 6. Data adapted from SEA-AD. Graph shows the relationship between brain pH and cognitive status. The red dot indicates normal brain pH.

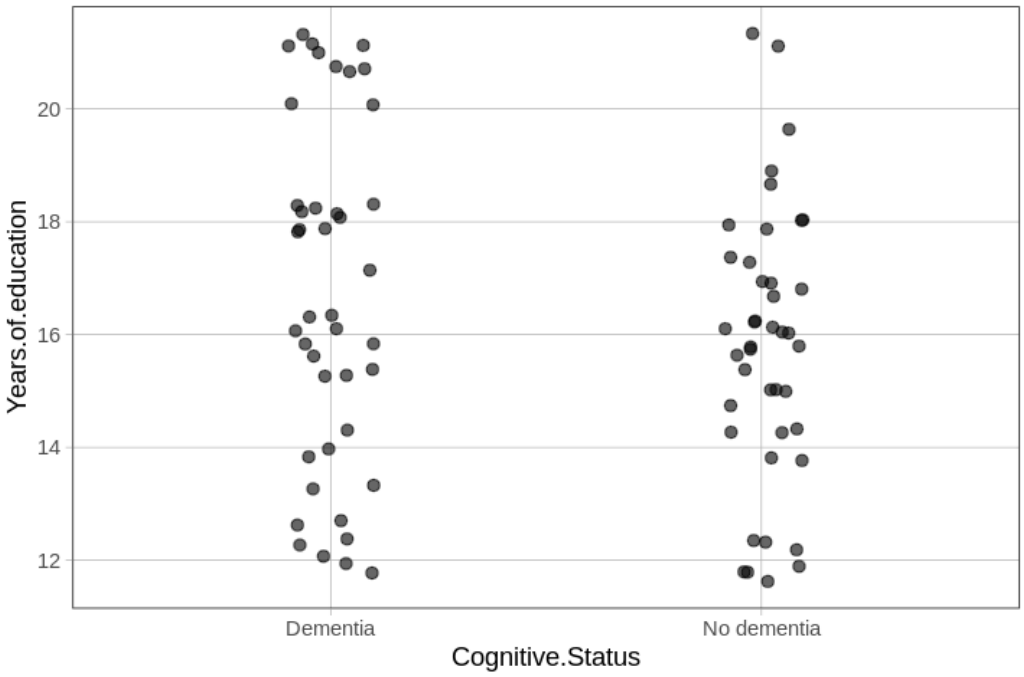

Figure 7. Data adapted from SEA-AD. Graph shows the relationship between years of education and cognitive status.

Of the 84 donors, there is a small group of highly affected donors that make up about 1/3 of the patients with dementia (14 out of 42 donors), who exhibit *severely* high pathology. Interestingly, these highly affected donors have systematically less nuclear RNA, have many heterochromatin regions, and experience steeper cognitive decline compared to the rest of the donors. The reduction in nuclear RNA may indicate that the cell being examined is less active and is deteriorating. Additionally, increased RNA in the cytosol than the nucleus may indicate that the mRNA in the cell is not getting broken down, meaning that there is some form of dysfunction going on within the cell. This could potentially be either a ribosomal issue (indicating that the cell is unable to manufacture proteins) or a lysosomal issue (indicating that the cell is not able to break down and get rid of waste products). It is also very difficult to stain for proteins in these donors as there is not much transcriptional information in their cells, which appear to be completely shutting down. The explanation behind why there is a group of donors with highly affected pathology is still unknown, although there may be a remote history of infection or traumatic brain injury that may have contributed to such high AD pathology. The hypothesis that a remote history of infection may increase an individual's likelihood of developing AD has been on rise in recent years. This is based on the idea that infection may increase AD pathology by triggering the production of amyloid clumps, which could indicate that amyloid serves a significant purpose of protecting the brain from infection (Abbott, 2020).

The SEA-AD examines precise cellular changes in distinct brain regions, as well as alterations occurring in response to selective populations of cell types undergoing degeneration. The first brain region examined in the Atlas was the medial temporal gyrus, which is also the first brain region that is severely affected by AD pathology. There are roughly 120 different neuronal types in the medial temporal gyrus. Of these 120, about a third are lost in the AD

donors primarily in layer 2/3 of the cortex. Layer 3 of the cortex is important for cortico-cortical connections, which relates to the previous discussion of the spread of neurofibrillary tangles via synaptically connected neurons. Some of these neuronal cell types include excitatory pyramidal cells and inhibitory interneurons such as somatostatin (SST) and parvalbumin (Pvalb). Interestingly, SST interneurons are the cells that are affected last by AD progression in the medial temporal gyrus. In fact, the loss of SST interneurons occurs at the end of the pseudoprogression of AD, meaning that this occurs during the stage of AD in which cognitive decline symptoms appear to temporarily improve, but is followed by further decline.

The SEA-AD comprehensively looks at different cell types and how they relate to the progression of AD. The cell map shows that neurons in the medial temporal gyrus structures, such as the hippocampus and the entorhinal cortex, are among the first to be affected and the first to die off in the progression of AD. Additionally, these neurons show very high levels of amyloid plaques and neurofibrillary tangles. The medial temporal gyrus has structures that are important for memory, attention, and spatial navigation. The SEA-AD also maps the effects of astrocytes and microglia. The Atlas shows that both astrocytes and microglia undergo significant changes in the pathology of AD. Specifically with microglia, they may become activated in a way that contributes to neuroinflammation and cell death. Specific neuronal cell types in specific areas, including intratelencephalic excitatory neurons, inhibitory neurons, and microglia subsets have issues with their gene expression and chromatin accessibility and manifest a change in cell populations, suggesting that these neurons may be especially vulnerable to AD progression (Travaglini et al., 2022). These issues of gene expression and chromatin accessibility may lead to additional consequences within the cell such as dysregulated protein production and compromised immune response functionality. Additionally, the altered function of these cell

types as a result of AD progression may result in cognitive decline, altered gene expression, and neuropathology that all manifest in AD.

While the SEA-AD cannot determine causation with respect to the differences in pathology between the donors, it is changing the way the scientific community looks at AD in terms of characterization, prevention, and treatment. By looking at AD through the lens of the most fundamental cellular level, we are able to better understand how this disease starts, develops, and manifests in the brain. AD researchers may use the information obtained from this Atlas in order to identify new therapeutic targets for AD. By better understanding the cellular and molecular changes that occur in different cell types affected by AD, scientists may be able to develop drugs that target key signaling pathways or specific cell types that are especially vulnerable to AD pathology.

Conclusion

Based on the research done thus far, the most effective method of diagnosing AD at a pre-clinical stage is by the detection of biomarkers such as amyloid plaques and neurofibrillary tangles through the use of brain imaging techniques and CSF analytics. While possessing the ApoE4 allele increases one's risk of developing AD, it is not a causative gene, and a wide array of other factors, including ancestry, environment, lifestyle, nutrition, and the overall natural aging process, contribute to the onset of the disease. Additionally, the correlation between the accumulation of tau pathology and cognitive decline could potentially be used to measure and detect other neurodegenerative disorders. To date, the two FDA approved treatments of AD in terms of reducing AD pathology are Aducanumab and Lecanemab, although there is a great deal of controversy surrounding the efficacy of these treatments and the validity of amyloid plaques as a therapeutic target. Currently, there are also medications being developed to specifically

target tau accumulation rather than targeting the removal of amyloid plaques, although none of these drugs have been approved as a treatment option for AD. The SEA-AD reveals new information regarding the cellular and molecular pathology of the disease and how it manifests in the brain, which may aid in developing new ways to prevent and treat the disease. By learning more about how AD progresses in the brain at various stages of the disease, new therapeutic approaches may be put in place in order to target cells most vulnerable to AD pathology to slow down disease progression over time. This will allow scientists to focus more on treating the cause of the disease rather than treating the symptoms at a much later stage. Based on current research, it appears that finding a cure remains many years in the future. However, it is important to not completely rule out the possibility of a cure but rather focus more on the technology at hand to detect AD early and prevent the disorder from getting worse before symptoms settle in and become irreversible.

The journey our brains and bodies endure during our last decades of life is a unique one. The deep complexities and difficulties that become of us are still new to the scientific community. From a neurobiological standpoint, changes in our neural activity and physiology are deeply connected to who we are as people. Not only is AD taking over the brains of millions of people, but also it is also taking over the world on social and economic fronts due longer life expectancy and the exponential rise in the aging population worldwide. This is why it is so important to urgently develop preventative measures against AD as well as other neurodegenerative disorders.

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References

Abbott, A. (2020). Are infections seeding some cases of Alzheimer's disease? *Nature*, 22-25.

Abyadeh, M., Gupta, V., Gupta, V., Chitranshi, N., Wu, Y., Amirkhani, A., ... & Mirzaei, M. (2021). Comparative analysis of aducanumab, zagotenemab and pioglitazone as targeted treatment strategies for Alzheimer's disease. *Aging and disease, 12*(8), 1964.

ALZFORUM (2023). U.S. FDA gives green light to Leqembi, aka Lecanemab. https://www.alzforum.org/news/research-news/us-fda-gives-green-light-leqembi-aka-lecanemab.

Alzheimer's Association (2022). 2022 Alzheimer's disease facts and figures. https://www.alz.org/alzheimers-dementia/facts-figures.

Alzheimer's Association. (2020). Federal alzheimer's and dementia research funding reaches $3.1 billion annually. https://www.alz.org/news/2020/federal-alzheimers-and-dementia-research-funding-r.

Alzheimer's Disease Research Center. (n.d.). Genetic risk factors. *University of Washington.* https://depts.washington.edu/mbwc/adrc/page/genetics.

Anoop, A., Singh, P. K., Jacob, R. S., & Maji, S. K. (2010). CSF biomarkers for Alzheimer's disease diagnosis. *International journal of Alzheimer's disease*, *2010*.

An, Y., Zhang, C., He, S., Yao, C., Zhang, L., & Zhang, Q. (2008). Main hypotheses, concepts and theories in the study of Alzheimer's disease. *Life Sci J, 5*(4), 1-5.

Auti, S. & Kulkarni, Y. (2019). Neuroprotective effect of cardamom oil against aluminum induced neurotoxicity in rats. Front. *Neurol*. *10*:399. doi: 10.3389/fneur.2019.00399.

Awada, A. A. (2015). Early and late-onset Alzheimer's disease: What are the differences?. *Journal of neurosciences in rural practice, 6*(03), 455-456.

Azam, S., Haque, M.E., Balakrishnan, R., Kim, I-S., & Choi D-K (2021) The ageing brain: molecular and cellular basis of neurodegeneration. Front. *Cell Dev. Biol*. 9:683459. doi: 10.3389/fcell.2021.683459.

Bivona, G., Lo Sasso, B., Gambino, C.M., Giglio, R.V., Scazzone, C., Agnello, L., & Ciaccio, M. (2021). The role of vitamin D as a biomarker in Alzheimer's disease. *Brain Sci. 11*, 334. https://doi.org/ 10.3390/brainsci11030334.

Cai, Z., Yan, LJ. & Ratka, A. (2013). Telomere shortening and Alzheimer's disease. *Neuromol Med, 15,* 25–48. https://doi.org/10.1007/s12017-012-8207-9.

Camussi, G., Deregibus, M. C., Bruno, S., Cantaluppi, V., & Biancone, L. (2010). Exosomes/microvesicles as a mechanism of cell-to-cell communication. *Kidney International*, *78*(9), 838–848. https://doi.org/10.1038/ki.2010.278.

Das, B., & Yan, R. (2019). A close look at BACE1 inhibitors for Alzheimer's disease treatment. *CNS drugs*, *33*(3), 251-263.

de Jesus, B. B., Schneeberger, K., Vera, E., Tejera, A., Harley, C. B., & Blasco, M. A. (2011). The telomerase activator TA‐65 elongates short telomeres and increases health span of adult/old mice without increasing cancer incidence. *Aging cell*, *10*(4), 604-621.

DeVos, S., Corjuc, B., Oakley, D., Nobuhara, C., Bannon, R., Chase, A., Commins, C., Gonzalez, J., Dooley, P., Frosch, M., & Hyman, B. (2018). Synaptic tau seeding precedes tau pathology in human Alzheimer's disease brain. Front. *Neurosci*. *12*:267. doi: 10.3389/fnins.2018.00267.

Di Micco, R., Krizhanovsky, V., Baker, D., & d'Adda di Fagagna, F. (2021). Cellular senescence in ageing: from mechanisms to therapeutic opportunities. *Nature Reviews Molecular Cell Biology*, *22*(2), 75-95.

Ellison, J. M. (2021). The history of alzheimer's disease. *BrightFocus Foundation.* https://www.brightfocus.org/alzheimers/article/history-alzheimers-disease.

Emrani, S., Arain, H. A., DeMarshall, C., & Nuriel, T. (2020). APOE4 is associated with cognitive and pathological heterogeneity in patients with Alzheimer's disease: a systematic review. *Alzheimer's Research & Therapy*, *12*(1), 1-19.

Engelen, A. (2009). Is vitamin D deficiency linked to Alzheimer's disease and vascular dementia? *Journal of Alzheimer's Disease.*

Farmer, B. C., Walsh, A. E., Kluemper, J. C., & Johnson, L. A. (2020). Lipid droplets in neurodegenerative disorders. *Frontiers in neuroscience*, *14*, 742.

Furcila, D., Domínguez-Álvaro, M., DeFelipe, J., Alonso-Nanclares, L. (2019). Subregional density of neurons, neurofibrillary tangles and amyloid plaques in the hippocampus of patients with Alzheimer's disease. Front. *Neuroanat*. *13*:99. doi: 10.3389/fnana.2019.00099.

Gauthier, S., Feldman, H. H., Schneider, L. S., Wilcock, G. K., Frisoni, G. B., Hardlund, J. H., Moebius, H. J., Bentham, P., Kook, K. A., Wischik, D. J., Schelter, B. O., Davis, C. S., Staff, R. T., Bracoud, L., Shamsi, K., Storey, J., Harrington, C. R., & Wischik, C. M. (2016). Efficacy and safety of tau-aggregation inhibitor therapy in patients with mild or moderate Alzheimer's disease: a randomised, controlled, double-blind, parallel-arm, phase 3 trial. *The Lancet*, *388*(10062), 2873-2884.

Hansen, R. A., Gartlehner, G., Webb, A. P., Morgan, L. C., Moore, C. G., & Jonas, D. E. (2008). Efficacy and safety of donepezil, galantamine, and rivastigmine for the treatment of Alzheimer's disease: a systematic review and meta-analysis. *Clinical interventions in aging*, *3*(2), 211–225.

Hansson, O., Grothe, M., Strandberg, T., Ohlsson, T., Hägerström, D., Jögi, J., Smith, R., &

Schöll, M. (2017) Tau pathology distribution in Alzheimer's disease corresponds differentially to cognition-relevant functional brain networks. Front. *Neurosci*. 11:167. doi: 10.3389/fnins.2017.00167.

Hippius, H. & Neundörfer, G. (2003). The discovery of Alzheimer's disease. Dialogues Clin *Neurosci*. 101-8. doi: 10.31887/DCNS.2003.5.1/hhippius. PMID: 22034141; PMCID: PMC3181715.

Huang, L. K., Chao, S. P., & Hu, C. J. (2020). Clinical trials of new drugs for Alzheimer disease. *Journal of biomedical science, 27*(1), 1-13.

Hussien, H. M., Abd-Elmegied, A., Ghareeb, D. A., Hafez, H. S., Ahmed, H. E., & Abd El-moneam, N. (2018). Neuroprotective effect of berberine against environmental heavy metals-induced neurotoxicity and Alzheimer's-like disease in rats. *Food and chemical toxicology*, *111*, 432-444.

Iqbal, K., & Grundke‐Iqbal, I. (2008). Alzheimer neurofibrillary degeneration: significance, etiopathogenesis, therapeutics and prevention. *Journal of cellular and molecular medicine*, *12*(1), 38-55.

Jack Jr, C. R., & Holtzman, D. M. (2013). Biomarker modeling of Alzheimer's disease. *Neuron, 80*(6), 1347-1358.

Jahanshahi, M., Nikmahzar, E., & Sayyahi, A. (2020). Vitamin E therapy prevents the accumulation of congophilic amyloid plaques and neurofibrillary tangles in the hippocampus in a rat model of Alzheimer's disease. *Iranian journal of basic medical sciences*, *23*(1), 86–92. https://doi.org/10.22038/IJBMS.2019.38165.9067.

Kato, T., Inui, Y., Nakamura, A., & Ito, K. (2016). Brain fluorodeoxyglucose (FDG) PET in dementia. *Ageing research reviews, 30,* 73-84.

Kavitha, C., Mani, V., Srividhya, S., Khalaf, O., & Tavera Romero, C. (2022). Early-stage Alzheimer's disease prediction using machine learning models. Front. *Public Health, 10*:853294. doi: 10.3389/fpubh.2022.853294.

Khacho, M., Clark, A., Svoboda, D. S., MacLaurin, J. G., Lagace, D. C., Park, D. S., & Slack, R. S. (2017). Mitochondrial dysfunction underlies cognitive defects as a result of neural stem cell depletion and impaired neurogenesis. *Human molecular genetics*, *26*(17), 3327-3341.

Kim, K. W., Woo, S. Y., Kim, S., Jang, H., Kim, Y., Cho, S. H., Kim, S. E., Kim, S. J., Shin, B., Kim, H. J., Na, D. L, & Seo, S. W. (2020). Disease progression modeling of Alzheimer's disease according to education level. *Scientific Reports, 10*(1), 16808.

Leoni, V. (2011). The effect of apolipoprotein E (ApoE) genotype on biomarkers of amyloidogenesis, tau pathology and neurodegeneration in Alzheimer's disease. *Clinical chemistry and laboratory medicine*, *49*(3), 375-383.

Littlejohns, T. J., Henley, W. E., Lang, I. A., Annweiler, C., Beauchet, O., Chaves, P. H., Fried, L., Kestenbaum, B. R., Kuller, L. H., Langa, K. M., Lopez, O. L., Kos, K., Soni, M., & Llewellyn, D. J. (2014). Vitamin D and the risk of dementia and Alzheimer disease. *Neurology*, *83*(10), 920-928.

Mahase, E. (2021). Three FDA advisory panel members resign over approval of Alzheimer's drug.

Martens, Y. A., Zhao, N., Liu, C.-C., Kanekiyo, T., Yang, A. J., Goate, A. M., Holtzman, D. M., & Bu, G. (2022). ApoE cascade hypothesis in the pathogenesis of Alzheimer's disease and related Dementias. *Neuron, 110*(8), 1304–1317. https://doi.org/10.1016/j.neuron.2022.03.004.

Mayo Foundation for Medical Education and Research. (2022). *Lumbar puncture (Spinal Tap)*. Mayo Clinic. https://www.mayoclinic.org/tests-procedures/lumbar-puncture/about/pac-20394631

Mizwicki, M. T., Menegaz, D., Zhang, J., Barrientos-Durán, A., Tse, S., Cashman, J. R., Griffin, P. R., & Fiala, M. (2012). Genomic and nongenomic signaling induced by 1α,25(OH)2-vitamin D3 promotes the recovery of amyloid-β phagocytosis by Alzheimer's disease macrophages. *Journal of Alzheimer's Disease, 29*(1), 51-62.

Nagele, R. G., Wegiel, J., Venkataraman, V., Imaki, H., Wang, K.-C., & Wegiel, J. (2004). Contribution of glial cells to the development of amyloid plaques in alzheimer's disease. *Neurobiology of Aging*, *25*(5), 663–674. https://doi.org/10.1016/j.neurobiolaging.2004.01.007.

National Registry of Clinical Trials (n.d.). *TRX0237 for the treatment of early and mild-moderate alzheimer's disease - full text view*. Full Text View - ClinicalTrials.gov. https://clinicaltrials.gov/ct2/show/NCT03539380?term=TRx0237&draw=2&rank=2 .

Novak, P., Kovacech, B., Katina, S., Schmidt, R., Scheltens, P., Kontsekova, E., Ropele, S., Fialova, L., Kramberger, M., Paulenka-Ivanovova, N., Smisek, M., Hanes, J., Stevens, E., Kovac, A., Sutovsky, S., Parrak, V., Koson, P., Prcina, M., Galba, J., Cente, M., Hromadka, T., Filipcik, P., Juraj, P., Samcova, M., Prenn-Gologranc, C., Sivak, R., Froelich, L., Fresser, M., Rakusa, M., Harrison, J., Hort, J., Otto, M., Tosun, D., Ondrus, M., Winblad, B., Novak, M., & Zilka, N. (2021). Adamant: a placebo-controlled randomized phase 2 study of AADvac1, an active immunotherapy against pathological tau in Alzheimer's disease. *Nature Aging, 1*(6), 521-534.

Novak, P., Schmidt, R., Kontsekova, E., Zilka, N., Kovacech, B., Skrabana, R., Vince-Kazmerova, Z., Katina, S., Fialova, L., Prcina, M., Parrak, V., Dal-Bianco, P., Brunner, M., Staffen, W., Rainer, M., Ondrus, M., Ing, S.R., Smisek, M., Ing, R.S, Winblad, B., & Novak, M. (2017). Safety and immunogenicity of the tau vaccine AADvac1 in patients with Alzheimer's disease: a randomised, double-blind, placebo-controlled, phase 1 trial. *The Lancet Neurology, 16*(2), 123-134.

Nuriel, T., Peng, K.Y., Ashok, A., Dillman, A.A., Figueroa, H.Y., Apuzzo, J., Ambat, J., Levy, E., Cookson, M.R., Mathews, P.M., & Duff, K.E. (2017). The endosomal-lysosomal pathway is dysregulated by APOE4 expression *in Vivo*. Front. *Neurosci, 11*, 702. https://doi.org/10.3389/fnins.2017.00702.

Padda, I. S., & Parmar, M. (2022). Aducanumab. In *StatPearls [Internet]*. StatPearls Publishing.

Piller, C. (2022). Blots on a field?. *Science (New York, NY)*, *377*(6604), 358-363.

Piller, C. (2022). Scientists tie third clinical trial death to experimental Alzheimer's drug [news]. *Science*.

R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Robbins, M., Clayton, E. & Kaminski Schierle, G.S. (2021). Synaptic tau: A pathological or physiological phenomenon? *Acta Neuropathol Communications, 9*, 149. https://doi.org/10.1186/s40478-021-01246-y.

Roses, A. D. (2006). On the discovery of the genetic association of Apolipoprotein E genotypes and common late-onset Alzheimer disease. *Journal of Alzheimer's Disease*, *9*(s3), 361-366.

Salvador, L., Singaravelu, G., Harley, C. B., Flom, P., Suram, A., & Raffaele, J. M. (2016). A natural product telomerase activator lengthens telomeres in humans: a randomized, double blind, and placebo controlled study. *Rejuvenation research, 19*(6), 478-484.

Sanes, J. & Holtzmann, D. (2021). The aging brain. In E. Kandel, J. Koester, S. Mack, & S. Siegelbaum (Eds). *Principles of Neural Science* (p. 1568-74). 6th edition. McGraw Hill Medical.

Santin, M., Vandenberghe, M., Herard, A-S., Pradier L., Cohen, C., Debeir, T., Delzescaux, T., Rooney, T., Dhenain, M. (2016). In vivo detection of amyloid plaques by gadolinium-stained MRI can be used to demonstrate the efficacy of an anti-amyloid immunotherapy. *Front. Aging Neurosci*. 8:55. doi: 10.3389/fnagi.2016.00055.

Seattle Alzheimer's Disease Brain Cell Atlas (n.d.). *Brain*. https://portal.brain-map.org/explore/seattle-alzheimers-disease.

Shi, M., Chu, F., Zhu, F., & Zhu, J. (2022). Impact of anti-amyloid-β monoclonal antibodies on the pathology and clinical profile of Alzheimer's disease: A focus on Aducanumab and Lecanemab. *Frontiers in Aging Neuroscience, 14*.

Sinha, P., & Barocas, J. A. (2022). Cost‐effectiveness of aducanumab to prevent Alzheimer's disease progression at current list price. Alzheimer's & Dementia: *Translational Research & Clinical Interventions, 8*(1), e12256.

Tagliavini, F., Tiraboschi, P., & Federico, A. (2021). Alzheimer's disease: The controversial

approval of Aducanumab. *Neurological Sciences*, *42*(8), 3069-3070.

Travaglini, K. J., Gabitto, M., Ding, Y., Ariza, J., Chakrabarty, R., Crane, P. K., Ferrer, R., Goldy, J., Grabowski, T.J., Guilford, N., Guzman, J., Hawrylycz, M.J., Hodge, R.D., Jayadev, S., Kaplan, E.S., Keene, C.D., Larson, E.B., Latimer, C.S., Levi, B., Mahoney, J., Melief, E.J., Mukherjee, S., Pham, T., Rachleff, V.M., Smith, K.A., Torkelson, A., Lein, E.S., & Miller, J. A. (2022). A multimodal atlas of the molecular and cellular changes to cortex driven by Alzheimer's disease. *Alzheimer's & Dementia, 18*, e060835.

U.S. Department of Health and Human Services. (n.d.). Alzheimer's disease diagnostic guidelines. *National Institute on Aging*. https://www.nia.nih.gov/health/alzheimers-disease-diagnostic-guidelines

U.S. Department of Health and Human Services. (n.d.). What happens to the brain in alzheimer's disease? *National Institute on Aging*. https://www.nia.nih.gov/health/what-happens-brain-alzheimers-disease.

Utz, J., Berner, J., Muñoz, L., Oberstein, T., Kornhuber, J., Herrmann, M., Maler, J., & Spitzer, P. (2021). Cerebrospinal fluid of patients with Alzheimer's disease contains increased percentages of synaptophysin-bearing microvesicles. Front. *Aging Neurosci. 13*:682115. doi: 10.3389/fnagi.2021.682115.

van Dyck, C. H., Swanson, C. J., Aisen, P., Bateman, R. J., Chen, C., Gee, M., Kanekiyo, M., Li, D., Reyderman, L., Cohen, S., Froelich, L., Katayama, S., Sabbagh, M., Vellas, B., Watson, D., Dhadda, S., Irizarry, M., Kramer, L., & Iwatsubo, T. (2023). Lecanemab in early Alzheimer's disease. *New England Journal of Medicine, 388*(1), 80-81. doi: 10.1056/NEJMe2214981.

Zhang, T., Ma, S., Lv, J., Wang, X., Afewerky, H. K., Li, H., & Lu, Y. (2021). The emerging role of exosomes in alzheimer's disease. *Ageing Research Reviews*, *68*, 101321. https://doi.org/10.1016/j.arr.2021.101321.

Zilberter, Y., & Zilberter, M. (2017). The vicious circle of hypometabolism in neurodegenerative diseases: ways and mechanisms of metabolic correction. *Journal of neuroscience research*, *95*(11), 2217-2235.

Appendix

Honors Symposium Speech

Panel title: Bodies in Distress

Presented May 20, 2023

The journey our brains and bodies endure during our last decades of life is a unique one. The deep complexities and difficulties that become of us are still new to the scientific community. From a neurobiological standpoint, changes in our neural activity and physiology are deeply connected to who we are as people, and this is especially prevalent in individuals with Alzheimer's Disease. Alzheimer's Disease (AD) is a neurodegenerative disorder that can lead to short-term memory loss, cognitive decline, and paranoia. 1 out of every 10 people over the age of 65 will develop symptoms, and this number increases to 1 in 3 after the age of 85. This is why, as the population continues to age and the burden on our care system grows, it is urgent to understand, treat, and cure this disease.

Alzheimer's is characterized by 2 primary neural pathologies: amyloid plaques and neurofibrillary tangles. As you can see in the top left picture *(point to figure),* both of these pathologies result in significant atrophy and cell loss, causing serious brain volume reduction.

Neurofibrillary tangles are formed due to dysfunction of the tau protein inside the cell. Tau is a protein naturally produced by the body that plays a crucial role in maintaining the structural integrity of a neuron and intracellular transport. Typically, this tau protein binds to microtubules inside the cell (which are small structures that help move things inside the cell), but in an AD patient, this protein detaches itself from the microtubule and sticks to other tau molecules in the cell, forming tangles.

As you may have heard on the news recently, there have been a few drugs developed targeting the removal of amyloid plaques. The focus of my presentation will be on addressing

these drugs and some of the issues that have arised as a result. Amyloid plaques are plaques formed by the clumping of amyloid precursor proteins that form in the synapses, ultimately resulting in the loss of connections between neurons. These plaques are formed due to the accumulation of amyloid beta, which is a protein that is normally produced in the brain in a soluble state but can adopt different states, ultimately forming amyloid plaques. These plaques cause a number of issues in the brain, such as neuroinflammation, issues with blood flow to the brain, and swollen cells *(point to figure* - this bottom left picture shows what amyloid plaques and neurofibrillary tangles look like in the brain on a cellular level compared to a healthy cell*)*. The progression of these pathologies are directly correlated with the severity of the progression of the illness, as seen in the top right picture *(point to figure)* ranging from preclinical to severe. The worse these pathologies become, the more severe the AD is and the more the neuronal integrity declines. Despite these pathologies being known for decades, treatments have been difficult to develop for a number of complex reasons.

For many years, amyloid plaques have been the primary focal point of AD treatment and drug development. The hope is that by removing these plaques, this will improve cognitive decline symptoms. In fact, the belief that amyloid plaques are the cause of AD symptomology have been so prevalent among the scientific community that they developed the "Amyloid Cascade Hypothesis." This hypothesis suggests that the formation of amyloid plaques is the initiating component of AD, which further leads to the formation of neurofibrillary tangles and cognitive impairment. However, there has been a lot of backlash and controversy regarding this hypothesis. The first study that came out claiming that amyloid plaques in the brain were the primary cause of AD was in 2006 by neuroscientist Sylvian Lesné of the University of Minnesota. In 2021, *15 years later*, it was found that this paper, along with almost 70 others from

Lesné, contained images that were tampered with. Basically, it was found that the results published in the original study had been edited in order to better fit their desired hypothesis. This has resulted in numerous issues, the biggest one being the fabrication that resulted in decades of research following a flawed hypothesis. This has led to major discrepancies in the scientific community that calls into question the validity of this hypothesis, as well as amyloid's role in AD. Despite the amyloid cascade hypothesis being called into question, research has continued in development and application for approval of plaque removing drugs, despite serious questions of its link to the primary cause of AD.

To date, there is no known cure, but there have been a few drugs developed to help slow down the cognitive decline symptoms that accompany AD. Recently, two drugs have been approved by the FDA as a treatment option: Aducanumab and Lecanemab (perhaps you have already heard of these). Both of these drugs target the removal of amyloid plaques using immunotherapies that target the plaques for removal by the patient's own immune system. This represents a breakthrough in how we target AD, but many scientists call into question the efficacy of these drugs. The approval of these drugs resulted in great controversy that led to 3 FDA advisory board members resigning over the approval of Aducanumab in 2021, with one of them saying that it was "probably the worst drug approval decision in recent US history." Both of these drugs underwent approval via the FDA's accelerated approval process. This process is granted in situations where there is uncertainty regarding the efficacy of a treatment, but there is anticipated clinical benefit, especially for a condition in which there are no currently available effective therapies. It is important to note that Aducanumab treatment costs around $56,000 per year per patient. It is quite obvious that there would be a significant financial gain made off of Aducanumab's approval, but the question regarding why the FDA would approve a drug that is

not effective remains unanswered. Because of this controversy, and the fact that the FDA is ultimately beholden to you, the voting public, it is worth digging into the process by which these drugs were approved.

In order for a drug to receive FDA approval, it must undergo 3 different phases of clinical trials *(point to figure)*. The phase I trial focuses solely on the safety of the drug. The phase II trial focuses on both safety and efficacy, but is primarily looking at the effectiveness of the drug. The phase III trial is where the drug is measured against the placebo in what is known as the gold standard, double-blind, placebo-controlled trial to ensure that it is significantly effective at treating the condition. For a drug to get approved, it must undergo at least 2 phase III clinical trials and yield at least 2 statistically significant results. If a drug has enough funding, it can undergo phase III clinical trials numerous times, and it only needs 2 positive results. So, technically, a drug can undergo, for example, 20 phase III trials, but as long as 2 of these trials are positive, the drug is eligible to be approved, AND, the other 18 trials that yielded negative results do ***not*** need to be published. In fact, there has been a long history of many drugs being approved and the negative results not being released to the public. This practice has been particularly egregious among many antidepressants commonly used today.

According to FDA documents, Aducanumab underwent two phase III clinical trials, both of which showed poor results. Both of these clinical trials were stopped early due to lack of efficacy concerns because the results produced by the studies showed no statistical significance in improving cognition in AD patients. However, an analysis of one of these studies showed positive results in the group that received the highest dose of the drug in which there was a 23% reduction in clinical decline compared to placebo. The FDA Nervous System Drug Advisory Committee deemed the approval followed by this outcome as questionable and premature,

especially given the negative results of the study that did not meet the clinical endpoint and the significant occurrence of adverse effects with the effective dose. *(pause)*

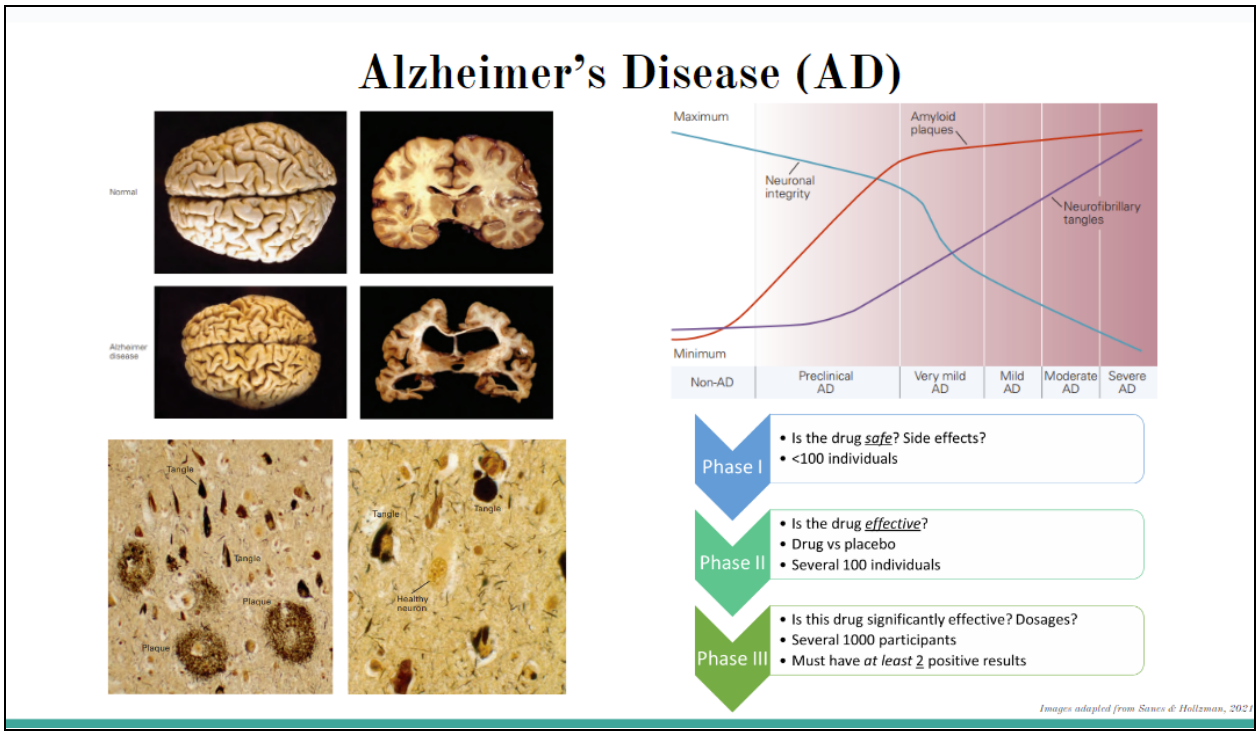
Lecanemab was another drug that was approved just a few months ago in January. During the phase II clinical trial, *89%* of participants had adverse effects after receiving the dose, which was *52 times higher* than the participants taking the placebo injection. Some studies have also found that Lecanemab caused some fatal side effects in patients, such as brain swelling and hemorrhage that resulted in 3 deaths linked to the drug. Overall, following the completion of the phase III clinical trial, it was concluded that Lecanemab reduced markers of amyloid in patients and resulted in a 27% cognitive decline improvement, but these results are not statistically significant when compared to placebo, and this is important to note. It is also worth saying that decline improvement means a slowing of decline and not improvement in symptoms. What this suggests is that it is not curing AD but instead, at best, slowing down the progression, IF it was a significant difference, which again, it was not.

While the cognitive impairment delay seen with the use of Lecanemab is not clinically significant, there are other issues surrounding the validity of how these results were reached. In order to measure cognitive decline and cognitive improvement, the participants were scored on a questionnaire called the Alzheimer's Disease Cooperative Study-Activities of Daily Living Scale, where lower scores indicate greater impairment. Participants who took the test before and after receiving the Lecanemab dose showed an increase in 1 to 2 points on the test. However, the researchers who made this questionnaire noted that differences within 2 points are not clinically meaningful. In other words, while there was slight improvement on the scores of the questionnaire, the improvement was so minimal that it was not statistically or clinically significant.

Following FDA approval of these drugs, there are a number of ethical concerns that arise. First, the high cost of these drugs. Not only do these high costs limit access to many individuals who may need treatment and may lead to disparities in access to treatment, it also raises a lot of questions regarding the financial gain that may be made off of the approval of these drugs. Second, these drugs have only shown limited efficacy and that they do not stop or reverse progression of the disease. Individuals who may be taking these drugs may still experience significant cognitive decline and functional impairment. Third, the clinical trials have indicated that there is a high risk of side effects, some of which may be serious and fatal. Fourth, the uncertainty of the long-term effects. These drugs are still in their early stages of use, and there is still a lot about the long-term effects of these drugs that we do not know. It is possible that these drugs could have unforeseen negative or even positive consequences for patients in the future. *We just don't know what they are.*

Based on the approval of Aducanumab and Lecanemab and the recent discovery of fabricated results, it is becoming increasingly likely that the removal of amyloid plaques may not be the cure. Therefore, there needs to be a shift by the scientific and medical community to broaden the current treatment approach of Alzheimer's. While the approval of Lecanemab did show a slight delay in cognitive impairment in early AD patients, there is still a lot that needs to be looked at regarding Lecanemab's safety and efficacy. Rather than focusing on the removal of amyloid plaques, there should be a shift to focusing on additional theories of disease progression to improve immunotherapy options. The bulk of scientific funding has focused on these two proteins, at the cost of other theories that are also supported by scientific evidence. Unfortunately, it is beyond the scope of this talk to get into these in any level of detail.

The various ethical and scientific issues that have arisen in the wake of these two new drugs will profoundly change the future of AD research. However, you, the public, are ultimately responsible for the direction of scientific funding and the process by which drugs are approved. When you see headlines like these *(point to slide)* that are making big claims about Alzheimer's, take the time to dig deeper. There is important journalism that raises questions about these claims, carefully analyzing the data for integrity, and holding our public officials to account on this. Without these diligent scientists and journalists, you wouldn't be hearing this talk today. While the ethicality of treatment options for AD to date have remained tentative, it is important to not lose sight of the bigger picture. The aim of the scientific community is to find a cure and help individuals age gracefully while preserving cognitive abilities and maintaining quality of life. With continued research and innovation, we can ultimately achieve our goal of finding a cure for Alzheimer's and improving the lives of millions of people affected by this devastating condition.


Alzheimer's Disease (AD)
Normal
Alzheimer disease
Tangle
Plaque
Healthy neuron
Maximum
Minimum
Amyloid plaques
Neuronal integrity
Neurofibrillary tangles
Non-AD
Preclinical AD
Very mild AD
Mild AD
Moderate AD
Severe AD
Phase I
• Is the drug safe? Side effects?
• <100 individuals
Phase II
• Is the drug effective?
• Drug vs placebo
• Several 100 individuals
Phase III
• Is this drug significantly effective? Dosages?
• Several 1000 participants
• Must have at least 2 positive results
Images adapted from Sanes & Holtzman, 2021


New Alzheimer's Drug Shows 35% Reduction in Cognitive Decline in Late-Stage Trial
HEALTH 13 May 2023 By ELEFTHERIA KODOSAKI, THE CONVERSATION
HEALTH AND MEDICINE
NEUROSCIENCE
New Alzheimer's Drug Halts Disease Progression In 47 Percent Of Trial Patients After 1 Year
The drug could soon be licensed for use, but some have called for caution until the full dataset is available.
LAURA SIMMONS
Editor and Staff Writer
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'Remarkable' drug reduces Alzheimer's decline, study shows
Experts say trial provides further evidence that attacking the build-up of proteins in the brain can slow the progression of the disease.
NEWS | 07 January 2023 | Correction 10 January 2023
FDA approves Alzheimer's drug lecanemab amid safety concerns
Reports of deaths potentially linked to the treatment have cast a shadow on what many hail as a landmark approval.