Alzheimer's Disease: From Pathology and Early Detection to Socioeconomic Impacts and Treatment

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ALZHEIMER'S DISEASE: FROM PATHOLOGY AND EARLY DETECTION TO SOCIOECONOMIC IMPACTS AND TREATMENT

An Interactive Qualifying Project submitted to the Faculty of the WORCESTER POLYTECHNIC INSTITUTE in partial fulfillment of the requirements for the Degree of Bachelor of Science by

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Authorship Page

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- Characteristics of β-amyloid and amyloid plaques
- Characteristics of tau protein and neurofibrillary tangles
- Calcium homostasis and mitochondrial functions
- Cellular effects
- Genes (presenilin 1, presenilin 2, β-amyloid precursor protein, and apolipoprotein E)
- Alzheimer's disease risk factors

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- CSF markers
- Plasma/serum markers
- Structural and functional imaging markers
- Combinations and comparisons of AD biomarkers

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- Current and Potential Treatments for AD
- Acetylcholinesterase Inhibitors
- Glutamate Receptors, NMDA, and Memantine
- Aβ Modulation
- Aβ Production: γ-secretase and β-secretase inhibition
- Aβ Aggregation, trace metals
- Immunotherapy
- Tau pathology, APOE-related
- Relation to Early Detection

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- Alzheimer's vs. Down Syndrome
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Abstract

The paper, composed for Worcester Polytechnic Institute, examines the various implications and possible solutions of Alzheimer's disease in society. It begins with a briefing on the numerous physiological characteristics of the disease and continues with a dissertation of the current treatments and possible future treatments, with the aid of early detection techniques. Furthermore, the paper also discusses the relationship of the disease with other disorders and assesses the socioeconomic impacts of Alzheimer's presently, with a conjecture on possible future techniques.

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Executive Summary

This Interactive Qualifying Project investigates the current fields of pathology, detection techniques, treatments, and prevention methods of Alzheimer's disease. Alzheimer's disease (AD) is a neurodegenerative disorder that occurs mainly in older people, and it is the most common cause of dementia. As of the 2009 Alzheimer's Report, there is an estimate of 36 million people living with dementia worldwide, and its prevalence is only increasing with an estimated 115 million diagnosed by 2050. There are currently no known cures or preventions, and the only definitive way to diagnosis Alzheimer's is through a post-mortem autopsy. The autopsy allows for the analysis of the excess presence of amyloid plaques and neurofibrillary tangles in the brain.

The presence of large amounts of amyloid plaques, made from β-amyloid proteins (Aβ), and neurofibrillary tangles, made from tau proteins, were the very first diagnostic characteristics of the disease. These were first visualized and determined by a young and accomplished German doctor named Alois Alzheimer in the brain slices of the very first patient with Alzheimer's disease, Auguste D. As the years progressed and the research into the disorder intensified, another possible characteristic of AD surfaced: the disruption of calcium homeostasis in neurons. This characteristic came to be identified as a possible cause when researchers established a greater understanding of the role calcium plays in memory formation and activation of apoptosis, otherwise known as cell death, as well as its continual presence in neurons of AD patients.

The combination of large concentrations of amyloid plaques and neurofibrillary tangles, along with the disruption of calcium homeostasis in the neurons, results in interference of normal neuronal functions that is caused by damages to the synapses.

Other causes include the destruction of the cellular trafficking system, the extensive production of reactive oxygen species leading to oxidative stress, the inability of the neuron to correct cellular damages, and later the activation of apoptosis. All of this manifests itself in the form of cortical atrophy, memory loss, decrease in cellular energy metabolism, smaller brain size, and psychiatric disturbances.

Although there has been great progress in understanding the characteristics of the disease and how it manifests itself, there has been limited understanding in the risk factors of AD. Nevertheless, there are diseases such as Type II diabetes and Down's syndrome, which have similar characteristics to Alzheimer's. There are several environmental, genetic, and evolutionary risk factors associated with the diseases, whether it is diabetes or Down's syndrome. As a result, it may be a disadvantage for the patients since they are at a higher risk for contracting the other two disorders in addition to having Alzheimer's. Conversely, the similarities may allow for a faster treatment and better opportunities to detect and treat Alzheimer's and other deadly disorders using the same tests. Not only is there a very limited knowledge in the possible risk factors of AD, but also there is also no detection technique that can 100% accurately diagnose the disease as well as differentiate it from other diseases.

The current detection techniques that show potential in distinguishing AD from normal controls and other types of dementia are biochemical markers and neuroimaging methods. In biochemical AD markers, there are two major categories: cerebrospinal fluid (CSF) and plasma markers. Studies report several CSF biomarkers showing consistent results in the concentrations observed in AD patients as well as in predicting the conversion from normal or mild cognitive impairment (MCI) patients to AD.

Furthermore, combinations of CSF markers, specifically Aβ42 and tau, prove particularly promising as potential diagnostic and prognostic biomarkers with accurate sensitivity and specificity. Plasma markers, on the other hand, provide either mixed results or no changes in the levels of several proteins. Although plasma markers are difficult to distinguish AD from controls and to predict progression from MCI to AD, some proteomic studies of plasma and serum are promising.

Along with biochemical markers, neuroimaging techniques are used increasingly to detect brain changes associated with AD, and thus have potential as markers of disease progression, monitors of therapeutic effects, and predictors of future dementia prior to symptoms. These imaging markers include computed tomography (CT), magnetic resonance imaging (MRI, functional MRI, positron emission tomography (PET), and single photon emission computed tomography (SPECT). Each imaging marker offers positive and negative predictive values and the appropriate sensitivity and specificity.

Yet, given the amalgamation of the various pathophysiological causes and mechanisms of the disease, it is unlikely that any of these markers will detect specifically for AD alone. Therefore, recent studies focus on developing compounds for the *in vivo* imaging of brain amyloid, neurofibrillary tangles, and activated microglia. Because AD pathology precedes the onset of AD symptoms by many years, "molecular" imaging agents would allow for early diagnosis and for the monitoring of disease progression and treatment efficacy. Therefore, it is likely that combinations of CSF/plasma and imaging measures will be required to accurately diagnose the disease early.

The field in Alzheimer's research focuses not only on understanding the

pathology of AD and detecting it at its earliest stage, but also on finding treatments for patients suffering from AD. Currently, there are a number of treatments available to patients suffering from Alzheimer's disease. The most common treatment for AD patients is two classes of drugs known as acetylcholinesterase (AChE) inhibitors and NMDA modulators. Although these treatments relieve many of the symptoms of AD and improve cognitive function in several patients, there is a major problem with the current standards of treatment. These therapies do not actually treat the underlying causes of the disease; they only offer a temporary reprieve from the debilitating conditions of AD. Therefore, scientists strive to find new, disease-modifying treatments for AD patients, as research has grown exponentially in recent years. A disease-modifying treatment is the ideal approach to Alzheimer's therapy. If AD was detected early in patients, even with the current standards of care, doctors would not be able to stop the disease from eventually taking its toll. If a disease-modifying therapy was successful, it is then possible that the disease could be prevented outright if it was detected early enough.

Research for potential treatments and even possible "cures" for Alzheimer's disease has exploded in the last decade. There is large amount of research devoted to the idea of Aβ modulation, which refers to altering toxic Aβ, which can be achieved through inhibition of secretase proteins in the brain. Aβ itself can also be modulated to prevent aggregation of these molecules into toxic oligomers, which contribute to neurodegeneration. These kinds of modulation treatments are expanding to include things such as removing trace metals from Aβ, which is an innovative field of research that has only grown recently. There is a large amount of research devoted to AD immunotherapy as well, but the mechanics of this treatment are not fully understood, which is a serious

issue with attempting to understand how this therapy would work clinically. In addition, there are lesser-known research areas devoted to treating things such as tau tangles and apolipoprotein E gene (APOE). Although not as high profile as Aβ modulation and immunotherapy, these areas are worth exploring as possible treatments. Yet many of these treatments are only minimally successful in early clinical trials, and their mechanisms are not fully understood. Unfortunately, the search for a true disease-modifying treatment for AD continues, and the world still remains relatively far away from having a commercial treatment.

One of the major negative impacts of Alzheimer's disease is the socioeconomic effect on humanity. Socially, the patient is unable to associate with anyone due to the neurodegenerative effects of AD, and thus is forced to live with uncertainty and solitude. Furthermore, the family of the patient has to deal with being away from their loved ones and having to pay a large sum of money just to allow the patient to live a relatively normal life. The lives of the caretakers are also impacted since they need to adhere to the patients' every need, as the patients are unable to perform simple daily tasks. Economically, there is an enormous impact of AD on a personal level and on the government. The research, care, and treatment for Alzheimer's accounts for a tremendous amount of money and it is one of the most expensive diseases to treat and follow up on. Therefore, the future treatments and prevention techniques need to be less expensive, so that the families are not constantly in a state of debt solely for the ones they love.

Prevention techniques have been developed in Alzheimer's, which include neurological tests and early detection. However, the techniques are not as effective as they should be. Being one of the deadliest diseases in the world, Alzheimer's needs to be

stopped at a genetic level. There are inhibitors that block the risk factors and prevent the onset early on. Some of the ones discussed include anti-amyloid therapy and the research performed by scientists at Alzheimer's Disease Neuroimaging Initiative (ADNI). Additionally, some of the techniques need to be improved and newer techniques need to be developed in order to completely stop the onset through the earliest possible detection of the disease. In the end there is a cascading effect of AD on the society as the development of the biomarkers leads to early detection, which ultimately leads to treatment of the disease. Moreover, all of the scientific factors correlate with the socioeconomic factors of AD as well. Ultimately, there need to be steps taken to minimize the negative impacts of AD while maximizing the efforts towards positive results.

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Chapter 1: Characteristics of Alzheimer's Disease

Alzheimer's disease (AD) is a very common cause of dementia among the elderly with its prevalence greatly increasing after the age of 80 years though in very few cases younger people can get AD as well. There are two types of AD: familial and sporadic. Familial Alzheimer's disease (FAD) involves gene mutations and is often passed down from parent to offspring, making it the main cause of early onset AD that occurs between the age of 40 and 60 years. Sporadic Alzheimer's disease, the most common form of AD, has no clear mode of causation and often occurs unpredictably, resulting in late onset AD that occurs after the age of 60. The most prominent physical trait of AD is cerebral atrophy, predominantly in the medial temporal lobe, extensive loss of neurons, and the formation of extracellular senile plaques and intracellular neurofibrillary tangles.

The cerebral atrophy characteristic of AD, which is the weakening and the decrease in brain size, is not enough to diagnose AD because it does not distinguish normal aging from the disease.[1] This is one of the reasons why currently the only definitive way to diagnosis Alzheimer's is through a post-mortem autopsy.[2] It is the microscopic examination of the brain tissues through a post-mortem autopsy that features the characteristics of the disease: "a cerebral cortex peppered with neurofibrillary tangles and senile plaques."[1]

Senile plaques, also known as amyloid plaques, are composed of β-amyloid proteins (Aβ) that is formed from the cleavage of β-amyloid precursor proteins (APP) and are present on the outside of the neurons. Neurofibrillary tangles, on the other hand, are composed of hyperphosphorylated tau proteins that have dissociated from it attachment on the microtubules and are present on the inside of the neurons.[3] Both amyloid plaques

and neurofibrillary tangles are present in low amounts in all aging individuals including normal adults and even in young adults. However when a certain density of amyloid plaques and neurofibrillary tangles accumulate in the brain, it progresses into AD.[1,3] Neurofibrillary tangles are present in many neurodegenerative diseases where it is assumed that they are closely involved in neuronal death but only amyloid plaques are found in AD, which is why many researchers considered amyloid plaques to be the cause of AD.[4]

In addition to amyloid plaques and neurofibrillary tangles, it has been shown that the neurons of AD patients also exhibit calcium disruption, mitochondrial dysfunction, metabolic interference, oxidative stress, and protein misfolding, all of which eventually leads to the activation of apoptosis, or cell death. All of these factors occur normally as an individual age. However, in AD they are all abnormally elevated or expressed.

Alzheimer's is a progressive neurodegenerative disease and because there is currently no cure, individuals with AD will have the disease for the rest of their lives. Therefore, neurons of the brain gradually die over a course of many years with fairly "small number of neurons dying at any one time". The neuronal death that is associated with AD is similar, but not identical, to the neuronal death associated with normal aging, which suggests that AD pathology is "not simply an acceleration of normal brain aging."[5] In the following sections, the two prominent characteristics of AD (amyloid plaques and neurofibrillary tangles) as well as the newly observed characteristic, calcium homeostasis disruption, will be discussed in further details.

1.1 Amyloid Plaques

The β-amyloid precursor protein (APP) is a transmembrane glycoprotein that extends the lipid bilayer of a cell and contains a β-amyloid (Aβ) section. This Aβ section is located within the membrane, extending from outside the cell to the inside of the plasma membrane.[3] APP is expressed in all cells in the body and is present not only on the plasma membrane but also on the endoplasmic reticulum (ER) as well as the Golgi apparatus. The protein comes in different sizes ranging from 695 to 770 amino acids with the larger N terminus on the exterior of the membrane while the smaller C terminus is in the interior. The most common form is the one that contains 695 amino acids and is mostly produced by neurons.[5] The protein is abundantly expressed in the brain and highly conserved throughout evolution, demonstrating the important role it plays in human development.[6] Figure 1 illustrates the proteolytic processing of APP that it can undergo.

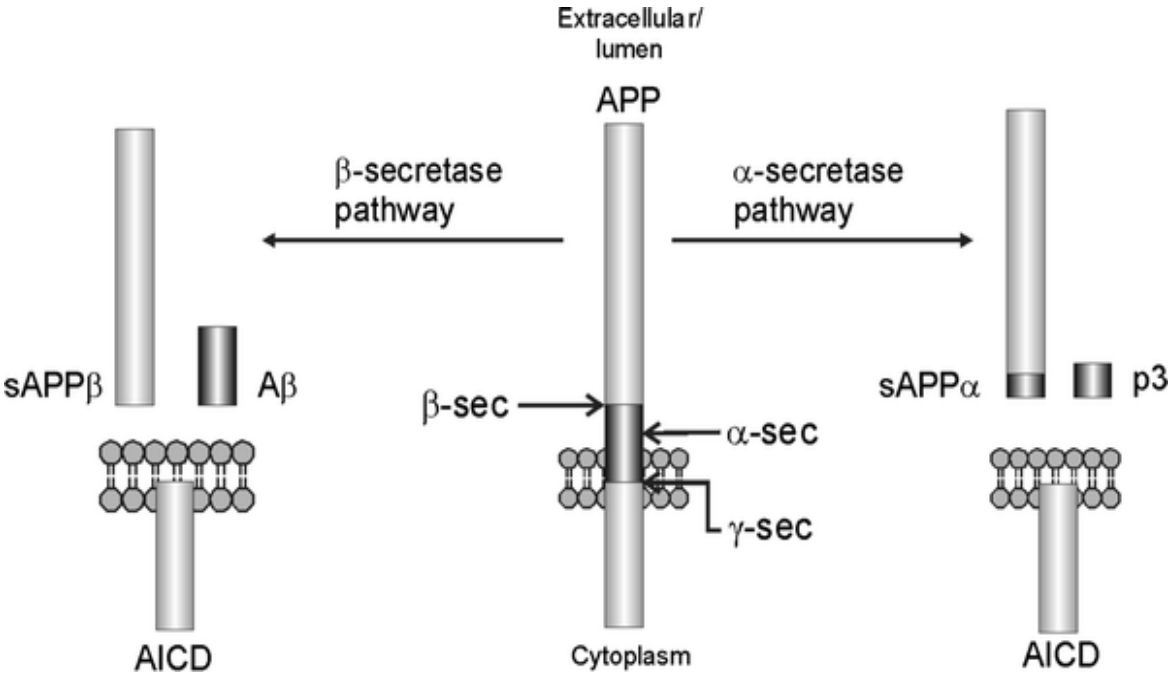


Figure 1: The Different Cleavage Pathways of Amyloid Precursor Protein (APP)[4]

Three different protease enzymes (α-secretase, β-secretase, and γ-secretase) cleave the β-amyloid precursor protein at different locations within the Aβ section. The enzyme β-secretase, also known as β-site APP cleaving enzyme (BACE1), cleaves the Aβ section that is located outside the cell while α-secretase cleaves the middle section, and γ-secretase cleaves the section that is inside the plasma membrane.[3,4] If α-secretase cleaves the Aβ section no Aβ is formed because it disrupts the entire Aβ section, dividing the Aβ section into a sAPPα and a p3 fragment.[4] However, if β-secretase cleaves the Aβ section and that is followed by γ-secretase cleavage of the products then Aβ is formed, producing amyloidogenic Aβ proteins that aggregate together and form insoluble plaques outside of the neurons.[3]

When α-secretase cleaves APP, it forms an extracellular secreted fragment, sAPPα, that is released from presynaptic terminals. sAPPα functions to increase synaptic plasticity, regulate neuronal excitability, promote learning and memory as well as enhance a neuron's resistance to oxidative stress and metabolic abnormalities. Since there is greater concentration of β-secretase and γ-secretase activity in AD, this promotes an increase in formation of Aβ and a decrease in formation of sAPPα. This decrease in sAPPα production would mean less amounts of sAPPα present to promote neurons resistance to oxidative stress and metabolic abnormalities and along with the increased concentration of Aβ, which exhibits its own neurotoxicity, further stimulates neuronal death.

When APP is cleaved by β-secretase, the fragment produced can be cleaved by γ-secretase to produce Aβ and an APP intracellular domain (AICD), or by caspase, an enzyme that mediates apoptosis, to produce a neurotoxic peptide to initiate cell death.

The AICD itself can also be further cleaved by caspase to produce the same neurotoxic peptide. The AICD acts as a gene expression regulator including genes involved in apoptosis and is formed regardless of what secretase pathway is undertaken.[4,5]

The enzyme γ-secretase is an integral membrane protein that cleaves "APP within the borders of the transmembrane domain" at multiple sites, producing many peptides of varying lengths from 39 to 42 amino acid residues. Even though γ-secretase can cleave APP at many sites, the majority of the Aβ produced are either 40 amino acids or 42 amino acids long. It is composed of 4 separate protein fragments: presenilin 1 (PS1), nicastrin, APH-1, and presenilin 2 (PS2).[7] The γ-secretase active site requires presenilin 1 (PS1) and can cleave several different proteins including APP and Notch-1. Notch-1 is a receptor on the cell that gets cleaved by γ-secretase when activated by a ligand and plays a role in regulating gene expression. This Notch signaling is especially important during development as it regulates cell fate.[5]

Of the two types of Aβ, the most common form is the 40 amino acid variant (Aβ40) and it is also the non-amyloidogenic one, meaning that it does not aggregate together to form the amyloid plaques.[6] Mutations in the presenilin (PS) genes shift the cleavage of APP to where more of the amyloidogenic Aβ42 is produced compared to Aβ40.[7] In normal neurons, the APP produces Aβ in the hippocampus, which facilitates learning and memory formation. In normal individuals, the Aβ peptides are produced at low concentrations with higher amounts of Aβ40 and lower amounts of Aβ42. In brains affected by AD, the Aβ peptides are produced at a high level with higher concentrations of Aβ42 and lower concentrations of Aβ40.[6] The 42 amino acid variant (Aβ42) is the longer form of β-amyloid and displays enhanced neurotoxicity because of its greater

ability to form insoluble amyloid plaques due to its size, making it highly amyloidogenic.[8] When they aggregate together along with glycosaminoglycan, a complex sugar polymer, they form the senile plaques that characterize AD.[1]

In the brain, APP and Aβ42 play an essential role in normal brain development. Studies have implicated that APP and its metabolites like Aβ42 are normally involved in cell signaling, synapse formation, cell adhesion, regulating neuronal survival, neurite extension, synaptic plasticity and ironically learning and memory.[5,6]

In experiments with transgenic animals that involved inhibiting APP or removing its expression, it caused impairments in avoidance learning and memory. In APP knockout mice, their spatial and avoidance learning as well as memory were impaired. In *Drosophila*, the removal of the homolog for APP caused "impaired avoidance learning that can be rescued by the expression of the human APP gene." When APP was bonded or blocked by antibodies or antisense-mediated blockade in chicks during the early phase of memory development, it disrupted inhibitory avoidance. On the contrast, in studies that involved administering secreted APP into the ventricular areas of the brain, it resulted in memory enhancement as well as a reduction of amnesia in the transgenic animals.[6]

Similarly to APP, Aβ42 also functions to facilitate memory formation by binding to nicotinic acetylcholine receptors (nAchr), a transmembrane protein that mediates rapid transduction to initiate memory development. In experiments involving Aβ42 where anti-Aβ antibodies (anti-Aβ) were injected into rats before they were trained to associate a mild foot shock with an avoidance of a certain area, it disrupted both their short term (STM) and long-term (LTM) memories compared to rats that had the control monoclonal

antibody (mAb). Both forms of memory were not restored until they were retrained 9 days later. These results are presented in graph A and graph B respectively in figure 2. However if the anti-Aβ were injected after they were trained, it had no effect on their memory retention as illustrated in graph C of figure 2.[6]

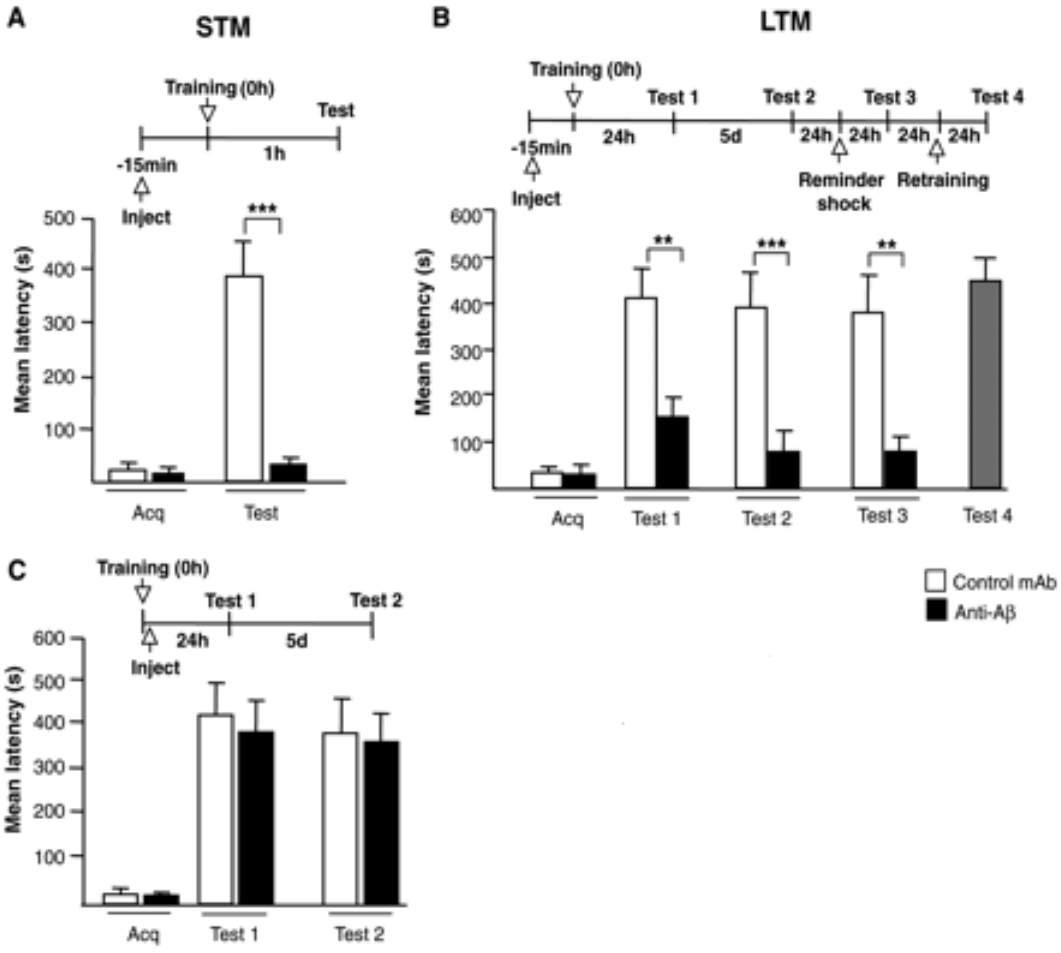


Figure 2: Avoidance Tests Conducted on Rats with Anti-Aβ Antibodies (anti-Aβ) Before or After a Training for Short-Term and Long-Term[6]

In contrast to the experiments discussed above that tested to see what would happen if the function of Aβ42 was inhibiting, another experiment was conducted to see what would happen to memory and learning if Aβ42 were injected into the rats. To test the effects of Aβ42 on amnesia, a memory test was conducted 24 hours after the training

on rats that had control monoclonal antibody (mAb) with Aβ42, mAb with scramble (sc) peptide, anti-Aβ with Aβ42, and anti-Aβ with sc peptide. Figure 3 illustrate the results of the test. The rats that had the control mAb with Aβ42 showed "significantly enhanced memory retention compared to those that received mAb and scrambled peptide." The rats that had anti-Aβ with scramble peptide had a substantial memory impairment compared to those that received anti-Aβ with Aβ42. In both cases the rats that had anti-Aβ along with either peptides had memory retentions that was both lower than the rats that had mAb with either peptides. The memory test was again conducted after 5 days and the same results were produced. Both experiments, the avoidance test and the memory test, show that Aβ peptides, including Aβ42 in low concentrations, facilitate both short-term and long-term learning and memory developments.[6]

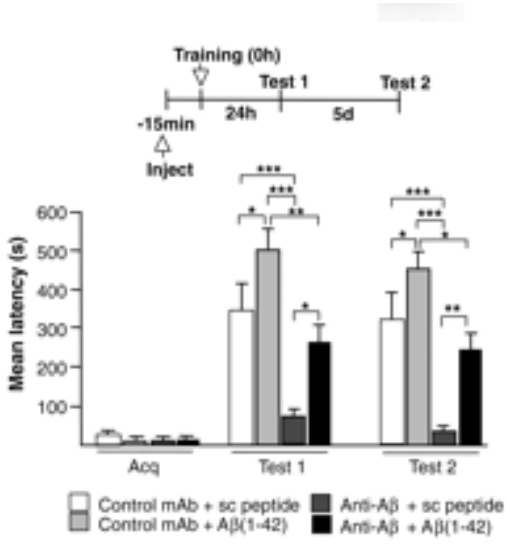


Figure 3: Memory Tests Conducted on Rats using Control Monoclonal Antibody (mAb), Scramble (sc) Peptide, Anti-Aβ Antibodies (anti-Aβ), and β-Amyloid42 (Aβ42)[6]

Even though APP and Aβ42 are important and necessary for facilitating learning and memory formation as demonstrated through the experiments discussed above, what causes them from becoming beneficial to becoming harmful to the brain is when neurons

can no longer control the amount of APP and Aβ42 produced, resulting in high concentrations of both proteins. In all cells, including neurons, when it is unable to function normally it cannot properly regulate and maintain its cellular processes such as correcting sudden mutations in DNA and proteins as well as proper protein formation. This inability to control APP and Aβ42 production can be caused by gene mutations and environmental factors. With the neurons not functioning properly, the APP and Aβ42 formed are not correctly folded or assembled. Therefore the cells are not only continually forming large amounts of the proteins but they are also misfolded and malfunctioning proteins. This accumulation of altered proteins is the most common pathogenic mechanism that several neurodegenerative disorders exhibit including AD.[9]

In addition to mutations in the APP gene, mutations in the Aβ peptide can also cause the misfolding of the protein, changing its conformation from the α-helix native structure to a β-sheet configuration, and foster self-aggregation, which leads to the formation of the insoluble accumulation of the plaques in AD individuals.[9,10] The misfolded protein can even interfere with the function of other normal proteins.[10] Cells have an ubiquitin-proteasome system (UPS) whose purpose is to degrade "misfolded, unassembled, or damaged proteins" by being bound to ubiquitin, a small protein that acts as a signal for degradation. When a misfolded protein is overly expressed, it is able to aggregate together forming a stable complex that resists the binding of ubiquitin thereby inhibiting UPS. If ubiquitin was mutated that would also inhibit UPS by making the protein unable to recognize misfolded or damaged proteins. Defects in ubiquitination of cerebral proteins as well as a large percentage of mutant ubiquitin were found in AD individuals.[9] Therefore, when Aβ is soluble and in low concentrations it has neurotrophic

properties that nourishes the neurons while when it is aggregated and in high concentration, Aβ has neurotoxic properties.[7]

1.2 Neurofibrillary Tangles

Tau protein is normally and abundantly produced in neurons as microtubule-associated proteins that bind to tubulin and promote microtubule assembly.[3] The protein is made of "alternatively spliced cytoplasmic proteins" that contains three or four microtubule-binding domains and it co-assembles tubulin with microtubules in order to stabilize the structure as well as helps form the "cross bridges between adjacent microtubules."[10]

If the tau proteins dissociate from the microtubules, they accumulate in the neuronal cell body and aggregate together in the cytoplasm forming paired helical filaments. These helical filaments are the components of the neurofibrillary tangles, one of the characteristics of AD. What causes the tau protein to detach from the microtubules is the hyperphosphorylation of the protein, most particularly at the sites that flank the regions of the microtubule binding domains.[3] Therefore, the tau proteins detachment from the microtubules destabilize the cytoskeleton and promote the formation of neurofibrillary tangles as illustrated in figure 4.[10]

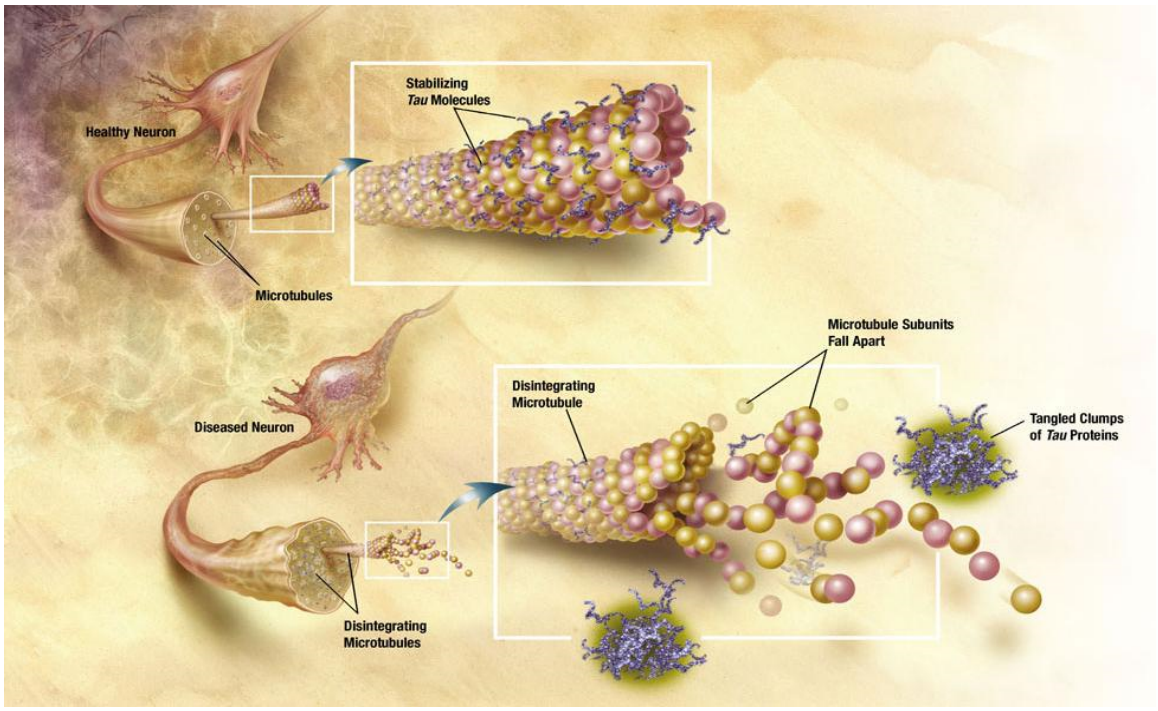


Figure 4: The Proper Function of Tau Protein and the Process of Neurofibrillary Tangle Formation[11]

What can promote the tau protein to be highly phosphorylated are mutations in the tau gene that can cause modification in the protein's function by either reducing the ability of the microtubules to form or by altering the protein's structure. The alteration of the proteins' structure can decrease tau's affinity to the microtubules thereby leading to the cytoskeleton instability. As a result, the freely floating tau proteins in the cytosol can become highly phosphorylated and aggregate into neurotoxic fibrils. When tau is phosphorylated it also becomes more resistant to proteases. Therefore, the tau protein cannot be degraded and it stays longer within the cell making it more readily to form paired helical filaments.[3]

When neurofibrillary tangles form, it shows that the neurons are unable to properly maintain its cytoskeleton, which is required to support the numerous complex cellular processes. The development of a few neurofibrillary tangles is normal within an

aging brain. However, it is the large amount of tangles and its distribution that contribute to the increased amount of neuronal death in AD.[1] What accounts for this high concentration of tangles in AD patients compared to a normal brain is the fact that phosphorylation of the tau protein is at a much higher extent in an AD brain than in a normal brain.[3]

Even though the high concentrations of tangles are present throughout the brain, some neurons in parts of the brain are more affected by the tangles than others, most notably in the hippocampus. The hippocampus is involved in memory storage and its prevalence in being affected by neurofibrillary tangles accounts for the symptoms of AD involving memory loss with daily activities, familiar faces, and loss of words.[1]

Along with mutations in the tau gene, altered calcium homeostasis and the resulting activation of kinases can also cause hyperphosporylation of tau as well as promote high oxidative modifications on the neurofibrillary tangles.[5,10] This continuous observation of high oxidative modifications in the brain of AD patients presented a new characteristic of AD that is slowly being recognized and that is calcium homeostasis disruption.

1.3 Calcium Homeostasis Disruption

Calcium ions (Ca^{2+}) are involved in transcription of genes, synaptic transmission, synaptic plasticity, exocytosis, and other cellular processes.[7] They also play a key role in storing and erasing memories. During the day, brief high concentration spikes of Ca^{2+} triggers the process of long-term potentiation (LTP), which is involved in temporary memory stores. The information in the temporary memory stores is then transferred to the permanent memory stores and erased when smaller elevation in Ca^{2+} activates the process

of long-term depression (LTD) during sleep. Figure 5 illustrates the process of memory storage. The high concentration of Ca^{2+} that is needed to activate LTP is derived from Ca^{2+} entering the NMDA receptors, which are Ca^{2+} channels on the cell, while the lower concentrations of Ca^{2+} needed to active LTD is the Ca^{2+} released from the ER by ryanodine receptors (RYR) and inositol 1,4,5-trisphophate ($InsP_3$) receptors. The high levels of Ca^{2+} cause protein phosphorylation of receptors and associated proteins and promote their insertion onto the plasma membrane to stimulate LTP. The low levels of Ca^{2+} cause dephosphorylation and the retrieval of those receptors and the associated proteins to activate LTD. When Ca^{2+} is released from the ER to generate a signal, a portion of the Ca^{2+} is taken up by the mitochondria, which act as cytosolic buffers. After the signal has been produced and the cell is in the recovery phase, the Ca^{2+} is returned back to the ER.[12]

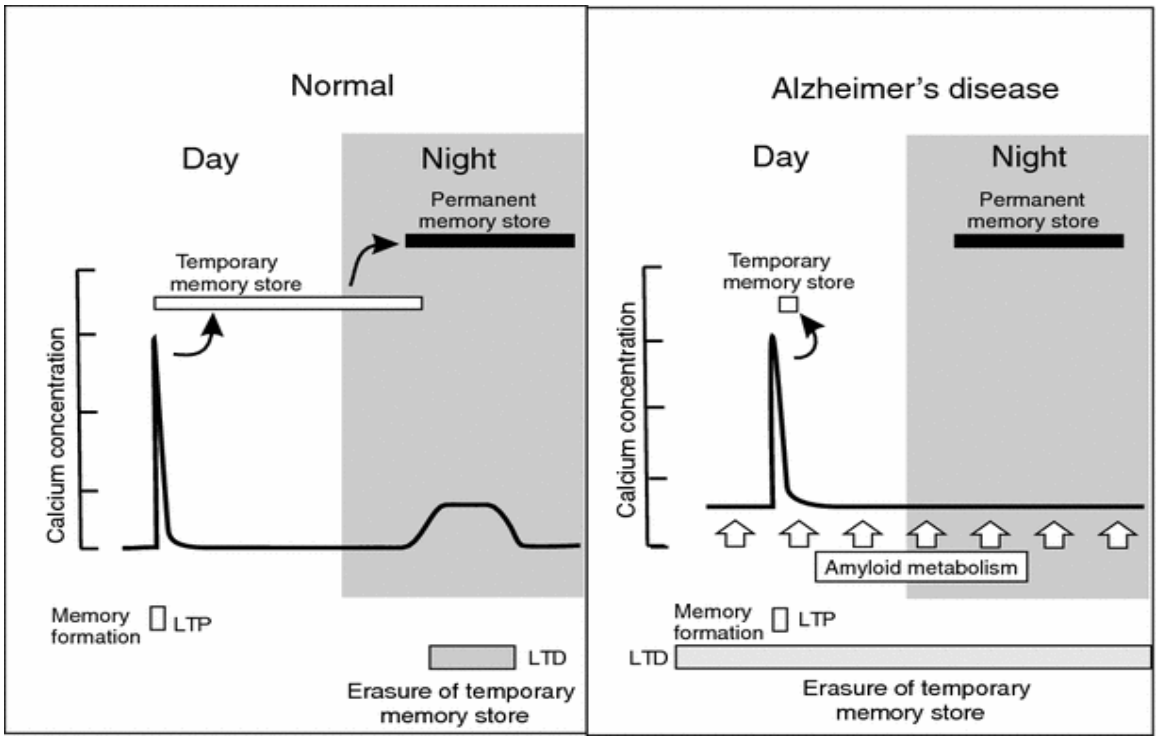


Figure 5: The Process of Storing and Erasing Memories in Relation to Calcium Concentrations in Normal and AD Brains[12]

Increased calcium level in neurons is normal as an individual age. However, in AD there is an constant abnormal disruption of calcium levels caused by the interference of the main intracellular calcium regulating stores (mitochondria and endoplasmic reticulum) and the calcium channels on the plasma membrane.[4,7] This constant elevated level of calcium also illustrated in figure 5. The abnormal production of amyloid plaques causes a change in Ca^{2+} homeostasis that affects the Ca^{2+} signaling pathways, thereby altering the mechanisms involved in learning and memory along with initiating the progressive decline in memory and the increase in neuronal death.

The APP intracellular domain (AICD), which is the peptide that is located inside the cell when APP is cleaved by γ-secretase, is a transcription factor. It promotes an increase production of ryanodine receptors (RYR) that function to amplify inositol 1,4,5-trisphophate ($InsP_3$) in order to release calcium from the ER. The AICD also decreases the transcription of the Ca^{2+} buffer calbindin, which functions to reduce Ca^{2+} signals that can be induced by β amyloids and heightened by mutated presenilin.[12]

When the ER calcium stores are destabilized, it leads to negatively influencing synaptic plasticity that results in memory deterioration and neurodegeneration.[7] The Aβ oligomers cause an influx of extracellular calcium into the cell and increase the level of calcium in the cytoplasm by weakening or thinning the plasma membrane or by forming small annular structures on the membrane that resemble pores. They can also stimulate the opening of calcium channels like NMDA receptors on the plasma membrane and RYR and $InsP_3$ receptors on the ER to allow the increase influx of calcium into the cytoplasm.[4] The increased production of oxidative stress caused by the continual formation of Aβ plaques also interferes with membrane calcium pumps and channels,

further promoting calcium influx into the cell.[5] This constant inflow of calcium into the neurons with no mechanism to relieve the stress, creates a permanent elevated resting level of Ca^{2+} that promotes the constant activation of LTD. This constant activation of LTD quickly and continuously delete memories in the temporary memory stores before they can be transferred to the permanent memory stores as illustrated in figure 5.[12] Based on this mechanism that is involved in the constant activation of LTD, it only affects the ability to learn new things. Therefore it would have no impact on knowledge already learned. However, the cerebral atrophy as well as the damages to the hippocampus and the frontal and temporal lobes of the brain that is caused by the high activation of apoptosis can effectively deteriorate the knowledge that was already learned.

Not only is calcium the main regulator of synaptic plasticity, it can also activate the process of apoptosis.[4] The increased amount of Ca^{2+} that is present in the cytoplasm promotes the mitochondria to take up as much calcium as possible in order to stabilize the levels. The large amount of calcium taken up by the mitochondria puts pressure on it causing the mitochondrial permeability transition pore (MTP) to open, collapsing the mitochondrial membrane potential, and releasing protein factors like cytochrome c to activate apoptosis.[12] In the process, this also generates free-radical oxidation that leads to more neuronal damages and later further promotes cell death.[7] Since accumulating Aβ significantly disrupts calcium levels, neurons that are located close to amyloid plaques have higher Ca^{2+} concentrations.[12]

Chapter 2: Cellular Effects of Alzheimer's Disease

Once the prominent characteristics of AD were determined and how they were created, one needed to know what effect does the amyloid plaques, the neurofibrillary tangles, and the calcium level disruption do to the brain. Why do they cause dementia in AD patients? What is the cellular effect that they cause in the brain and how does that translate into the traits that people observe in AD individuals? These are the questions that provide the purpose into researching the disease and through many studies researchers have determined the reason why and how AD is the way it is.

In Alzheimer's patients, the disease is first exhibited as mild cognitive impairment that gradually develops into more "severe amnesia accompanied by apathy and stupor," thereby affecting the ability to perform daily living tasks. In the later stages, the patients become bedridden and must be "dependent on others for all basic living activities."[4,13] Along with losing their cognitive abilities, their memories, and exhibiting personality changes, Alzheimer's patients also experience psychiatric disturbances such as delusions, depression, agitation, hallucinations, sleep disturbance, and behavioral problems.[5,13]

The disease involves the death of neurons that function to process and store information. The regions in the brain that are involved in memory and learning processes such as the frontal and temporal lobes are smaller in size in an AD brain compared to those same regions in a normal brain due to the deterioration of the synapses and massive cell death.[5] Along with a smaller brain size, AD patients also have large decreases in cellular metabolism in the mitochondria throughout the brain.[5,7]

Definitive diagnosis of AD involves postmortem examination of the brain and it must contain an abundant amount of senile plaques and neurofibrillary tangles in order to

be classified as AD.[5] Amyloid plaques and neurofibrillary tangles are present mainly in areas of the brain that are associated with memory, learning, and emotional behaviors such as the hippocampus and the neocortex of the brain and their numbers increase as the disease progresses.[4,5] What triggers cell death may include high production of Aβ leading to increased extracellular plaques, the disruption of the microtubules paving way to the formation of neurofibrillary tangles, large amounts of cellular damages including the DNA, elevated intracellular calcium levels, and increased oxidative stress.[5]

2.1 Amyloid Plaques

One of the noticeable traits of AD is the high formation of Aβ42 resulting in large accumulation of plaques. The Aβ peptides first form dimers, then oligomers, and protofibrils before they form plaques.[12] Research has shown that Aβ are the most toxic when it is a soluble oligomer since they can diffuse into the synaptic clefts and interfere with synaptic functions, as well as being able to form the insoluble plaques that surround neurons.[5,10]

The oligomers aid in memory impairment by inhibiting long-term potentiation because they can increase Ca^{2+} entry into cells by functioning as either a channel or by activating channels in the plasma membrane through receptor operated channels.[5,12] Along with memory decline, the increased formation of Aβ leads to the death of neurons because the protein is directly toxic to the cells and enhances their vulnerability to metabolic and oxidative stress as well as excitotoxicity, a constant stimulation of a neurotransmitter.[5] Not only can Aβ oligomers diffuse into the synaptic cleft and form plaques this way, but also APP can move along the axon and accumulate at presynaptic

terminals in high levels as well. This results into an additional buildup of Aβ at the synapses.

The presence of Aβ proteins injures the synapses and axons that are associated with the plaques.[5] The damaged and dilated dendrites and axons near the amyloid plaques show signs of "altered intracellular transport and slowed nerve conduction that contributes to compromised information processing in the limbic and association cortices."[10] The synapses also contain many of the proteins that are involved in the commencement and implementation of apoptosis. Since many of the Aβ are located at the synapses and they can induce apoptosis, the site for initiation of neuronal death usually happens at the synaptic clefts.[5]

Along with the ability to initiate apoptosis, the aggregated Aβ in the synaptic clefts can also accelerate chronic destructive inflammatory processes by activating microglia and astrocytes. This stimulates the glial cells production of cytokines, which leads to increased expression of APP and later destruction of the nerve cells due to high levels of microglia-activated free radicals produced and damages to the membrane.[7]

There are more glial cells (astrocytes, oligodendrocytes and microglia) in the brain than neurons and they are subjected to the same conditions as neurons, experiencing the negative effects and abnormalities in the presence of Aβ plaques and neurofibrillary tangles. In astrocytes, the Aβ plaques disturb calcium regulation, impair glutamate transport, and promote the production of pro-inflammatory cytokines thereby influencing synaptic dysfunction and neuronal death. White matters that surround neurons act as an insulator and facilitate fast conduction of action potential and are composed of axons and oligodendrocytes. Oligodendrocytes are susceptible to damages and are often destroyed

by Aβ, leading to the deterioration of white matter, which is very prominent in AD individuals. Microglia cells participate in the immune system by engulfing debris and respond to infection and injury to the brain. In AD, they surround amyloid plaques and deteriorated neurons in order to remove them from the brain and in the process may produce inflammatory cytokines and toxins that aid in the neurodegenerative process.[5]

2.2 Neurofibrillary Tangles

The purpose of tau protein is to promote microtubule assembly, which is one of the key components of the cytoskeleton that functions to transport organelles, proteins, and maintain cell integrity.[3] Therefore, the malfunctioning of tau affects axonal transport.[5] The tangles that accumulate in the cells disrupt axonal and dendritic transport, alter neuronal metabolism, and cause structural changes in the cell bodies and the neurites.[10] Neurons that contain neurofibrillary tangles have high levels of calcium, which also cause over activation of Ca^{2+}-activated kinases and Ca^{2+}-dependent proteases. This leads to large levels of activated apoptotic proteins.[5]

2.3 Calcium Levels and Mitochondrial Dysfunction

The malfunction and deterioration of synapses may also involve Aβ-induced oxidative stress caused by the impairment of membrane ion, glucose, and glutamate transporters as well as the interference with mitochondrial function by an oxidative stress mediated mechanism.[5]

The presence of Aβ plaques promote the formation of free radicals in the mitochondria, increase the number of reactive oxygen species (ROS) in the cell, "[disrupt] the dendrite apparatus" and finally activate apoptosis and necrosis of the neurons.[7] When apoptosis is activated, it promotes the production and activation of

proteins whose function is to increase the permeability of the ER and the mitochondria. This causes calcium to be released from the ER and cytochrome c and apoptosis-inducing factors to be released from the mitochondria. The release of calcium activates caspases and along with endonucleases they cleave many different proteins like ion channels, cytoskeleton, and DNA resulting in cell death.[5]

Oxidative stress is especially prominent in neurons that are surrounded by senile plaques and neurofibrillary tangles, exhibiting oxidatively modified lipids, proteins, and DNA. The source of oxidative stress comes from the redo-active metals like Cu^{+} and Fe^{2+} and Aβ plaque generation. When Aβ oligomers interact with Cu^{+} or Fe^{2+}, it generates H_2O_2.[5]

Free radicals such as hydrogen peroxide (H_2O_2), hydroxide (OH^{-}), and oxide ions (O_2^{-}) are normally produced in the cell as a result of oxidative phosphorylation. Cells have several ways to detoxify and convert the free radicals to harmless products using many different enzymes. If the cell can no longer detoxify, the free radicals wreak havoc within the cell, altering many different classes of macromolecules and DNA. Because mitochondrial DNA does not have histones or a DNA repair system, the oxidative stress is especially damaging.[14]

When the mitochondrial permeability transient pores (MPTPs) opens, it initiates apoptosis by collapsing the electrochemical gradient and activating caspases and apoptosis-inducing factors. Significant uptake of calcium by the mitochondria, excessive mitochondrial energy metabolism reduction, and the activity of ROSs can cause the opening of MPTP and initiate apoptosis in AD.[7]

The functions of key enzymes in energy metabolism like pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and cytochrome c oxidase are reduced in the mitochondria of neurons.[5] Due to the collapsing of the electrochemical gradient and the deterioration of the mitochondria, many of the mitochondrial DNA and cytochrome c oxidase is found in the cytoplasm along with swollen and decreased numbers of mitochondria and lack of microtubules in the neurons.[14]

The neurons with defective mitochondria (low levels of cytochrome c oxidase) would have impaired intracellular calcium buffering, further excessive production of free radicals, inhibited glucose metabolism, greater sensitivity to IP_3-mediated calcium release, and high calcium concentrations in the cytosol.[5,14] In the regions of the brain involved in cognition, the neurons lacking mitochondria would contain high levels of Aβ and have decreased hypoxia tolerance.[5]

Chapter 3: Genetics

Genes may play a role in AD, specifically for familial Alzhiemer's disease (FAD). Through the use of "linkage analysis of familial AD" four genes that are responsible for the disease were identified: three causative genes [β-amyloid precursor protein (APP), presenilin 1 (PS1), and presenilin 2 (PS2)] for FAD and one predisposition gene [apolipoprotein E ε4 (APOEε4)] that is more specific for sporadic AD. Mutations in these genes all cause an increase in the production of Aβ especially for the "highly amyloidogenic 42 amino acid variant (Aβ42)", and/or an increased deposit of amyloid plaques.[3,8]

Sporadic AD is much more common than familial AD, which indicates that gene mutations are not a common cause of AD.[5,9] Familial AD happens mostly in young adults between 40 to 60 years old while sporadic AD happens mostly in older adult ages from 65 years or older.[7] Even though gene mutations are not a common cause of AD, the mutation still represents "an acceleration of the disease process that occurs gradually with advancing age."[10] For the mutations that do occur, Alzheimer's requires mutations on multiple genes in order to be manifested in an individual and is therefore considered a "genetically heterogeneous disorder" and "not a single-gene disease."[3]

3.1 Amyloid Precursor Protein

Mutations in APP involve "one or two amino acid changes within or immediately adjacent to the Aβ" region which increases its cleavage by β-secretase and γ-secretase.[5] Mutations in the APP gene on chromosome 21 have been identified near or at the cleavage sites of both β-secretase and γ-secretase, promoting the increased production for the long form of β-amyloid, Aβ42, which aggregates more readily than the shorter form

of β-amyloid, Aβ40.[1,8] However, these particular mutations are rare in FAD.[8] Mutations that occur in the middle of the Aβ region on APP "enhances the oligomerization and fibril formation" of the Aβ peptides.[10]

3.2 Presenilin 1 and Presenilin 2

The majority of the mutations that are connected with FAD are located in the "two PS genes, particularly in the PS1 gene."[8] Presenilin is an intramembrane aspartyl protease that is present on the membranes of multiple organelles including the ER, Golgi apparatus, and endosomes.[10,12] Presenilin is involved in multiple functions including calcium regulations, unfolded protein response, APP processing as well as Notch signaling, which is a key process in cellular differentiation and releasing intracellular domains to signal in the nucleus.[10] Normally when presenilin is in the ER, it controls the passive leak of Ca^{2+} but when it is processed and located in the Golgi and the endosomes they interact with γ-secretase to form the γ-secretase complex.[12]

Presenilin binds to the catalytic site on γ-secretase and forms a presenilin-γ-secretase complex that participates in the cleavage of APP. Therefore, presenilin mutations affect the proteins' function, preventing the proteolytic processing of Notch and "the normal γ-secretase processing of APP to release Aβ." Mutations in PS1 or PS2 change the cleavage specificity of γ-secretase creating more of the minor cleavage at the Aβ42 residue and less of the major cleavage at the Aβ40 residue.[10] Cells with mutations in PS1 or PS2 lead to a 1.5 to 5-fold increase of Aβ42 compared to cells that had no mutations in PS1 or PS2.[9] If the PS1 gene was deleted, then it would cause a reduction of γ-secretase cleavage, which would results in a decreased amounts of Aβ formed.[8]

Genetic analysis has also shown that the age of onset is related to the position and type of amino acid substitutions in the PS1 mutations. The study involved taking blood samples from 70 FAD subjects and their DNA analyzed to detect where the amino acid substitutions were at and compare that to the subjects' age of onset.[9]

Presenilin that are located on the ER control the passive leak of calcium and therefore calcium homeostasis.[12] Sarcoplasmic ER Ca^{+2}-ATPase (SERCA) pumps are located on the ER and function to pump cytosolic calcium into the ER to maintain a low level in the cytosol. Presenilin functions to regulate SERCA pumps in order for them to work normally and the regulation of SERCA affect Aβ formation. In cells without presenilin, there is an elevated level of calcium in the cytosol and weak ER calcium stores.

When wild-type presenilin is overexpressed or when the genes are mutated, it causes an overfilling of the ER calcium stores caused by the upregulation of the SERCA pumps. This overfilling enhances the $InsP_3$-mediated calcium release by increasing the sensitivity of the $InsP_3$ receptors to presenilin and this only gets worse when the presenilin genes are mutated.[15] This dual and conflicting effect may be a way for the cell to try to respond to the overfilling of the ER by releasing calcium and at in the same time try to respond to the high intracellular concentration of calcium by storing the excess in the ER. This back and forth movement of calcium to try to fix the excess concentration in the cell results in a neuron that cannot solve any of the underlying issues. In the end the "increase release of intracellular calcium from the ryanodine or inositol 1,4,5-trisphosphate ($InsP_3$) channels on the ER" is what overpowers the overloading of the ER and is the most harmful to the cell and the brain by leading to memory deterioration and

activation of apoptosis.[4,7] Therefore, presenilin is directly involved in filling the ER stores and indirectly involved in emptying it of calcium, which explains why a mutation in the gene can cause both an increase in the ER stores and an altered calcium homeostasis that resulted from "an increase amount of Ca^{2+} being released from the ER".[12,15]

Another important proteolytic function of PS involves two signaling molecules (IRE1 and ATF6) that are part of the unfolded protein response (UPR), which is a signaling pathway that initiates the proper folding of misfolded or unfolded proteins. Both IRE1 and ATF6 are ER transmembrane proteins that undergo proteolytic cleavage to allow themselves to enter the nucleus upon initiation of UPR. When PS1 is mutated, it impairs the UPR by reducing the expression of chaperone molecules, which are proteins that properly fold misfolded proteins, in the ER, causing the accumulation of unfolded proteins such as APP and Aβ.[9]

3.3 Apolipoprotein E

An important gene that plays a role in sporadic AD is the apolipoprotein E gene (APOE), which is "firmly established as the major risk factor other than age for the development of AD."[1] The apolipoprotein E gene is the susceptibility genes, which means that if an individual has this gene he has a higher chance of developing AD, thereby making him susceptible to the disease. The gene has 3 allelic forms (ε2, ε3, and ε4) and the lipoprotein that is produced is involved in synaptic repair in response to tissue injury as well as maintenance of neuron structures and cholinergic functions.[1,5]

An individual that has the ε4 isoform has a higher chance of getting AD.[5] The chance of developing the disease sharply increases when APOEε4 and one or two of the alleles are inherited and enhances the levels of amyloid plaques in the brain. The

mechanism is not involved in increased Aβ production but instead stabilizes and decreases the clearance of the fibrillar Aβ deposits.[10] Along with diminishing removal of Aβ, the ε4 allelic form is involved in raising oxidative stress, promoting Aβ aggregation and amyloidgenic processing of APP, and interfering with neuronal plasticity.[5]

Chapter 4: Possible Risk Factors of Alzheimer's Disease

Through numerous amounts of studies and research being done on Alzheimer's, a great deal of progress has been made in understanding this disease. Yet there is still so much that is unknown. The mechanism as to how the disease works is slowly being deciphered, however the reason as to why the disease happens, or even what factors may trigger it are still not fully understood. Although there is so much information and data that needs to be understood, there is a general idea as to what are the risk factors for Alzheimer's. Some of the factors may be indirect causes of Alzheimer's, a result of the disease, or just a symptom that happened to be present but has no relationship to the disease. Nevertheless, there are still risk factors that need to be looked at and understood. The table below gives a general overview to the risk factors presented.

Table 1: Possible Risk Factors for Alzheimer's Disease

Possible Risk Factors	Reasoning
Down's Syndrome	Extra copy of chromosome 21 results in an increase production or mutation of APP leading to a higher concentration of Aβ[16]
Type II Diabetes Mellitus	Insulin resistance promotes increase secretion of Aβ and hyperphosphorylation of tau proteins as well as inhibiting Aβ degrade[17]
Stroke	Lack of blood flow damages the brain[18,25]
History of Head Trauma	Damages the brain with it being more serious when loss of consciousness accompanies the head injury[20]
Depression	Causes neuronal death in regions of the brain like the hippocampus[13]
High Blood Pressure	Decrease blood flow to the brain resulting in hypoxia injuries and later cognitive decline[23,24,25]
Dietary (high consumption of calories, cholesterol, saturated fats, and red meat as well as low consumption of fruits and vegetables)	High levels of cholesterol, calories, and saturated fats contributes to heart disease like high blood pressure,[2] promotes the production of Aβ42,[5] aid in insulin resistance,[2] and neuronal degeneration;[5] high levels of red meat increases the level of iron;[26] low levels of fruits and vegetables provide low levels of antioxidants[2]
Vitamins (low levels of vitamin B, A, C, and E)	Low levels of vitamin B increases the level of homocysteine;[2,5] low levels of vitamin A, C and E gives low levels of antioxidants[2]
Metals (high levels of iron, copper, and zinc)	High levels of iron, copper, and zinc leads to high oxidative stress, increase production of Aβ and neurofibrillary tangles, and later neuronal death[26,27]
Sedentary Lifestyles	Decreases neurogenesis, hippocampal synaptic plasticity, and long-term potentiation[28,29]
Chronic Stress	Increases the production of APP and apoptotic enzymes along with enhancing senile plaques accumulation and tau phosphorylation[28]
High Alcohol Consumption	Promotes oxidative damage in the brain leading to brain atrophy and causes mitochondrial dysfunction and deterioration[2]
Low Education Levels	Less network of synapses and inefficient use of neural networks as well as a less ability to recruit networks[30]

4.1 Down's Syndrome

Down's syndrome is a chromosomal disorder where an individual is born with an extra copy of chromosome 21, resulting in a total of 3 copies, which is why it is also called Trisomy 21. The disease starts from birth and causes malformation and mental retardation throughout the life of the individual. What correlates AD with Down's syndrome is that not only do both diseases cause brain dysfunction but people with Down's syndrome also exhibit abnormal buildup of Aβ in the brain. The reason for this is because the gene for APP is located on chromosome 21. With an extra copy of chromosome 21, Down's patients have a higher chance of having mutations on the APP gene, thereby increasing their chance of producing malfunctioning APP that can later result in misfolded and highly amyloidogenic Aβ. Even if there was no mutations in the APP gene, the extra copy of the chromosome would still contribute to an overexpression of APP and later Aβ, giving rise to an increased amount of amyloid plaques in the brain.[16] As with high productions of anything, the increase concentration of the protein would allow for a higher chance for something to go wrong.

4.2 Type II Diabetes Mellitus

Diabetes mellitus, or simply diabetes, is a disorder where the body produces little to no insulin, a hormone that functions to control the level of sugar in the blood. Therefore an individual with diabetes has high levels of glucose in the blood or hyperglycemia. There are two types of diabetes: type I and type II. Individuals with type I diabetes are considered insulin dependent since their body cannot produce any insulin while individuals with type II diabetes are considered non-insulin dependent since their body is still able to produce insulin just in inadequate amounts. Sometimes in type II

diabetes, even though the body is still making insulin, the cells are not using it efficiently and have lowered responses to the hormone, resulting in what is called insulin resistance. Therefore, the insulin is not being used by the cell and is left circulating and accumulating in the blood along with high blood sugar.

What connects diabetes with AD is that insulin resistance is "associated with β-Amyloid-accumulation and hyperphosphorylation of tau-protein," the two characteristics of AD.[63] Studies have shown that insulin increases the amount of Aβ by inhibiting its degrade by insulin-degrading enzymes and can stimulate the secretion of Aβ "by the enhancement of its trafficking from the ER and trans-Golgi network, the main site for Aβ generation, to the plasma membrane."[17] It has also been noted that AD patients have large decreases in glucose metabolism throughout the brain that is attributed to disruption in the insulin signal transduction.[7,17] This impairment in the insulin signal transduction cascade, which is also observed in type II diabetes, prevents glucose from being transported from the blood into the cells, thereby providing the cells, including neurons, with inadequate energy to perform properly, eventually leading to apoptosis.[17]

4.3 Stroke

Stroke happens when the brain suddenly cannot receive nutrients and oxygen due to the loss of blood or inadequate blood supply. The lack of blood flow to the region of the brain causes damages that can lead to neuronal death. Studies indicate that stroke increases the risk of getting dementia, whether or not the stroke was symptomatic, and the risk only increases with each successive stroke. Not only does the number of strokes experienced contribute to the risk but also the location of where it happens, such as the hippocampus, as well as the intensity of the stroke can play a role.[18,19] It has been shown

that the risk of developing AD in relation to stroke increases if the individual also exhibits vascular problems such as high blood pressure, heart disease, and diabetes.[19]

4.4 Head Trauma

While any damage to the brain is unfavorable, there has been no conclusive evidence that indicates head trauma causes Alzheimer's disease in individuals. Some studies show that there is a correlation between head trauma and AD while other studies show that there are no correlations between the two. However, there is evidence indicating that men with head injuries have a higher chance of getting AD than women with head injuries. What can cause this discrepancy may be due to female hormones, particularly progesterone and estrogen. In an animal model, the female rats would have considerably smaller contusions in the brain than in male rats, which may suggest that the hormones play a neuroregenerative and neuroprotective role in the brain.[20] As with any injury, the more serious the head trauma, such as ones that causes unconsciousness, the more damage it can create to the brain.

4.5 Depression

Depression is very common in Alzheimer's patients as well as being one of the first symptoms expressed. However it is difficult to show whether depression is a result of having AD or is a cause of AD.[21] Some studies indicate that depression is a risk factor, while others show that AD causes depression, and still others say there is no association between the two.[22] Depression does negatively influences the brain by being directly involved in hippocampal atrophy, thereby interfering with information recall and memory performance, one of the key signs of AD. Therefore, it has a toxic effect on the brain that decreases cognition.[13]

4.6 High Blood Pressure

There is increased evidence that indicates good heart health and good brain health are intertwined. An individual with high blood pressure has a higher chance of getting a heart attack, stroke, and an aneurysm, a swelling of the artery walls. While high blood pressure does not cause Alzheimer's disease, it can make the brain more vulnerable to dementia.[23] Hypertension decreases the blood flow to the brain causing cerebral infracts, which are localized cell deaths, and medial temporal lobe atrophy.[24] The microinfarctions of the cerebral white matter result in demyelination, the loss of the myelin covering on neurons, and the elevated blood pressure promotes increased plaque and neurofibrillary tangle formation in the hippocampus and the neocortex. All of the resulting injuries caused by the hypoxia, which is the deficiency of oxygen, later stimulates cognitive decline in the individual.[25]

4.7 Dietary

High consumption of saturated fats, cholesterols, and calories contribute to cardiovascular diseases such as high blood pressure, which that can affect the brain since the systems in the body are highly connected. High levels of saturated fats can aid in insulin resistance and further promote the development of diabetes.[2] Cholesterol, on the other hand, has been indicated to play a more direct role in the brain by stimulating amyloidogenic processing of APP. In both cultured cells and APP mutant transgenic mice, the high levels of cholesterol increased the production of Aβ. The cholesterol levels can also disturb signal transduction and membrane fluidity in the neurons, thereby promoting neuronal degeneration. With high levels of cholesterol, it is highly incorporated into lipid rafts on the plasma membrane, which are known to contribute to

the APP processing, synaptic signal transduction, and the activation of apoptosis. When increased amounts of cholesterols are incorporated into the lipid rafts, this also indicates that there is an increased level of oxidative stress present.[5]

High consumption of red meat increases the level of iron in the body and that can lead to increase production of reactive oxygen species.[26] Low intake of fruits and vegetables provide lack of antioxidants such as carotenes that can combat oxidative stress like the formation of peroxide. There are many evidences that support the fact that high consumption of dietary antioxidants can also lower the risk of stroke and cerebrovascular diseases.[2] In animal models that had dietary restrictions, the animals showed prolonged lifespan and greater resistance to neuronal deterioration. Even though there has been accumulating evidences that suggest dietary factors may impact the development of AD, there is no causal or direct relationship between the two. However, there might be an indirect relationship between dietary factors and AD.[5]

4.8 Vitamins

A low level of vitamin B results in a high level of homocysteine, a precursor in making methionine and cysteine, in the brain.[2,5] The homocysteine is active in the brain and can act as a neurotoxin in high concentrations by impairing DNA repair in the neurons located in the hippocampus and making them more vulnerable to Aβ toxicity. High consumption of vitamin B lowers the risk of heart disease as well as stroke thereby lowering the risk of getting AD.[2] Vitamins A, C, and E, on the other hand, are antioxidants that help suppress the inflammation signaling cascade and oxidative stress as well as hinder the formation of nitrosamines, a carcinogen that helps forms cancerous cells. Along with these benefits, antioxidants are also known to lower the risk of stroke

and cerebrovascular diseases. Therefore a high intake of vitamins is beneficial to the body with dietary antioxidants being more beneficial than supplemental antioxidants. Although there are numerous studies illustrating the positive effect of antioxidants, there is no consistent data showing that vitamins help with lowering cognitive decline and AD.[2]

4.9 Metals

Both iron and copper are necessary for proper growth and development. Iron is essential for oxygen transport in hemoglobin and both metals are components of other proteins and enzymes. However in high amount they can catalyze the production of toxic hydroxyl radicals and reactive oxygen species that can lead to neurodegeneration. Moreover, copper is also involved in amyloidogenic formation since both APP and β-secretase have a binding site for the metal. β-secretase needs to bind to copper in order to be activated to cleave APP and form Aβ. Therefore high concentrations of copper can result in increased β-secretase activity in the brain. Tau also associates with copper by binding to it to form the neurofibrillary tangles.[26] Along with copper, zinc can also bind to Aβ and promote its aggregation as well as produce reactive oxygen species.[27] Although copper may play many essential roles in AD, there are conflicting studies as to whether its increased amount may contribute to AD pathology.[26] Nonetheless iron, copper, and zinc do promote the formation of reactive oxygen species that increase oxidative stress.[26,27]

4.10 Sedentary Lifestyles

In many animal studies involving transgenic mice, exercise has improved their learning and memory abilities as well as decreased Aβ plaques and increased

hippocampal synaptic plasticity, volume, and neurogenesis. Exercise stimulates gene expression such as increasing the production of brain-derived neurotrophic factors (BDNF) in the hippocampus. Brain-derived neurotrophic factors are involved in many intracellular signaling pathways that initiate neurogenesis and neuronal survival. The neurotropic factors also stimulate synaptic plasticity and long-term potentiation (LTP), both of which play a role in memory and learning.[28] Exercise also decreases tau phosphorylation, APP cleavage, the number of plaques through the production of plasmin, and apoptotic signaling in the brain.[28,29] Besides promoting neuronal survival and improving cognitive functioning, exercise increases the cerebral blood flow and promotes the development of capillaries in the brain, allowing for improved circulation of nutrients, glucose, and oxygen.[29] Therefore a sedentary lifestyle does not provide any of the benefits that accompany an active lifestyle.

4.11 Chronic Stress

Increased amount of recurring stress can lead to cognitive decline caused by promoting cortical and hippocampal apoptosis and decreasing neurogenesis. Along with that are defects in dendritic branching and long-term potentiation, reduced BDNF levels as well as impaired memory and learning. In relation to AD pathology, stress can enhance senile plaques accumulation, tau phosphorylation, APP production, and apoptotic enzymes. Essentially stress produces the opposite effect of increased physical activity. Even though there are a few studies that illustrate stress is involved in dementia, there is no causal relationship suggesting that stress causes AD.[28]

4.12 High Alcohol Consumption

Alcohol is a neurotoxin and a high concentration of it can lead to oxidative damage to the brain. Studies show that rats with recurrent exposure to intoxicating levels of alcohol had developed neuronal mitochondrial dysfunction and deterioration. In humans, a moderate alcohol consumption results in brain atrophy, less white matter, and a lower brain weight. However, it also contributes to less infarcts, strokes, better endothelial function, increased concentration of high-density lipoprotein (HDL), and decreased platelet adhesiveness. This dual effect illustrates that alcohol acts as both a neurotoxin and helps lower cerebrovascular disease regardless of what type of alcohol it may be. However, there have been increased studies indicating that wine is much more beneficial than all the other alcoholic beverages simply because it contains antioxidants that can help combat oxidative stress. Even though alcohol may exhibit this dual effect of negatively affecting the brain while also lowering cerebrovascular disease, a high intake of alcohol does cause it to be more of a neurotoxin that far outweighs the benefits and is therefore considered to contribute to a higher risk of dementia including AD.[2]

4.13 Low Education

Many studies involving education indicate that increased schooling can protect individuals from developing Alzheimer's disease. Patients that were diagnosed with AD showed decreased dementia when they had additional education. The additional education did not reduce the AD pathology, but instead aided in increasing the patients' ability to resist decline in cognitive function, illustrating that the same number of amyloid plaques would have less effect on cognition with increased education. The brain may use pre-existing neural networks and cognitive processing attained through past experiences

and challenging tasks like education more efficiently and improve the ability to recruit networks as a way of compensating and coping with the pathological damage, thereby postponing clinical manifestations.

There are many studies demonstrating that increased education as being protective against AD but there is no indication that a low level of education is a risk factor for AD.[30] However, it can be assumed that an individual with a low education will have fewer benefits attained than an individual with a high education, like dense network of synapses, efficient use of neural networks, and the ability to recruit networks. Therefore an individual with a low level of education would have a higher risk of experiencing AD.

Chapter 5: Biochemical Markers

Research has shown that the pathology of AD starts to become active even before showing signs of neurodegeneration. Therefore, the most effective way to treat AD would be to detect and diagnose AD at its earliest possible stage. Currently, the diagnosis for possible AD is a neuropsychological exam that tests for memory and other cognitive functions. There are numerous types of neuropsychological tests that are relatively sensitive and specific for diagnosing AD and distinguishing AD from other neurological disorders. These evaluations can also track and document the progression of AD and describe the psychiatric symptoms associated with the disease. However, these tests are mostly used in assessing the efficacy of prospective AD clinical drugs. Furthermore, other neuropsychological tests are less well developed. Therefore, they continue to remain an area of controversy on their reliability and validity. Other methods for diagnosing AD are magnetic resonance imaging (MRI) or single photon emission computed tomography (SPECT), one of the numerous imaging methods that can detect vascular dementia. Genetic analyses demonstrate mutations in the amyloid precursor genes are for familial AD, and the presence of the APOEε4 allele is proven to be associated only with sporadic AD. In addition, these factors are only found in a small number of AD cases and thus cannot be used as a diagnostic test. Nonetheless, AD can only be confirmed after a post-mortem examination of the brain. The post-mortem examination checks for the presence of large numbers of plaques and tangles. However, the presence of plaques and tangles is also seen in healthy, elderly people but to a lesser extent. Therefore, a better strategy to confirm AD would be to use a biomarker that would detect and diagnose AD at it earliest state.

A biomarker is any detectable biological feature that provides information about its source. A biomarker can be any or all of the detection modalities from imaging to chemical. Characteristics of an ideal diagnostic or prognostic biomarker include detecting a fundamental feature in the pathology of AD that is different from other dementias, being reliable and valid in confirmed AD cases, as well as being inexpensive and minimally invasive. The guideline for a biomarker to reflect the neuropathologic characteristic of AD should have a sensitivity of at least 85% and, to differentiate AD patients from controls of the same age and patients with other forms of dementia, a specificity of at least 75%.[31]The ideal biomarker would be an easy and inexpensive blood test that would reflect AD in proteins, nutrients, and DNA. However, there is no biomarker that can differentiate whether a patient has AD or another form of dementia because not a single biologic marker has specificity towards AD.

Since AD occurs due to various pathophysiological causes and mechanisms, it may be that the different mechanisms that cause AD are different in each patient, and thus each biomarker can only be found in subpopulations of patients and not in all AD patients.[32] In addition, different tests might be effective at different AD stages, which range from early to late stages. There might be some fallbacks to each test such as the patient's mood, side effects of treatments, or the presence of another illness.[31] The results of the test might also be detected late and may be dismissed mistakenly.[31] Therefore, there is an urgent need for biomarkers to provide insight into the physiology, severity, and stage of AD. Currently, there are 3 main categories of biomarkers being investigated: genetic biomarkers, biochemical biomarkers, and neuroimaging biomarkers.

Genetic AD biomarkers do not seem to take on a major role as a diagnostic marker because they only account for 2-5% of all AD cases, which are typically associated with the rarest form of AD: familial AD.[31] Therefore, the following section delves into the current biologic markers in serum (also known as plasma) and cerebrospinal fluid (CSF) that test for molecules prominent in AD pathology oxidative stress, inflammation reactions, cholesterol homeostasis (maintenance and regulation of cholesterol), plaque formation and vitamin levels in AD patients.

5.1 Alzheimer's Disease Pathological Alteration Markers

Because neurofibrillary tangles and Aβ plaques are characteristics in the pathology of Alzheimer's disease, it is elementary that the proteins associated with them can be potential biomarkers for AD. These proteins are amyloid-β (Aβ), tau protein, glial fibrillary acidic protein (GFAP), and amyloid precursor proteins (APP).

5.1.1 Amyloid β-Protein

Aβ protein is a prominent feature in the pathology of AD. Because Aβ can be detected in both CSF and plasma, it serves as the most convincing candidate biomarker for AD. Aβ occurs in two main forms, Aβ40 and Aβ42 amino acids, depending on the C terminus.[33] Several studies demonstrate Aβ-protein is decreased in CSF in AD patients compared to other types of dementia (Table 2), which remains unclear. However, decreased CSF Aβ concentrations are observed at moderate severity of the disease, suggesting a possible use as marker in early diagnosis. However, its use in differential diagnosis is limited and it can only be differentiated by 60% from other types of dementia.[32]

Investigations of Aβ42 in CSF then came about with the discovery that Aβ peptide forms the main component of AD plaques, which is composed primarily with a length of 42 amino acids (Aβ42) and is secreted by cells.[34] Several experiments show a reduction of Aβ42 by approximately 50% in AD patients compared to normal controls of the same age (Table 2).[31] The diagnostic sensitivity and specificity levels ranged between 80% and 90%, although it is not clear why Aβ42 is reduced in AD patients. Compared with other types of dementia, the specificity level was only approximately 60%. An autopsy study demonstrates an inverse correlation between Aβ42 levels in the CSF and the number of plaques. It was also recently shown that subjects with a positive signal in amyloid positron emission tomography (PET) studies with Pittsburgh Compound B (PIB), a molecular imaging agent, had the lowest Aβ42 values in the CSF.[31] Nevertheless, future research need to take into account the considerable daily fluctuations in Aβ levels in the CSF.

In several experiments, the concentration of Aβ, including Aβ42 and Aβ40 (another variant form of Aβ peptide), in plasma are not consistent with each other, giving contradictory results (Table 2).[32] The broad overlap in both types of plasma Aβ concentrations between AD and control subjects suggests that plasma Aβ cannot accurately distinguish sporadic AD from control cases, and even more so between AD and mild cognitive impairment (MCI) subjects, which is the transitional region between normal aging and AD. Furthermore, several studies have shown plasma Aβ levels do not correlate with the severity of the disease. Thus, plasma Aβ measures are not sensitive or specific enough to be considered candidate biomarkers for AD.

CSF analyses show that Aβ42 levels are decreased in AD patients, but have fluctuating levels in plasma in AD and MCI subjects. Earlier research shows that in AD patients there are no correlations found between Aβ concentration in CSF and in plasma. Yet one analysis observes a clear correlation between CSF and plasma level for both Aβ40 and Aβ42 in healthy individuals and found no such correlation in AD and MCI individuals. The findings suggest that the normal equilibrium between Aβ concentrations in CSF and in plasma is disrupted with the start of amyloid deposition in the brain.[35]

5.1.2 Tau protein

Tau protein is the main component involved in the intraneuronal changes in AD, as neurofibrillary tangles are formed when tau protein is dissociated from its binding sites on the microtubules. Total tau protein (t-tau) shows an increase in concentration in AD by 300% in several reports (Table 2). The specificity and sensitivity levels are between 80% and 90%.[32] Levels of CSF tau protein are elevated in AD patients and could be distinguished from vascular dementia, frontotemporal dementia (FTD), alcoholic dementia, or depression of Parkinson's disease, indicating its use as a marker for early diagnosis.

The combination of Aβ42 and total tau could differentiate AD from healthy controls with a sensitivity of 85% and a specificity of 86% (Table 2).[32] However, this combination could not differentiate AD from other degenerative dementias. Yet, several studies demonstrate that the ratio of Aβ (Aβ42/Aβ40) combined with the increased tau concentration give an increased specificity and sensitivity, and could distinguish between AD patients and other neurological patients and controls. This leads to the conclusion that this specific combination is a good marker for early diagnosis. However, tau protein

measurement in CSF should have some standardization on genetic and geographic differences, and "the use of CSF limits the possibility to develop the assay for routine diagnostic purposes or as a population screening method."[32]

In AD patients, tau protein is present in a pathologic, hyperphosphorylated form.[32] Research suggests hyperphosphorylated tau (p-tau) is promising in distinguishing AD from FTD, with sensitivity and specificity rates of 85% to 90% (Table 2).[32] Although a combination of various p-tau subtypes did not provide improved results in distinguishing between groups, reports show that high p-tau concentration correlates with a decline in cognitive performance and conversion to AD. They also correlate with disease progression as measured structurally by the rate of hippocampal atrophy.[32] In addition, a report on CSF p-tau in MCI subjects proved to be a powerful candidate of AD even for a short observation period of 1 to 2 years. Nonetheless, it is promising for the clinical use of p-tau by general practitioners as consultants to inform patients as soon as possible.

A combination of all 3 markers (t-tau, Aβ42, and p-tau) proved to be useful in optimizing prediction in a varied MCI population during a longer observation period. The assay used is Luminex XMAP technology that is based on flow cytometry that counts and examines molecules in order to determine different biomarkers simultaneously with only a small volume of CSF.

5.1.3 Glial Fibrillary Acidic Protein

Glial fibrillary acidic protein (GFAP) is the "major intermediate filament cytoskeletal protein expressed mainly by astrocytes" and it is found in increased tissue concentrations in several neurodegenerative diseases.[32] Various diseases have shown an increase of GFAP in CSF and in serum (Table 2). However, it should be noted that during

normal aging, levels of GFAP expression increase in the brain and in CSF. Nonetheless, GFAP autoantibodies could be detected in serum and in CSF of patients with several types of dementias. Thus, GFAP autoantibodies may be markers with high specificity for brain damage or other types of dementia.[32] However, no anti-GFAP autoantibodies are observed in presenile dementia, suggesting limited applicability as an early diagnostic marker.[32]

5.1.4 Platelet Amyloid Precursor Protein Isoforms

The amyloid precursor protein (APP) is involved in the proteolytic process of Aβ protein in order to yield Aβ42 and Aβ40. Investigations report reduced isoform ratios of APP in platelets, which are irregularly shaped cell fragments, in AD patients (Table 2).[33] The diagnostic sensitivity and specificity levels range between 85% and 90%. These reports find that the reduction in the APP isoform ratio correlate with the severity and progression of the disease, but not with age.[33] One particularly promising approach in CSF studies is the detection and quantification of BACE, one of the key enzymes responsible for the pathology of amyloid cleavage of APP. Therefore, a significant increase in BACE concentration in the CSF of AD patients compared to healthy controls would help in diagnosis of AD.

Table 2: Summary of Concentration of Pathological Alterations Markers in Serum and CSF of AD Patients

	Marker	AD vs. Normal
CSF	Aβ	- (9)
	Aβ autoantibodies	= (2)
	Tau	+ (14)
	Hyperphosphorylated tau	+ (1)
	Combination tau	+ (5)
	Combination tau with Aβ42/Aβ40 ratio	- (1)
	β-secretase (BACE1)	+ (1)
	GFAP protein	+ (4)
	GFAP autoantibodies	+ (2)
Serum/plasma	Aβ	+ (3) = (2)
	Aβ40	+ (with age and initial stages)(2)
	Aβ42	+ (with age and initial stages)(1) - (1)
	GFAP autoantibodies	+ (4)
	Aβ autoantibodies	= (2)
	Platelet APP isoforms	- (2)

+: Increased levels observed in AD patients compared to controls
=: No difference observed in levels of AD patients compared to controls
-: Decreased levels observed in AD patients compared to controls
#: Number of studies conducted

5.2 Cholesterol Homeostasis Markers

The maintenance and regulation of cholesterol is essential in the brain. Excess cholesterol, particularly extracerebral cholesterol, in the brain needs to be removed because it does not contribute to the brain cholesterol content.[32] The transport mechanism of cholesterol is not entirely clear, but it is speculated that APOE and 24S-hydroxycholesterol, an oxidized product, facilitates in its removal.

5.2.1 Cholesterol

Cholesterol in plasma yield inconclusive results due to conflicting outcomes conducted by several studies. Increased cholesterol levels were associated with an

increased risk of AD in several investigations, although no association was found in one study, and established AD cases had lower cholesterol levels in other studies (Table 3).

5.2.2 24S-Hydroxycholesterol

24S-hydroxycholesterol is produced through enzymatic oxidation for the removal of cholesterol from the brain. Several analyses observe increased CSF levels of this oxysterol during early stages of AD and decreased levels in the more advanced stages (Table 3). These results suggest that 24S-hydroxycholesterol could possibly be a stage marker of neurodegenerative diseases. Because it is "present almost exclusively in the brain," it might be of use as a biological marker with "high specificity for alteration in brain cholesterol constituency."[32] However, its use as a single marker might be limited in individual patients because there are large overlaps between patients and controls. 24S-hydroxycholesterol in plasma represents a balance between production in the brain and metabolism in the liver.[33] However, plasma levels show a weak correlation with CSF levels in that 24S-hydroxycholesterol levels in AD plasma are inconsistently increased compared to that of in AD CSF (Table 3).[33]

5.2.3 Apolipoprotein E

APOE plays a major role in lipid transport and cholesterol homeostasis.[32] It mediates cholesterol transport to repair and remove excess cholesterol from dying cells.[32] Experiments indicate increased APOE CSF expression in astrocytes in the frontal and temporal cortex of AD patients (Table 3).[32] However, no clear differences or correlations have been found in CSF and plasma concentrations. Although the mechanism for APOE is not entirely clear, the APOEε4 allele is one of the main risk factors currently known for increased risk of AD, getting AD at an early age, increased amyloid plaque load, and

elevated levels of Aβ40 levels in the AD brain.[33] Research shows that a defect in or close to the APOE gene could be responsible for the increased susceptibility to AD.[33]

Subjects with the APOEε4 risk allele were found to have the highest concentrations of APOE in CSF (Table 3). There was no consistent association of plasma APOE protein levels with diagnosis. Plasma experiments documented elevated APOE levels in AD, no difference, or reduced levels relative to controls (Table 3).[33] These conflicting results of APOE concentrations in CSF and in serum may be only limited to large groups, and therefore have limited value for diagnosis of individual patients.[33] Nevertheless, researchers postulate that there may be a correlation between APOE concentration or the number of ε4 alleles and tau protein concentration.[32]

5.2.4 Lipoprotein Lp(a)

Lipoprotein Lp(a) is a low-density lipoprotein-like particle containing the plasminogen-like APOE.[33] Because APOE was detected in primate brain, Lp(a) particles (which can also carry APOE) was suggested to be involved in cerebral lipoprotein metabolism.[33] Serum Lp(a) levels were not associated with cognitive decline over 3 years within an elderly population, although AD patients had higher levels of Lp(a) in a cross-sectional study, which is a descriptive study that observes and provides data on an entire population that consists of different subgroups (Table 3).[33]

Table 3: Summary of Concentrations of Cholesterol Homeostasis Markers in Serum and CSF of AD Patients

	Marker	AD vs. Normal
CSF	Apolipoprotein ε4	+ (1) = (1) - (1)
	24S-hydroxycholesterol	+ (2)
Serum/plasma	Apolipoprotein ε4	= (3) - (2)
	24S-hydroxycholesterol	+ (1) - (2)
	Cholesterol	+ (4) = (1) - (2)
	Lp(a)	+ (1) = (1)
	Homocysteine	+ (6) = (1)

+: Increased levels observed in AD patients compared to controls
=: No difference observed in levels of AD patients compared to controls
-: Decreased levels observed in AD patients compared to controls
#: Number of studies conducted

5.3 Oxidative Stress Markers

Literature research has shown that oxidative stress plays a significant role in the process of AD. The brain has high oxygen consumption and is rich in polyunsaturated fatty acids that are easily oxidized.[32] Yet, decreased cerebral perfusion in AD patients leads to a decrease in oxygen and in glucose delivery to the brain, resulting in energy deprivation.[32] This then causes oxidative stress in the brain. Therefore, oxidative stress in AD can be detected by an increase in protein carbonyl content and lipid, protein, and DNA oxidation products.

Isoprostanes are likely candidates as oxidative stress (OS) markers, specifically markers of lipid peroxidation, which are particular for a neurodegenerative disease. They are derived from the free radical mediated peroxidation of polyunsaturated fatty acids.[32]

Isoprostanes, specifically F2-isoprostanes, are found to increase in the CSF of MCI and AD brains of subjects over time (Table 4). Other isoprostane-like compounds include F-4 isoprostanes that are shown to be elevated in the "temporal and occipital [lobes of the brain], but not in the parietal lobes of AD patients."[32] Another variant of isoprostanes, 8,12-isoprostane-F2α-VI shows an increase in CSF as well in blood and urine of AD patients (Table 4). 8-hydroxy-2'-deoxyguanosine is a DNA oxidation product that is shown to increase in concentration in the CSF of AD patients compared to controls (Table 4). No overlaps were observed between the two groups, suggesting an interesting CSF marker that can distinguish AD patients from controls.[32] Another oxidative stress is 3-nitrotyrosine because it is seen in various neurodegenerative diseases. Analyses have shown increased levels of 3-nitrotyrosine in CSF, but cannot be detected in serum due to breakdown of nitrated protein and different phases (Table 4).[32] CSF isoprostane markers perform better than memory tests because of their diagnostic precision. CSF isoprostane markers even improve the results obtained with hippocampal volumetric analysis to distinguish AD from the other dementia types.

F-2-isoprostanes are increased in plasma also; however this was contradictory to another group (Table 4). This might reflect the differences in measurement techniques. 8,12-isoprostane-F2α-VI was observed to have no change in concentration in the plasma of AD patients (Table 3), conflicting with the increased concentration in CSF. Another product of lipid peroxidation, 4-hydroxynonenal, is also elevated in AD plasma (Table 4). One difficulty in detecting isoprostanes in plasma is that they can be oxidizes easily. Overall, the limit to the use of isoprostanes as biomarkers is the very demanding analysis

method that is required to detect it. Therefore, isoprostranes should still be regarded as a merely scientific approach.[32]

Table 4: Summary of Concentrations of Oxidative Stress Markers in Serum and CSF of AD Patients

	Marker	AD vs. Normal
CSF	8-hydroxy-2'-deoxguanosine	+ (1)
	F2-isoprostanes	+ (6)
	F4-isoprostanes	+ (1)
	3-nitrotyrosine	+ (3)
	8,12-isoprostane-F2α-VI	+ (1)
Serum/plasma	8,12-isoprostane-F2α-VI	+ (1)
	F2-isoprostanes	= (3)
	4-hydroxynonenal	= (1)

+: Increased levels observed in AD patients compared to controls
=: No difference observed in levels of AD patients compared to controls
-: Decreased levels observed in AD patients compared to controls
#: Number of studies conducted

5.4 Vitamins

Vitamins are known to be modulators of cognitive performance. Vitamins A, B6, B12, C, and E are important antioxidants that are possibly related to cognitive deterioration. Vitamin E is known to break major chains in biological membranes, which are high in lipid content and polyunsaturated fatty acids of the brain.[32] Homocysteine, a sulfur containing amino acid, and folic acid are also known to be associated with cognitive impairment, but their mechanisms are not clearly understood.

A CSF study observes the levels of vitamin E to be significantly low, while another one finds no change (Table 5). The same results of no difference in the levels is observed in vitamin A and C. Overall, a low vitamin level might indicate the susceptibility of a patient to oxidative damage (Table 5). Several studies observe reduced serum or plasma levels of vitamins A, C, and E in AD and vascular dementia patients, even when controlled for nutritional status (Table 5). Other clinical researches observe no

difference in the levels of vitamin C and E in plasma (Table 5). "Increased levels of homocysteine in serum in combination with decreased levels of vitamin B12, B6, or folic acid are deemed a possible risk factor in AD"[32] as epidemiologic reports indicate a relation among them in the aspect of cognitive performance. More insight is needed for this relation. Also, it must be considered that vitamin B12 and folic acid deficiencies are common among the elderly. Biasing factors such as age, education, and gender should also be taken into account. After adjustment of those biasing factors in one CSF report, it was observed that the levels of the vitamins A, C, and E did not change in both AD and normal patients (Table 5). Yet, another research group still observed a decreased level of vitamin E in dementia patients. Thus, the intake of vitamins or dietary habits can affect the levels of vitamin markers.

Table 5: Summary of Concentrations of Vitamins in Serum and CSF of AD Patients

	Marker	AD vs. Normal
CSF	Vitamin A	= (1)
	Vitamin C	= (1)
	Vitamin E	- (1) = (1)
Serum/plasma	Vitamin B6	- (1)
	Vitamin B12	- (1) = (1)
	Folic acid	- (2)
	Homocysteine	- (1)
	Vitamin A	- (3)
	Vitamin C	- (4) = (1)
	Vitamin E	- (6) = (1)

+: Increased levels observed in AD patients compared to controls
=: No difference observed in levels of AD patients compared to controls
-: Decreased levels observed in AD patients compared to controls
#: Number of studies conducted

5.5 Inflammatory Markers

Reports indicate that proteins such as interleukin-1(IL-1β), interleukin-6 (IL-6) and tumor necrosis factor (TNF-α), usually associated with inflammatory processes, are found in senile plaques. IL-1β, IL-6, and TNF-α are cytokines that participate in inflammatory reactions Normally, microglia and astroglia (which are both glial cells in the brain) and even neurons produce interleukins at low levels.[32] However, if the brain is under oxidative stress, then microglia are activated and produce high amounts of IL-1β and TNF-α. Furthermore, the concentrations of inflammatory-related proteins change in AD patients.

CSF and plasma studies of inflammation markers produce conflicting results (Table 6). One analysis shows that there is an increase in several interleukins in CSF, another shows decreased levels, and yet another shows no change at all in the levels. Inflammatory molecules variably increased in CSF and plasma AD include interleukin (IL)-1, tumor necrosis factor-α, IL-6, IL-6 receptor complex, α 1-antichymotrypsin, and transforming growth factor-β; these were unchanged in other reports, as were other cytokines including IL-12, interferon-α, and interferon-γ (Table 6). Problems can arise due to low levels of interleukins and low sensitivity of the ELISA assays (biochemistry assay that detects the presence of a substance) and due to using control groups with other neurological diseases. Another problem is that studies focus on different stages of the disease. One group focused on severely affected patients, while another on early-onset AD patients. Also, investigations were done in smaller groups, thus contributing to the differences when comparing to larger groups.

α-antichymotrypsin (α-ACT), a glycoprotein that inhibits the activity of certain enzymes, showed higher concentrations in more severely affected patients. Thus, α-ACT may prove to be a marker in monitoring the progression of the severity of AD symptoms. Haptoglobin (Hp), an iron transporting protein involved in the process of oxidative stress and an acute-phase protein, show an increase in some of the lower weight isoforms in AD patients, indicating a promising discriminative role between AD and controls.[32] Acute-phase proteins are proteins that increase or decrease their concentrations in plasma in response to inflammation. There is still a large overlap of Hp concentrations between AD and controls. In addition, the method 2D gel electrophoresis is used to separate the proteins by charge or size, but it is laborious as a diagnostic practice. In order to have better analyses and consistent results, it is suggested to use sensitive assays and large patient groups with the similar stages of AD.[32] Other markers related to inflammation have been investigated: lithostathine, glycoprotein 130, and cleavage products of kininogen in CSF; mannan-binding lectin and proteinase kallikrein in both serum and CSF; histamine, anti-GFAP, anti-NGF, anti-histone, and anti double-stranded DNA antibodies in serum.[32]

Table 6: Summary of Inflammatory Proteins in Serum and CSF of AD Patients

	Marker	AD vs. Normal
CSF	IL-6	+ (2) = (8) - (1)
	IL-6 receptor	= (1) - (2)
	Glycoprotein 130	= (1) - (1)
	IL-1β	+ (2) = (6)
	IL-1β receptor II	+ (1)
	TNF-α	+ (1) = (3)
	TNF-α receptors	= (3)
	α-ACT	+ (3) = (2)
	Haptoglobin	= (1)
	Haptoglobin fragments	+ (2)
	Lithostathine	+ (1)
	Proteinase kallikrein	+ (1)
	Mannan-binding lectin	- (1)
	Cleavage products of kininogen	- (1)
Serum/plasma	IL-6	+ (5) = (4)
	IL-6 receptor	- (1)
	IL-1β	+ (1) = (5)
	TNF-α	+ (3) = (4) - (1)
	α-ACT	+ (5) = (2)
	Haptoglobin	= (2)
	Haptoglobin 2-1	+ (1) = (4)
	Proteinase kallikrein	+ (1)
	Histamine	+ (1)
	Anti-glial fibrillary acidic protein	+ (1)
	Anti-nerve growth factor	+ (1)
	Anti-histone	+ (1)
	Anti-double stranded DNA	+ (1)
	Mannan-binding lectin	= (1)

+: Increased levels observed in AD patients compared to controls
=: No difference observed in levels of AD patients compared to controls
-: Decreased levels observed in AD patients compared to controls
#: Number of studies conducted

5.6 Novel Markers

Other proteins worth mentioning are L-glutamine, L-aspartate, glutamic oxaloacetate transaminase (GOT), and glutamic pyruvate transaminase (GPT). L-glutamine and L-aspartate are one of the most abundant amino acids in CSF.[36] L-glutamine occurs at higher levels in AD CSF than in normal CSF. L-glutamic acid is derived from L-glutamate and thus occurs at higher levels as well. L-aspartate occurs at significantly lower levels in AD CSF than in normal CSF. GOT and GPT are two transaminases that removes the amino group from the amino acid leaving behind an α-keto acid and converting it into an amino acid. In AD, frontal, parietal, and occipital cortices, GOT is present at "higher levels (about 1.5 times higher) than in normal brain cortices."[36] No significant difference for GPT activity between AD and normal. Because CSF receives amino acids from brain tissues and because GOT catalyzes the conversion of L-aspartate to L-glutamine, the high concentration of L-glutamine and the low concentration of L-aspartate found in AD CSF might be a consequence of the higher activity of GOT that occurs in the AD brain.[36]

Another research group has discovered a different path of detecting AD. They identified Aβ40 and Aβ42 in lenses from people with and without Alzheimer's at concentrations that are the same in the brain and Aβ40 in the primary aqueous humor at concentrations that are the same in CSF. Yet, Aβ accumulate in lenses specifically in the cataracts of AD subjects and not in controls. The mechanism by which AB accumulates in this compartment is unclear.[35]

They find that Aβ binds to αβ-crystallin, an abundant cytosolic lens protein, and promotes lens protein aggregation, extracerebral amyloid formation, and supranuclear

cataracts, which are located just above the center of the nucleus of the lens. This was observed from doing a double immunogold electron microscopic examination of lenses from people with AD showing Aβ and αβ-crystallin immunoreactivity within single cytosolic aggregates. They examination also shows high-affinity binding and coaggregation of Aβ and αβ-crystallin.[35] This could be measured non-invasively and tracked optically. Two technologies that apply this noninvasive quantitative measurement are quasi-elastic light scattering (QLS) and fluorescent ligand scanning (FLS). The company Neuroptix is commercializing these technologies and combining them into a screening system that can be used in a doctor's office.

QLS emits an IR laser beam in certain sections of the eye and collects the light scattered at various angles by using a device called a photon-counting detector and correlator assembly. It yields an estimate of the molecular size of the scattering elements by measuring the intensity of the scattered light as a function of time. In AD, protein aggregates have a high molecular weight that is large enough to scatter light. Therefore, by monitoring the light scattering, you can monitor the process of protein aggregation, its size and shape.

FLS is a complement method that validates if these suspect proteins are indeed Aβ proteins. Topical applications of Aβ-specific molecules are applied to the patient's eye. These molecules then bind to the amyloid aggregates and emit light at a certain wavelength. Namely, the molecules are methoxy-X04 and methoxy-X34 due to their strong binding to amyloid and their high fluorescence. These emissions are measured quantitatively in specific locations to confirm the presence of amyloid. If the binding increases over time, then there is more fluorescence, proving to be a positive diagnosis of

AD. Clinicians can then monitor the progression of the disease by measuring the levels of fluorescence. QLS is only dimly visible to the human eye, which means it is well-tolerated and safe to patients. Both technologies are currently being combined to be used in clinical settings.

Chapter 6: Neuroimaging Biomarkers - Structural and Functional Imaging

As mentioned earlier, a biomarker can be any detectable biological feature that provides insight about its source. This is not exclusive only to molecules that can provide information on concentration levels in AD patients but also neuroimaging of these molecular and volumetric changes in the brain. Therefore, a neuroimaging biomarker detects a biological feature in the brain through the use of imaging modalities. Neuroimaging AD biomarkers are divided into 2 categories. One is structural imaging – which includes computed tomography (CT) and magnetic resonance imaging (MRI) – and the other is functional imaging – which includes single photon emission tomography (SPECT) and positron-emission tomography (PET). These neuroimaging techniques are used nowadays to detect different types of brain changes associated with AD, and thus have potential as markers of disease progression, monitors of therapeutic effects, and predictors of future dementia prior to symptoms. Each imaging marker offers positive and negative predictive values with the appropriate sensitivity and specificity, suggesting each one as a promising AD biomarker.

6.1 Computed Tomography

Computed tomography (CT) is a medical imaging method that uses tomography to image sections created by computer processing. Because atrophy (partial or complete wasting away) in the medial temporal lobe is an early sign of AD, CT can volumetrically measure this medial temporal lobe atrophy (MTA). One study on simple CT linear measurements of the brain documented atrophy factors, specifically ratios of particular areas of the brain, that contribute most significantly to AD diagnoses.[37] In combination with other clinical factors such as apolipoprotein ε4 genotype, AD classification was

achieved with 90.2% accuracy. As a result of the inexpensiveness, availability, and simplicity of linear measurements, CT linear measurements can be valuable in the assessment of AD patients.[37]

6.2 Magnetic Resonance Imaging

Although CT has observed atrophy of medial temporal regions, magnetic resonance imaging (MRI) is superior to CT in AD studies due to its greater accuracy, manipulability, and precision.[38] MRI measures *in vivo* brain morphometry, or brain shape and size, by capturing gray and white matter atrophy as well as cerebrospinal fluid spaces. Gray matter is a component of the central nervous system (CNS) that consists of neurons, dendrites, unmyelinated axons, and glial cells. White matter is also part of the CNS that consists mostly of glial cells and myelinated axons. Measurements of MRI atrophy have accuracies between 70% and 90% in AD depending on the type of MRI method used.

6.2.1 Methods

There are several commonly used methods to extract information from MRI images. These are volumetry, quantitative voxel-based techniques, boundary shift integral (BSI), T1ρ, and other less common methods discussed in the following section. Visual assessment is a method worth mentioning because it is fast and efficient in analyzing MRI scans. However, the drawback to it is that it does not capture the fine incremental grades of atrophy.[39] On the other hand, volumetry is a more powerful strategy and it is the most common cross-sectional quantitative measurement used in AD when "changes in different individuals are measured cross-sectionally."[39]

6.2.1a Volumetry

MRI traces and quantifies the volume of medial temporal lobe structures such as the hippocampus or entorhinal cortex. Hippocampal volumetry and entorhinal cortex volumetry are beneficial because both "measure a known anatomic structure that is closely related to the pathological manifestation of the disease and is also functionally related to one of the paramount early clinical symptoms, memory impairment."[39] Hippocampal volumetry is the leading method in determining the hippocampal volume. Yet, it is time-consuming and involves a great deal of manual work. The large amount of manual work in using hippocampal volumetry is compensated through automation protocols, which uses control systems and information technology to reduce human work in the measurements of volumetry. However, these automation protocols still need to be comprehensively automated. However, it is the best-established structural biomarker for AD to date. A recent analysis proves that hippocampal volume can detect approximately 73% of the conversion from MCI to AD.

Volumetry of the entorhinal cortex, which lies adjacent to the hippocampus, at the MCI stage can improve prognostic efficacy by a few percentages as compared to hippocampal volumetry. However, it is more laborious than hippocampal volumetry as no automated procedures are available yet and no sufficient data were obtained to assess whether it adds on another benefit such as distinguishing AD from other forms of dementia.

In addition, several experiments using both cross-sectional methods (visual assessment and hippocampal volumetry) demonstrate that atrophy observed using MRI can predict the likely progression to AD with good accuracy. Though it is not known if

the accuracy of using both hippocampal volumetry and visual assessment is acceptable for use as a potential biomarker, it provides an example to find other possible combinations of MRI methods that can predict the conversion to AD with higher accuracy.[39] As a result of detecting pathological changes that occur before the onset of clinical symptoms, these imaging MRI methods can serve as biomarkers that aid in the prediction of conversion from MCI and normal to AD.[39]

6.2.1b Quantitative Voxel-Based Techniques

Quantitative voxel-based methods are other strategies that assess atrophy over the entire 3D MRI scan. These are advantageous over visual assessment and volumetric methods because using all of the spatial information in a 3D MRI provides a more accurate disease measurement compared to using only using a single region of interest.[38] Quantitative voxel-based methods include voxel-based morphometry (VBM), deformation-based morphometry (DBM), boundary shift integral (BSI), and tensor-based morphometry (TBM). The term *voxel* is a volumetric pixel representing a value in 3D space. *Morphometry* is the volume of an object measured by drawing regions of interests on the image of the object and calculating the volume of the object from those regions of interests.

VBM and DBM utilize the concept of morphometry which is measured in voxels to visualize the pattern of neurodegeneration. Methods for VBM additionally provide statistical analyses to test for group-wise comparisons between cross-sectional MRI scans of AD and those of normal controls.[38] VBM demonstrates reduction in the cortical gray matter in the region of mediotemporal, laterotemporal, and parietal associative areas in AD patients. However, it is difficult to determine this reduction in each AD individual

because it is always based on group statistics. On the other hand, DBM transforms brain volume to a standard template brain to eliminate the large difference in the brain anatomy of AD patients. These differences in reduction, which form deformation fields, provide statistical information to distinguish between AD patients and healthy controls by 80%. Thus, DBM might be used for individual risk prediction and VBM might be used for group-wise differences between AD and normal subjects.

6.2.1c Boundary Shift Integral

In addition to assessing atrophy in regions of or the entire brain at one point in time, BSI tracks structural changes in the brain over time in order to monitor the progression of the disease. BSI quantifies the "global percentage change in brain volume between two MRI scans and determines the total volume through which the surface of the brain has moved between scans acquired at two time points."[39] This is especially helpful in tracking the disease in MCI patients and normal controls because atrophy rates can predict the conversion to AD in both groups. The measurements commonly used for evaluating or tracking disease progression are increased in ventricular volume and decrease in brain volume over time.[39] These measurements are more sensitive in capturing changes over time compared to cross-sectional measurements because all scans of the same subject are registered together in order to reduce inter-scan variability.[39]

6.2.1c T1ρ

T-1-rho (T1ρ) is an alternate "contrast [MRI] mechanism that visualizes the early pathological changes using the spin lattice relaxation time constant in the rotating frame."[40] MRI is so dynamic and flexible that variable image contrast can be achieved by using different pulse sequences and by changing the imaging parameters. Because senile

plaques and neurofibrillary tangles are seen in early AD, these are expected to alter bulk water T1ρ relaxation times. Results from this experiment show that T1ρ MRI increased by 6% in AD patients compared to controls. Follow-up reports and longer evaluation periods need to be conducted in order to examine whether normal and MCI patients with high values will progress to AD or not. However, T1ρ experiments have the potential to provide information on low frequency motions in biological systems. For this reason, it is possible for T1ρ MRI to probe protein content in an indirect manner in tissues such as cartilage, brain, and blood.[40] Thus, measuring and comparing T1ρ values with changes in brain volume demonstrate T1ρ's potential value as an important AD biomarker.

6.2.1d Other Methods

While BSI monitors only spatial shift in the brain surfaces, TBM further provides a 3D profile of voxel-level brain degeneration. TBM uses 3D voxel-based methods to observe how the disease progresses in the brain as a result of the underlying pathological changes. Another method worth mentioning is analysis of cortical thickness. For this method, the cortical thickness of the neocortical and entorhinal cortex is 90% distinguishable between AD and normal subjects. Further investigations need to be conducted because: 1)this method has not yet been evaluated in independent groups and 2)the accuracy of predicting conversion to AD in MCI subjects has yet to be studied. Another method would be imaging structural changes of the cholinergic nuclei in the basal forebrain that occur in early AD. MRI-based method showed a signal reduction in the region of lateral and medial nuclei of basal nucleus.

6.2.2 Other Applications

In addition to using MRI methods to differentiate AD from normal patients, other applications of using MRI methods include measuring the efficacy of therapeutics, screening in clinical trials, differentiating from other dementia subtypes, and extrapolating mechanisms into the disease process of AD. MRI measurements can allow clinical research to be conducted in smaller sample sizes than is usually possible using traditional clinical instruments such CT measurements or neuropsychological exams. Currently, AD biomarkers have not yet been "validated as surrogate endpoints for regulatory purposes and therefore cannot be used as the primary indicators of efficacy."[39] However, in a few clinical trials these have been found to be potentially useful in capturing the pharmacodynamic effects.[39] In addition to evaluating therapeutic efficacy, atrophy on MRI can be used to selectively choose at-risk MCI subjects for clinical trials.[39] MRI is also routinely used at two stages in clinical trials and used for safety screening during the investigation.[39]

Because the pathology of AD does not always match the clinical expression of the disease and has considerable clinical heterogeneity, MRI markers can aid in the differentiating AD diagnosis from other dementia types. "The absence of significant medial temporal lobe atrophy in dementia with Lewy bodies [abnormal aggregates of protein that develop inside nerve cells] and vascular dementia, significant frontal lobe atrophy in behavioral variant frontotemporal dementia, or pronounced asymmetrical temporal lobe atrophy in semantic dementia can be used to separate these non-AD dementias from AD."[39] The differential diagnosis of dementias using MRI will be particularly helpful when therapeutics become readily available.[39] In addition, using MRI

as an independent biomarker of neurodegeneration helps in understanding relationships between cognition and neurodegeneration in AD.[39] This has led to insights into disease mechanisms in AD. The conclusion that neurodegeneration is more associated with cognitive decline was derived from several MRI analyses.[39] Thus, structural MRI is a powerful biomarker in the stage and intensity of the neurodegenerative aspect of AD pathology.

6.3 Functional Magnetic Resonance Imaging

Functional magnetic resonance imaging (fMRI) allows measurement of brain activity during cognitive tasks at high resolution without any radiation exposure.[35] It investigates changes in the functional connectivity between regions of an activated network. By examining changes across the network supporting cognitive function, fMRI shows promise as a biomarker of AD. Several fMRI investigations observe altered activation in AD as well as in MCI.[38] Several research groups have also tested and quantified the diagnostic value of various fMRI paradigms (activation/deactivation/resting state) in AD.[42] Research focusing on resting state fMRI report diagnostic values with sensitivity and specificity well above 70% (Table 7).[42] Only values from AD patients and healthy controls (HC) are included in the table because they were reported across all studies.[42]

Table 7: Overview of Studies that assessed the Diagnostic Value of Resting State fMRI (rs-fMRI) Candidate Biomarkers[42]

Study	fMRI candidate biomarker	Patients and subjects	Diagnostic value
Li et al. (2002)	rs-fMRI (COSLOF-Index)	AD (*N* = 14) vs. HC (*N* = 13)	Sensitivity: 80% Specificity: 90%
Greicius et al. (2004)	rs-fMRI- ICA components of the DMN	AD (*N* = 13) vs. HC (*N* = 13)	Sensitivity: 85% Specificity: 77%
Koch et al. (2010b)	rs-fMRI- Combined functional connectivity (ROI based) and coactivation magnitude (ICA-based)	AD (*N* = 15) vs. HC (*N* = 21)	Sensitivity: 100% Specificity: 95%
Chen et al. (2011)	rs-fMRI- ROI-based pairwise product moment coefficients	AD (*N* = 20) vs. Non-AD group (=pooled aMCI and HC (*N* = 35))	Sensitivity: 85% Specificity: 80%
Supekar et al. (2008)	rs-fMRI- Small world network properties (clustering coefficient)	AD (*N* = 21) vs. HC (*N* = 18)	Sensitivity: 72% Specificity: 78%

In differentiating AD from other dementia types, one report finds an almost inverted picture in frontotemporal dementia (FTD) compared with AD patients when assessing intrinsic brain activity during resting state using fMRI.[43] It was found that in the FTD group, the connectivity in certain brain regions increased, and in other areas it decreased. The opposite picture was observed in AD patients, with decreased connectivity in areas that had increased connectivity in the FTD group, and increased connectivity in areas that decreased connectivity in the FTD group. These results are highly promising and should encourage further fMRI-based comparisons of AD with other neurodegenerative diseases.

Overall, functional activation and connectivity in the brain constitute a new group of potential dynamic candidate biomarkers and surrogate biomarkers in AD. Nonetheless, fMRI needs further stepwise validation. Incorporation of CSF and plasma markers as well as integration of physiological signals (e.g. respiration) should be routinely implemented in fMRI analyses in order to add value to the diagnostic case.[42] In general, confirmation of its potential value as a biomarker can be attained through longitudinal studies (studies that can be repeated over long periods of time with the same variables) and long-term population based observations.

6.4 Positron Emission Tomography and Single Photon Emission Computed Tomography

Positron emission tomography (PET) is used to study the metabolic activity in cortical regions of the brain. It is one of the leading methods for *in vivo* diagnosis of early AD. The disadvantages of PET are that it is not widely available and it is very costly. It is less validated for differential diagnosis, but has an established automated analysis. In

PET, there are 2 markers used to detect the cortical metabolism. ^{18}F-2-deoxy-2-fluoro-D-glucose (FDG) is used as a marker to examine regional cerebral metabolism.[38] Observed changes using FDG-PET show reduced pattern of cortical uptake in several regions of the brain such as temporoparietal, entorhinal, posterior cingulate, hippocampal complex, medial thalamic regions, and mammillary bodies. Decreased cortical uptake "reflects metabolic deficits due to synaptic dysfunction and probably tau-mediated neuronal injury."[39] $H_2{}^{15}O$ is another marker used in PET studies to measure regional cerebral blood perfusion, and in turn cerebral activation. Analyses have demonstrated that AD and MCI brains have patterns of activation that differ significantly from normal controls when performing the same task.[38]

Single photon emission computed tomography (SPECT) is similar to PET, also measuring cortical metabolism. Most SPECT studies show a decreased regional cerebral perfusion in AD compared to controls.[38] Furthermore, one study that examined the accuracy of SPECT in differentiating AD from FTD found that the diagnostic differentiation of FTD from AD using SPECT alone was less accurate than clinical diagnosis. It was found that the sensitivity is only 65% and specificity is only 72%.[44] Record studies show that a combination of using SPECT and clinical diagnosis is more accurate than either diagnostic tool alone, with an increase of sensitivity to 84% and an increase in specificity to 84%.[44]

Both PET and SPECT are imaging methods that are also used for *in vivo* evaluation of brain function in health and in disease.[45] By "binding radioactive molecules to water, neurotransmitters, or products of intermediary metabolism and imaging the distribution of radioactivity within the brain, PET and SPECT images can provide

information on neural or synaptic function, the status of neurotransmitter systems, or specific metabolic processes within the brain under controlled conditions."[45] Therefore, the use of several markers that bind to specific AD pathology enables PET and SPECT to visualize these characteristics.

6.5 Molecular Markers Used in Neuroimaging Methods

A promising approach is imaging receptor-binding of specific transmitters. Recent research focuses on developing compounds for the *in vivo* imaging of brain amyloid, neurofibrillary tangles, and activated microglia. Because AD pathology precedes the onset of AD symptoms by many years, "molecular" imaging agents would allow for early diagnosis and for the monitoring of disease progression and treatment efficacy.[38] Five amyloid PET ligands and two microglial PET ligands have been tested in AD patients and have yielded promising results.

Amyloid imaging agents are [^{18}F]FDDNP, Pittsburgh compound B (PIB), ^{18}F-BAY94-9172, ^{11}C-SB-13, and ^{11}C-BF-227. The microglial imaging agents are [^{11}C](R)PK11195, [^{123}I]iodo-PK11195, and [^{18}F]FE-DAA1106. FDDNP-PET experiments demonstrate increased retention of [^{18}F]FDDNP in the brains of AD patients. FDDNP has been proven to bind to neurofibrillary tangles *in vivo* and prion plaques *ex* vivo as well.[38] PIB-PET, the most successful of the imaging amyloid agents, observes increased retention of PIB in the brains of AD patients as well as, specifically in the frontal, parietal, temporal, and occipital cortices and striatum. Additionally, PIB studies report Aβ plaques in a subset of cognitively normal controls and detect cerebral amyloid angiopathy, which is a form of angiopathy in which amyloid deposits form in the walls of blood vessels of the CNS. The other three PET amyloid imaging agents also demonstrate

an increased retention in AD patients. Similarly, PET markers of microglial activation also demonstrate an increased retention in AD patients. Although microglial activation is not specific to AD, activated microglia is closely associated with neurotic plaques, providing a marker for inflammatory processes. Therefore, due to the fact that activated microglia are associated in inflammatory mechanisms in neurodegenerative diseases, the unique pattern of ligand binding within AD brains along with the use of other more specific markers of AD may prove to be useful for the diagnosis of AD.[38]

Chapter 7: Combinations and Comparisons of AD Biomarkers

The major AD biomarkers that are typically considered for clinical trials are CSF Aβ42, CSF t-tau, FDG-PET, PIB-PET, and MRI. A few comparisons among major AD biomarkers by summarizing the results from several studies provide insight into the usefulness of combining specific markers to accurately diagnose AD.

7.1 Combinations of MRI Methods

One study tested if there was a correlation between "different methods of measuring rates of brain atrophy from serial MRI with corresponding clinical change in normal elderly subjects, with mild cognitive impairment (MCI), and probable AD."[41] The results of this study indicate that normal subjects who converted to MCI or AD had higher atrophy rates than those who remained stable. Specifically, MCI participants who converted to AD had higher rates than those who remained stable, and fast AD progressors had higher rates than their slow counterparts. It was found that "atrophy on MRI was detected more consistently than decline on cognitive tests/rating scales" because the tests were frequently unreliable.[41] Some MCI and AD subjects saw improvement in cognitive performance. Therefore, it is probable that using the rate of change from MRI analyses coupled with standard clinical/psychometric measures may be used as a marker of disease progression in AD in order to produce more meaningful measures than using either measure alone.

7.2 MRI vs. CSF

Several CSF and MRI reports compare the diagnostic and prognostic accuracy of both and attempt to characterize the associations between the two biomarkers in the same set of subjects (Table 8 – please refer to Appendix M on page 165). The earlier reports

concentrate mainly on the associations between CSF and MRI biomarkers, whereas the more recent ones investigate the association between these biomarkers and cognition. For example, one study suggests CSF Aβ signifies a specific molecular pathway whereas CSF tau, p-tau, and MRI may reflect the disease stage or intensity of AD.[39] As mentioned before, BSI tracks structural changes in the brain over time and is therefore able to monitor the progression of the disease However, MRI appears to be a more stable indicator of neuronal loss in comparison with the CSF measures because the brain volume quantification with MRI is not similar to the daily turnover of a soluble protein measured using CSF. Nevertheless, the majority of the analyses conclude that although MRI and CSF provide independent diagnostic information, the combination provides better discrimination of AD than either one does alone. Research also demonstrates that both biomarkers are good predictors of MCI progression to AD. However, the associations between both of the biomarkers are inconsistent across studies. This could be due to measuring the biomarkers at different stages of the disease, thereby providing different results. Another source of inconsistency comes from the large variability in the methodologies used such as the assays performed and different MRI methods.

7.3 MRI vs. FDG-PET

MRI atrophy is observed in the medial temporal lobes, whereas decreases of FDG-PET uptake are observed in the posterior cingulate and parietal lobes. Studies investigate FDG-PET and MRI in the same group of subjects and compare both markers on the basis of diagnostic and prognostic accuracy (Table 9 – please refer to Appendix N on page 166). FDG-PET is found have better discrimination accuracy than MRI. Still, a couple of recent ADNI investigations found that both FDG-PET and MRI have similar

performance and have largely overlapping value for discrimination.[39] This overlapping information between FDG-PET and MRI remains to be investigated in large study populations.

7.4 MRI vs. PIB-PET

Studies investigate both PIB-PET and MRI in the same group of subjects and the effect of Aβ plaques as measured by both on cognition (Table 10). The majority of reports have found a correlation between baseline MRI and PIB-PET measures. PIB-PET and MRI analyses have found that "longitudinal changes are much more pronounced on MRI and that longitudinal change in PIB-PET is minimal."[39] These analyses have shed light on the understanding that Aβ accumulation increases, as detected using PIB-PET, in the brain and is therefore an upstream process in AD, whereas, neurofibrillary tangles and neurodegeneration are considered to be downstream pathological processes that progressively worsen in the presence of Aβ plaques and which then directly lead to cognitive decline.[39]

Table 10: Summary of Combined MRI and PIB Studies in AD[39]

Study	Subjects	Associations
Archer *et al*., 2006	9 AD	Positive correlation between rates of whole-brain atrophy and regional PIB uptake
Jack *et al*., 2008	20 CN, 17 MCI, 8 AD	[a]Proportional odds to separate all groups: PIB: 0.75; MRI: 0.84; MRI + PIB: 0.86. Global PIB and MRI were correlated with each other as well as with clinical measures.
Jack *et al*., 2009	21 CN, 32 MCI, 8 AD	Longitudinal annual change was observed only in MRI and not in PIB. Change in MRI was associated with change in cognitive measures.
Mormino *et al*., 2009	37 CN, 39 PIB + MCI	PIB and MRI were correlated with each other as well as with episodic memory.
Scheinin *et al*., 2009	13 CN, 14 AD	During 2 years, only longitudinal MRI change was observed but not in PIB.
Strorandt *et al*., 2009	135 CN	PIB was associated with cross-sectional brain atrophy and longitudinal cognitive decline.
Bourgeat *et al*., 2010	92 CN, 32 MCI, 35 AD	In CN, PIB retention in the inferior temporal region and hippocampal volume were strongly correlated.
Chetalat *et al*., 2010	94 CN (49 subjective cognitive impairment), 34 MCI, 35 AD	Global atrophy and regional atrophy were strongly related to PIB load in CN subjects with subjective cognitive impairment but not MCI and AD.
Driscoll *et al*., 2010	57 CN	In CN, current PIB load was not related to longitudinal MRI changes in the preceding years.

7.5 FDG-PET vs. SPECT vs. MRI

One report evaluated and compared FDG-PET, SPECT, and MRI to predict conversion to AD in patients with MCI (Table 11).[46] Results showed that FDG-PET performed statistically better than the other two methods.

Table 11: Weighted Summary of Sensitivity and Specificity for Each Modality[46]

Modality	Sensitivity	Specificity
FDG-PET	88.8%	84.9%
SPECT	83.8%	70.4%
MRI	72.8%	81.0%

Chapter 8: Current and Potential Treatments for Alzheimer's Disease

The battle against Alzheimer's is one that has been fought for a long time, and has so much information and documentation that it is extremely difficult to process and condense it all. However, in the presented chapters, a good overview on where the field of research is in regards to current and possible treatments for Alzheimer's is provided. Although it is not common knowledge, there are indeed drugs currently on the market to treat AD patients. These therapies are sold commercially and are used in clinical treatments for those suffering from AD. However, these treatments are not perfect; they do not slow neurodegeneration, nor can they eradicate the disease fully and restore cognitive function permanently. Thus, the current treatments for AD are simply temporary reliefs from the disease.

The current "gold standard" for treatment in AD is a group of drugs that treats the symptoms of AD and neurodegeneration, but not the disease-causing aspects of the condition. There is ongoing research into drugs and other potential treatments in which people are working to develop a disease-preventing or disease-modifying treatment for AD. The ideal goal would be to develop these types of therapies, but unfortunately these potential treatments are incredibly difficult to go through later phases of clinical testing. Although many treatments show great promise in initial testing periods on mouse models, the human biology is very different and successes become much rarer. Many drugs simply do not reach a point where they are reliable enough for market release. Unfortunately, this is not a very big surprise considering the complicated nature of AD. Its neurodegenerative effects, and the fact that our understanding of AD is still quite

limited as to what the "true cause" of the disease may be, has slowed the progress of therapeutic developments.

The following section of the paper will be devoted to research on the potential treatments of AD, as well as a discussion on the current standards of treatment for AD patients and where the field of therapy research is currently moving. In order to understand where the field of research will go in the future, it must be understood where the world is currently with respect to understanding and treating the disease.

8.1 Acetylcholinesterase Inhibitors

There are a number of treatments currently on the market for AD. The "gold standard" of treatment is a group of drugs known as acetylcholinesterase (AChE) inhibitors. As mentioned above, these drugs are not disease-modifying, but they do improve cognitive function and temporarily guard against the neurodegenerative symptoms of AD. However, to presume that these treatments are not effective for those suffering from AD would be incorrect. These treatments are still critically important for those who suffer from AD. Although disease-modifying strategies will be discussed in-depth later on, it is worth mentioning them now to highlight the differences between their approach and the current "gold standard" for treatment, which are AChE inhibitors.

Many disease-modifying strategies would potentially treat typical pathological hallmarks of AD. They typically focus on Aβ peptides and the pathologies associated with them, as were discussed earlier. Some of these strategies focus on amyloid plaques and neurofibrillary tangles. The negative effects of these are well-documented, but it should be noted that "cognitive impairment and behavioral abnormalities are not directly caused by [these] pathological hallmarks."[47] Therefore treating amyloid plaques and

neurofibrillary tangles, which would modify the disease, would still not treat the loss of synaptic connections and damage caused by Aβ. However, AChE inhibitors are still extremely therapeutic for AD patients, though disease-modifying treatments would still be incredibly worthwhile to research.

AChE inhibitors are useful treatments for AD patients because of how they work with respect to Alzheimer's therapy. The cholinergic system is a system of nerve cells that uses the neurotransmitter acetylcholine to transmit signals. This system is heavily involved in the regulation of memory and learning in the brain, and is heavily degenerated in those that suffer from AD. Studies showed that "Aβ peptides are able to reduce choline uptake, inhibit acetylcholine releases", which alters the efficiency of the cholinergic system and results in cognitive harm for the AD patient.[47] Therefore, many treatments designed to counteract AD seek to enhance cholinergic function, usually through inhibitors of AChE. The function of these treatments is to inhibit the acetylcholinesterase enzyme from breaking down acetylcholine, which therefore increases the duration in which acetylcholine can act as a neurotransmitter.

Unfortunately, AChE inhibitors can be extremely harmful. There can be severe neurodegenerative side effects associated with using these types of drugs to treat any condition, let alone Alzheimer's. Some AChE inhibitors, when in high quantities, can trigger acetylcholine to continue to act for long periods of time, which causes permanent contraction of certain bodily muscles. AChE inhibitors are, in fact, used as incredibly dangerous chemical weapons in some cases, including the infamous nerve agents used in WWII. But the current FDA approved AChE inhibitor drugs used to treat Alzheimer's are all reversible inhibitors and can be regulated in the system. In other words, the method of

action for AChE inhibitors can be reversed and will not have permanent negative effects on the body of a patient who is using it for therapy.

There are currently four AChE approved drugs that have been developed and marketed to treat AD. They are known as Tacrine, Donepezil, Rivastigmine, and Galantamine, and were developed in that order (i.e. Tacrine is the "oldest" approved treatment). Again, these drugs do not prevent or reverse the progression of AD but they do provide temporary improvement in cognitive function, have been a commercial success, and are currently considered a "gold standard" for AD therapy.[48] Recent studies showed that AChE inhibitors could be "effective in protecting cortical neurons from glutamate-induced neuronal death."[49] The fact that AChE inhibitors could act on glutamate allows for transition into a much newer development, which is the fifth and final FDA approved treatment for AD. This treatment is known as the NMDA-receptor modulator Memantine.

8.2 Glutamate Receptors, NMDA, and Memantine

Glutamic acid is an incredibly important amino acid in the body, as it is the basis for an extremely prominent neurotransmitter. The carboxylate anions and salts of glutamic acid are known as glutamates. Glutamate is an extremely important neurotransmitter in the body; it is known as the "principal excitatory neurotransmitter in the brain."[50] Glutamate, like acetylcholine, is a central molecule in aiding in memory and learning. However, in many AD patients, research showed that glutamate can cause severe problems within the nervous system. "Glutamic overstimulation may result in neuronal damage, a phenomenon that has been termed excitotoxicity. Such excitotoxicity ultimately leads to neuronal calcium overload and has been implicated in

neurodegenerative disorders" including AD.[50] According to research, "Almost all neurons in the CNS carry the N-methyl-D-aspartate (NMDA) subtype of ionotropic L-glutamate receptors, which can mediate post-synaptic Ca^{2+} influx."[51] In addition, "excessive activation of NMDA receptors may enhance vulnerability of neurons in a manner consistent with AD neuropathology" due to the excitotoxicity and imbalance of neuronal and synaptic Ca^{2+} that results.[51] Thus, the NMDA receptor has been implicated in Alzheimer's pathogenesis, particularly dementia and memory degeneration, and is the target of the drug therapy Memantine.[51]

Memantine is known as an "NMDA modulator", or an "NMDA receptor antagonist." It is an uncompetitive NMDA receptor antagonist, meaning it binds within the ion channel to "block" it, and was studied and tested as a possible therapy for the neurodegenerative systems linked to dementia including AD. When it binds to the NMDA receptor, it is able to inhibit the receptor and thus slow the influx of Ca^{2+} ions, which protects against neuronal excitotoxicity. Memantine is a fairly recent treatment that has been developed and there are still ongoing tests about its potency, but it has been FDA approved and released as a therapy treatment for AD patients as it improves their cognitive function. Despite the method of action for therapy using this "NMDA modulator" approach is distinctive from the cholinomimetic mechanism addressed above, the end result is similar with improvements in cognitive function. In addition, one can see that there appears to be a link between the action of such AChE inhibitors and the functions of Memantine as stated above, indicating that the actions of the therapy treatments all perform on a linked system. This linkage between AChE inhibitors and Memantine could help us better understand the true nature of AD pathology.

Despite the effectiveness of Memantine in terms of providing therapy to AD patients, it also suffers from the same downfalls as AChE inhibitor therapies. Though it provides relief and a temporary counter to neurodegeneration and the loss of cognitive function that AD patients suffer, it is simply not viable as a permanent solution. Memantine, like the AChE inhibitors, is not a disease-modifying drug; therefore, it does not prevent or treat the underlying causes of AD, nor does it reverse any existing neurodegeneration. While these current standards of treatment are important for improving cognitive function and thus helping the quality of life for AD patients, they simply cannot be considered as a long-term solution to the problem of Alzheimer's disease. Thus, research is moving to look into other possible treatments that are actually disease-modifying with respect to AD therapy.

Chapter 9: Research and Possible Future Treatments for Alzheimer's Disease

The fact that the current "gold standard" of treatment for AD does not actually work in a disease-modifying manner has caused the current field of AD therapy research to branch out in several different ways. The research is mainly looking into possible treatments that do indeed modify the disease and attempt to inhibit AD progression. The role of Aβ in the progression of AD pathogenesis is well documented, and the focus of current inquiries has largely been on the toxic Aβ peptide. Current strategies aim to target the production and clearance of these peptides in order to prevent their aggregation in the brain. In addition, some research has focused on the treatment of neurofibrillary tangles containing p-tau protein, which is another significant marker of Alzheimer's pathology, though these explorations have not been developed and studied as much as research focusing on Aβ. Both of these features of AD have been discussed at length in previous chapters, and since they are two of the largest and well-documented aspects of Alzheimer's disease, it is extremely important to examine them in terms of developing a possible treatment and therapy.

There are other less known directions that research has been going towards recently that also deserve to be mentioned in regards to possible future therapies for AD. One such area of investigation is the use of anti-inflammatory drugs in order to help treat AD, though this is a very nebulous area and not much is known about it. Anti-inflammatory drugs are typically used in studies that are focused on modification of Aβ. Additionally, approaches to AD therapy focused on APOE are discussed. Yet ultimately there is not much research devoted to these potential treatments in comparison to the

major areas of study noted above. Ultimately, the wide majority of research is focused on modulating Aβ in some way, and that is where the bulk of the focus will be.

9.1 Aβ Modulation

Aβ, specifically Aβ42, has an immense role in the pathology of AD, and produces an incredible number of negative effects in the brain. Therefore, with the shift in Alzheimer's research aimed at possible disease-modifying approaches, it is only natural that Aβ would become the focus of a large portion of research. However, study on treatments of Aβ is further divided into a number of potential areas of investigation. One significant area of inquiry is devoted to the modulation of Aβ production, where enzymes such as β and γ secretases are targeted in an attempt to slow or halt Aβ production in order to prevent negative developments including neurotoxicity that goes along with Aβ production. In addition to preventing Aβ production, there are also other areas of study that are devoted to preventing Aβ aggregation into harmful plaques and possible clearance of Aβ from the system, though this area of research is still very new and no real effective drugs have been developed.

Both of these are challenging aspects of research to conquer due to the blood brain barrier resisting movement of drugs in the brain. Additionally, the activation of enzymes can be a difficult task. Yet, they are still attractive areas of inquiry. One area of research worth mentioning is the role of trace metals in AD pathology, and how potential treatments revolving around Aβ aggregation could relate to them. Another one focuses on immunotherapeutic approaches for treating AD through vaccination of Aβ42. Finally, there are also a few less known treatments, revolving around the tau protein and the APOE gene. These treatments are not very well known, but they are worth discussing,

since the field of research for Alzheimer's treatment is extremely broad and any leads on potential treatments are worth looking into.

9.1.1 Aβ Production Research

Research devoted to modulation of Aβ production is a wide field and there are many camps within this field investigating different aspects to altering Aβ production as means of countering AD by modulating and interfering with Aβ production. There are two major fields of study within this line of thinking, and they both focus on the enzymes related to Aβ. One area of investigation is devoted to inhibiting γ-secretase specifically, focusing on the role of anti-inflammatory drugs with relation to γ-secretase inhibition and modulation. The second distinct area of examination in this area of inquiry revolves around modulation and alteration of β-secretase, which also plays a major role in AD pathology. Though these areas of exploration are all extremely attractive with respect to AD therapy, there are unfortunately a number of problems associated with developing them as well.

9.1.1a γ-Secretase Inhibition

γ-secretase is an enzyme that was noted very early in Alzheimer's inquiries as a potential therapy target, but it is a very difficult enzyme to try to inhibit.[52] Since it plays an immense role in Notch cleavage in the brain, which is a function that is absolutely necessary for development. Thus, simply deleting the PS1 gene responsible for faulty γ-secretase cleavage in AD patients would lead to extremely hazardous complications. The link between γ-secretase and Notch cleavage did slow this field of research for a time, because scientists were not sure how to inhibit action of γ-secretase but keep Notch cleavage functioning correctly. Recent explorations have gone into an interesting

direction, where non-steroidal anti-inflammatory drugs (NSAIDs) have been studied as a potential treatment for γ-secretase.

There have been many inquiries in the last decade focused on the role of NSAIDs with respect to γ-secretase and Alzheimer's disease. This area of investigation was built off of observations that chronic users of NSAIDs had a reduced prevalence of AD.[53] This area of research attempts to treat AD by reducing the inflammatory response in the brain, which results from of neurotoxicity. In addition, Aβ42 reduction can, in theory, be mediated by direct interactions between these compounds and γ-secretase or its substrates.[52] One risky aspect of NSAID research is that although these compounds can generally avoid interfering with γ-secretase's Notch cleavage mechanism, they are generally used to inhibit cyclooxygenase (COX), which is an important biological enzyme, as it is responsible for the formation of important biological mediators. NSAIDs typically inhibit COX to relieve pain and inflammation, but therapies do not wish to overly inhibit this important enzyme. Luckily, research has shown that a subset of NSAIDs, including common ibuprofen, can lower toxic Aβ42 production independently of COX inhibition.[53] This is a very significant development, but scientists still speculate whether or not COX is upregulated in AD patients.[54] More research will have to be devoted to that possible link, but as of now, these scientists are working under the assumption that COX inhibition and Aβ42-lowering activities operate under separate pharmacological pathways.[54] Particularly, there have been NSAID derivatives that can "potently lower Aβ42 levels but lack COX inhibitory activity."[54]

Although this is an exciting field of research, and there has been a large area of study devoted to the action of NSAIDs on γ-secretase, the understanding of all the

mechanisms involved is not complete. Furthermore, γ-secretase inhibiting drugs can be developed, but without a full understanding of the inhibition mechanism, it can be difficult to move these drugs through clinical trials. Many of them do not even reach clinical trials, as they are found to result in suppression of the Notch signaling.

There are some current drugs which, although they are very potent against Aβ42 production and do not inhibit other functions of γ-secretase, are not easy to clinically test. This is due to their low brain permeability and COX inhibition, which could potentially harm elderly AD patients.[54] γ-secretase inhibition is a very exciting, yet complicated field, one that is still not fully understood.

9.1.1b β-Secretase Inhibition

A second area of research by which scientists seek to inhibit the production of Aβ42 is through the modulation of β-secretase. β-secretase modulation and inhibition can potentially be a great candidate for AD examination since, like γ-secretase, it plays a very early part in AD pathology through its role in Aβ42 production. However, its natural biological function is not entirely clear, and in research where BACE1 gene has been knocked out in mice, they appeared to suffer no noticeable negative effects.[52] This is another reason why β-secretase is a promising area of study, as it does not carry the weight of something akin to the Notch risk of γ-secretase. In fact, even transgenic mice engineered to overexpress APP did not develop any amyloid deposits when BACE1 was knocked out.[55] Although there could be underlying behavioral effects due to the knockout of the BACE1 gene in mice that were observed, this successful knockout without any severe harmful implications gives many researchers the hope that this could be a pathway to a successful AD treatment if BACE1 could be easily inhibited.

Despite all of this research, there is a major problem with attempting to inhibit BACE1 *in vivo* and it has been documented that developing such a drug has been incredibly challenging.[55] One of the reasons being that the size of a possible inhibition drug. This inhibition drug must be small enough to cross the blood brain barrier. Very few drugs, which are both small enough to pass through and yet effective enough to achieve inhibition, have been produced. In addition, the specificity needed to produce a desirable result is not fully understood. There are questions as to whether or not scientists must exclusively work with BACE1, or if they can cross-inhibit with the similar, but far less dangerous BACE2 protease. In conclusion, although this field is very promising, it is extremely complex and even less understood than the field of γ-secretase inhibition. More research must be undertaken before any real conclusions about this method versus other methods of AD therapy can be made.

9.1.2 Preventing Aβ Aggregation

As it has been documented, one of the main aspects of Alzheimer's pathology is the aggregation of Aβ, particularly Aβ42, into oligomeric plaques. While in the past, many scientific groups believed that only the large aggregates, known as the "mature plaques," could induce toxicity, current research have been looking into the early, small aggregates of Aβ as also playing a role in neurotoxicity. Studies have demonstrated that these small oligomeric assemblies can induce some synaptic dysfunction.[52] Even though this is a field of study that is very much unknown, it is still an attractive one to explore. However, there are issues with attempting to find solutions using this method.

One serious problem with attempting to develop methods to prevent Aβ comes from the fact that no consensus regarding the mechanism of oligomer assembly has

emerged.[52] Without a full understanding of how the aggregates assemble, it is a challenge to attempt to produce something that interferes with the unknown mechanism. In addition, "the exact nature of the pathogenic oligomeric species remains unclear."[52] If these mechanisms become understood, developing a drug that will interfere with Aβ peptide would be an extremely promising area of study for AD research. Currently though, there are very few drugs that have made it to clinical testing, and much discussion on the topic is limited to speculation.

9.1.2a Trace Metals and Aβ

An interesting field of study to mention is the apparent influence of trace metals may influence Aβ pathology and current attempts to interfere with toxic Aβ species The idea is that impaired metal ion homeostasis can cause synaptic dysfunction and influence AD.[56] Indeed, there is "considerable evidence that free zinc in the extracellular fluid induces amyloid deposition."[57] AD is also "characterized by elevated brain iron levels and accumulation of copper and zinc in cerebral Aβ deposits (e.g. senile plaques)."[58] In particular, copper can yield toxicity, either through direct toxicity when binding with Aβ or through an oxidative process, which may make the Aβ easy to precipitate with zinc.[57] Moreover, "Copper (and iron) can also promote the neurotoxic redox activity of Aβ and induce oxidative cross-linking of the peptide into stable oligomers."[58] Thus it can be seen that "both ionic zinc and copper are able to accelerate the aggregation of Aβ, the principle component of [Aβ] deposits" and stabilize the structure.[58] In addition, copper oxidation of Aβ can result in the "generation of hydrogen peroxide by soluble but oxidized forms of Aβ."[57] Thus, trace metals play an extremely important role in the aggregation of toxic Aβ in the brain. The role of metal imbalance in the brain has been studied intensively in

recent history, and has become a great area of interest with respect to Alzheimer's disease.

Using the "Metal Hypothesis of Alzheimer's Disease," which emphasizes the incredibly important role of metals in AD, researchers have begun developing therapies to inhibit the action of trace metals in Aβ oligomer production. It is noted that zinc chelators can inhibit Aβ plaque deposition and when combined with copper chelators, can "reverse Zn/Cu-induced aggregation of synthetic Aβ *in vitro*, [and] inhibit Aβ-mediated hydrogen peroxide formation."[57] Research and testing on some chelator drugs are underway, including a compound known as clioquinol that can cross the blood-brain barrier and bind to zinc. This has resulted in a dramatic reduction of the number of amyloid plaques in transgenic mice.[57] The potential treatments seem to be lesser known among Alzheimer's studies, but they are very worth looking into and companies such as Prana have been working to take advantage of this hypothesis. In addition, a compound known as PBT2 is in clinical trials and it has been shown to decrease Aβ levels by sequestering zinc, which otherwise could be "protease resistant Aβ:Zn aggregates."[56] The New York Times conducted an interview with Dr. Rudolph Tanzi, a leading researcher in the field of Alzheimer's therapy. Dr. Tanzi explains this particular treatment as something that "sucks metals out of the amyloid deposits."[59] He clarifies that "If you suck copper and zinc out the [sic] amyloids, they fall apart."[59] Thus, these chelators also work by attempting to prevent Aβ aggregation by sequestering trace metals, and by helping to degrade the already present amyloids by removing these trace metals from Aβ.

9.1.3 Aβ Degradation and Its Challenges

Traditional thinking is that many forms of Aβ are completely insoluble. However, recently, there has been an increase in research attempting to determine ways to degrade and clear Aβ through a number of proteases and enzymes.[52] Cleaving and degrading Aβ could be an interesting way to attack Aβ toxicity. However, like many other potential AD treatments, there are struggles in attempting to develop a reliable method of Aβ degradation. Once again, the blood brain barrier remains a significant blockade to potential therapy treatments as it is difficult for many treatment drugs to cross this barrier safely. In addition, being able to activate an enzyme is much more difficult than inhibiting an enzyme. Potential treatments using this method will have to either move enzymes to the periphery where they could be acted upon, or use a cascade path in which the potential degradation enzyme is no longer inhibited by a currently active inhibitor enzyme.[52] This is an extremely exciting field of research for AD, but one that has many barriers to progress.

9.2 Immunotherapy Possibilities

The field of immunotherapy is vast and nearly overwhelming one with respect to worldly diseases. This field has been extended towards the realm of AD therapy in the last decade. It is an area of study that has absolutely exploded within the last decade, which makes it even more difficult to know exactly where to start looking for possible treatments that focus on immunization as a primary means of treating AD pathology. The paper that is often credited as being the first paper to really discuss the possibility of vaccinating for Aβ42 is one by Schenk et al in 1999, and the industry has moved forward using this paper as a baseline.[52,60] Dr. Rudolph Tanzi describes it as a similar treatment to

a vaccination "where you actually inject the amyloid so that you have an immune response to it, and that promotes clearance of the amyloid."[58] Interestingly, the immunization worked very well, yet the mechanisms of this action are not fully understood, which has stalled this area of research for a short time. Despite that, hypotheses have been developed to explain how AD immunotherapy could work and this has grown into a very important field for AD therapy research.

The major hypothesis for AD immunotherapy, which could provide insight as to the processes involved, revolves around a "mechanism based on microglial activation and phagocytosis,"[52] in which a small proportion of antibody reaches the CNS. This antibody, distributed peripherally, would bind to amyloid deposits and trigger phagocytosis of the amyloid.[52] Although that is the hypothesis that is most well-known, there have been other hypotheses proposed for the action of immunotherapy with regards to AD treatment. In addition to the phagocytic hypothesis, one report indicates that, "antibodies can resolve *in vitro* aggregated Aβ fibrils."[52] Specifically, studies have shown that "monoclonal antibodies (mAbs) specific for N-terminal epitopes of the Aβ peptide disaggregated preformed Aβ fibrils and neutralized their neurotoxic effects."[60] While there have been promising results for this hypothesis in initial testing, it is not truly understood "how small amounts of antibody would dissolve existing insoluble fibrils in the brain."[52] Another proposed action of immunotherapy is a method of Aβ clearance performed by "Aβ mid-region monoclonal antibody 266," which have been found to reduce amyloid levels.[52] This antibody works not by binding to insoluble plaques, but rather through "passive administration."[52] It is believed that it captures soluble Aβ and "produces a net flux of Aβ from the CNS to the periphery."[52] This occurs in "concentrations sufficient to

produce detectable cerebrospinal fluid levels", and it is believed that this mechanism can "lead to decreased … amyloid load."[52] Even though it is not fully understood, this proposed clearance mechanism has been modeled and shows promising results during *in vivo* testing in some transgenic models. Studies have shown that in transgenic mouse models immunotherapy with regards to toxic Aβ has provided beneficial effects on cognitive performance and that "an immune response against Aβ reduced the amyloid plaques and associated dystrophic neuritis."[60]

Nonetheless, there are ongoing debates on which hypothesis and antibody should be researched further, and that these are primarily based around the epitope conformation of antibodies used for treatments. Despite the small molecular size of Aβ, it is possible to "raise antibodies to distinct [among] aminoterminal, mid-region, carboxy-terminal and possibly conformational epitopes." This results in a number of different antibodies being used for testing, each with a unique effect. All three proposed hypotheses discussed revolve around antibodies which each recognize different epitopes of Aβ, and none of the antibodies perform in the same way. It is hypothesized that the specific choice of epitope "affects the predominate mechanism of action and determines which Aβ isoforms are cleared."[52] One reason for the debate over which epitope and hypothesis should be tested more is because certain epitope choices can determine a number of possible liabilities and complications that could arise from treatment. Specifically, many Aβ-specific antibodies result in intracerebral hemorrhage when administrated. Yet this hemorrhaging only appears to occur in specific plaque-binding antibodies; antibodies which act in a different manner. For example, the 266 antibody do not show this effect. Researchers must thus take possible side effects into consideration when discussing possible immunotherapeutic

treatments. The risk of side effects arising when using certain antibodies serves to highlight one concern in a long list of setbacks with attempting to move forward in an immunotherapeutic manner for AD therapy.

Many immunotherapy tests that are done clinically perform fairly well in their early trials. Therapy approaches generally do well in early clinical trials, with improvement being shown in a large number of transgenic models. Unfortunately, they have almost universally run into problems during Phase II and III testing. "Although several Aβ immunotherapeutic are currently in clinical development, there are no definitive data on the efficacy of any immunotherapeutic approach, and no Phase III trial has been completed yet."[52] This indicates that, even if Phase III trials have been performed for immunotherapeutic treatments, there has been no Phase III success for any of the proposed treatments. It is likely that this is due to a number of problems with attempting to apply *in vivo* models of action in transgenic mice to the immune system of humans. There are a number of problems that arise with immunotherapy procedures, including possible autoimmune responses and the presence of the blood-brain barrier, which works to prevent the introduction of many drugs into the brain.[60] The blood-brain barrier is a layer of endothelial cells which works to prevent the leakage of blood from circulatory vessels into the brain. This layer of cells also prevents against diffusion of small and large molecules into the brain tissue and fluid. Thus, a drug which is able to bypass this barrier is extremely rare and difficult to develop. There is also a concern over whether or not immunotherapies truly do aid in Alzheimer's. However, this is primarily due to conflicting results when analyzing post-mortem trial patients, who either have continued to progress to end-stage AD or have had significantly reduced functional

decline. Research will have to continue in order to give a better estimation of whether or not immunotherapeutic truly aid in treatment for those suffering from AD. Despite the drawbacks of this area of study, it is still a hopeful one, and could provide an answer for the treatment and prevention of AD if the mechanisms involved were truly understood.

9.3 Tau Pathology Approaches

The involvement of tau in Alzheimer's pathology, particularly its role in creating neurofibrillary tangles when it is hyperphosphorylated, is well documented. Tau is a soluble microtubule-binding protein that plays a major role in stabilizing microtubules in axons.[52] These tubules are used for both cell growth and axonal transport, which serves critical function in the brain. However, problems can arise when tau becomes hyperphosphorylated, aggregates in the brain, and becomes insoluble. These aggregates greatly contribute to neurotoxicity by being unable to support axonal transport. The presence of tau tangles and the presence of tangle load have a distinct correlation with cognitive dysfunction in not only AD, but other cognitive disorders as well. Thus, it provides an interesting angle for those wishing to treat AD, with two strategies primarily being approached: inhibition of tau aggregation and blockade of tau hyperphosphorylation.

Inhibition of tau aggregation is a desirable strategy since tau aggregates are incredibly detrimental to brain cells and tissues and it is seen as a possible preventative treatment, to inhibit tau aggregation before toxicity spreads. However, there are issues with attempting to follow up with this therapy, and "anti-aggregation approaches pose a lot of challenges."[52] Specifically, the blood-brain barrier provides a large and difficult blockade for therapy molecules. In addition, attempting to "[find] molecules with drug-

like properties that specifically disrupt protein-protein interactions over large interaction surfaces" is incredibly difficult.[52] Despite this, the research continues and efforts have been initiated to attempt to find possible therapies using this strategy.

The second primary strategy used to provide AD therapy with respect to tau is a strategy focused on reducing tau hyperphosphorylation. These areas of study are more widely pursued and yet it also contain problems that scientists must struggle to overcome. There are questions on whether or not hyperphosphorylation is truly critical to tau pathology or if there is another possible cause.[52] In addition, there is no broad consensus on what key pathogenic kinase should be inhibited in order to prevent this hyperphosphorylation, or even if such a single kinase "culprit" exists.[52] Additionally, generation of a small-molecule inhibitor of such an enzyme would be incredibly challenging as there could be severe side effects with chronic inhibition of tau. Finally, the blood-brain barrier once again provides a challenge for drugs to attempt to pass through into the brain.

Although there are major issues with exploring tau-focused therapies for AD, there are some clinically advanced trials being conducted in this area of study. "At present, the clinically most advanced tau-directed therapy is methylionium chloride … which has been reported to dissolve tau filaments from AD brains *in vitro* and to prevent tau aggregation in cell-based models."[52] These findings have allowed the company TauRx Therapeutics to initiate a Phase II clinical trial for mild and moderate AD patients. Though these tests are ongoing, researchers believe that proper treatment could possibly arrest AD disease progression. This would be an immense step forward for AD research as a whole as preventing progression entirely would be a disease-modifying strategy and

would provide a basis for further therapy. Despite the fact that there are many problems with using a tau-based approach to AD therapy, this is an extremely promising field of study.

9.4 APOE-Related Therapies

Therapies focusing on the role of APOE in the brain have been researched as well. As discussed earlier in chapter 3, APOE plays a role in the development of AD in many patients. The gene that encodes APOE has been identified as a large genetic risk factor for late-onset AD, and specifically, it is the ε 4 allele that carries the highest increase in risk with respect to other alleles of the APOE gene.[52,61,62,63] In fact, examination has shown that the "risk for AD increased from 20% to 90% and mean age at onset decreased from 84 to 68 years with increasing number of APOEε4 alleles."[62] This is incredibly significant and studies such as the one conducted by EH Corder et al[62] have led researchers to conclude that the APOEε4 gene is "a major risk factor for late onset AD and, in these families, homozygosity for APOEε4 was virtually sufficient to cause AD by age 80."[62] With APOEε4 being such a large risk factor, in principle it could be a huge target for potential therapeutic treatments attempting to alter or correct the action of this gene in order to prevent or treat AD in many patients. While this approach makes sense, in theory, research on an APOE-centric treatment has moved very slowly.

Unfortunately, research relating to APOE-related treatments to help prevent or treat Alzheimer's disease has moved rather slowly, despite the fact that APOEε4 has been identified clearly as a risk factor.[52] This is due to a number of reasons, primarily because the biological effects of APOE and its isoforms are very complex, and that APOEε4 is a specific risk factor for a number of other conditions. While it is known that APOEε4

disrupts cholesterol and brain lipid metabolism, regulates synaptic functions, and "contributes to AD pathogenesis,"[65] it is not known if there is a specific pathway of molecular action relating to AD.[52] There are two lines of research related to APOEε4 research relevant to AD. Although different, the two research directions focus on APOEε4 modulation.

One area of exploration is focused on the notion that APOEε4 could have gained some toxic properties relative to APOEε3.[52] Those who believe this stance to be true study APOEε4 in an attempt to mitigate its toxic effects. An approach that is used is to inhibit a neuronal protease that generates a toxic APOEε4 fragment.[52,64] Another approach would be to use molecules known as "'structure correctors' to convert [APOEε4] to an ['APOEε3-like'] molecule."[52,65] This method of action binds a specific molecule to APOEε4 in order to "block the intramolecular domain interaction that is characteristic of this isoform,"[52] which would convert the structure to one akin to APOEε3, which is much less likely to take on a pathogenic state. There are other areas of examination that believe that the problem with APOEε4 is that it has lost a beneficial function of APOEε3 with respect to the amyloid pathway, and is proven with results suggesting that enhancing APOE could benefit those who carry alleles for APOEε3.[52]. Although this is a growing field of research, there are no reported progressions of APOE-related treatments into clinical trials.[52]

Chapter 10: Discussion of Treatments and Early Detection

It has been established that the current standards of care and treatment for those that suffer from Alzheimer's disease are not entirely sufficient. While the current treatments do relieve some symptoms of cognitive dysfunction, and are thus very important for those suffering from the disease, they are not sufficient to prevent the progression of AD permanently. Because the current standards of treatment only help with the symptoms and do not change the underlying causes of the disease, they are not considered preventative or disease-modifying. Currently, there are many possible answers to this solution, but the search continues for a disease-modifying approach to AD therapy.

It is important to note that, granted, this area of study appears to have many faults, it is still growing extremely rapidly. Over the past decade, the understanding of Alzheimer's has expanded to a far greater degree. The many possible treatment options outlined in the previous chapter are a result of this explosion of study into AD. As understanding of the mechanisms of AD grows, so too does research areas focused on treating the underlying causes of AD. To understand the biological mechanisms that go into the disease provides a base for the development of possible treatments and cures. Nevertheless, AD is an extremely complex disorder with many parts involved. However, the mechanisms of the disease are much more fully understood now than they used to be. This expansion of knowledge has thus provided an avenue for those seeking to use disease-modifying approaches in order to treat AD. Treatments such as those focused on modifying or preventing Aβ aggregation, modification of tau, alteration of APOE, and immunotherapy of Aβ, all have their roots in understanding the biology of the disease.

The field of research for Alzheimer's treatment does have a number of struggles. Particularly, the blood brain barrier serves as a formidable obstacle to many researchers attempting to provide therapy through drugs that would work in the brain. In addition, the mechanisms of actions for some potential therapies are not fully understood, and this provides a blockade to some potential treatments, which require this understanding in order to move forward. Questions about some mechanisms of AD also have slowed development of some therapies because although the understanding of AD has grown exponentially in the recent decade, there are still some mysteries. Still, this large, all-encompassing area of disease-modifying treatments is extremely promising, and will prove to be at the forefront of Alzheimer's research for a long time. Particularly, this will happen if steps are taken in order to develop means of detecting AD early in those who may be at risk.

The notion of a disease-modifying approach to AD goes hand in hand with the idea of detecting Alzheimer's early. As discussed in Chapters 8-9, the current standards of care are not sufficient even without reliable early detection techniques. For those determined to be significantly at risk for Alzheimer's or those in the early stages of AD, the current symptomatic treatments would still not be enough to prevent these patients from proceeding into the later stages of AD. Thus, developing disease-modifying techniques for Alzheimer's treatment is just as critical as developing a reliable means of detecting the disease early in patients. If a disease-modifying treatment was tested and found to be a great means of slowing or even stopping the progression of AD altogether, and combine it with early detection techniques available, then it would be possible to use these treatments in order to greatly slow or possibly prevent the disease in those who may

develop it thereby curing them of AD. This would be an outstanding breakthrough in Alzheimer's research. There are many different areas of study revolving around potential detection techniques as well as potential treatments, but as these two areas of research continue to move forward, the world moves closer to developing a reliable way to prevent and even cure Alzheimer's disease.

Chapter 11: Correlation with Other Diseases

Neurodegenerative disorders have crippled the society since the evolution of man. Alzheimer's disease, as explained in the aforementioned chapters, has been a major culprit, especially causing dementia in older patients. As with all of the major disorders, Alzheimer's disease (AD) has been related to several epidemic-like diseases around the world. Whether or not there is a perfect correlation between AD and these other disorders is uncertain; however research has developed models which may be able to explain the complicated yet severe relationship.

In the presented chapter, the relationship of AD with two main diseases is discussed: Type II Diabetes and Down's syndrome. This includes briefly examining the origins, explaining the different types of similarities and differences, and finally providing possible evidence for detection and diagnoses. There are several other disorders related to AD, which will be mentioned briefly; however the main focus will remain on the two major diseases mentioned above.

11.1 Type II Diabetes Mellitus

One of the diseases that is medically interrelated to AD is type II diabetes mellitus (T2DM). Mainly, caused by insulin resistance due to hypercortisolaemia, type II diabetes has been around for ages. High dietary saturated fat and high cholesterol levels cause T2DM. Furthermore, it can also be genetic or be induced by low levels of physical and social activities. However, it has been determined that the above factors can also induce AD as well. It has been shown that exercise and physical activity not only are associated with cholesterol levels, but are also associated with cognitive impairment and function.[66] Both of the diseases are rapidly becoming an epidemic and have created several problems

for society. Usually occurring in adults, these diseases are very well known to the common public and extensive research has been done to try to find a cure for them.

There are several perspectives that can relate T2DM to AD, one of them being from an evolutionary standpoint. Natural selection states that the genes from the most favorable species will be transferred to their offspring. Therefore, the genetic contribution of the population would also include genes from T2DM and AD patients. Since both of the diseases are known to be partly genetic, they can be reproduced due to the post-reproductive ideal, which disregards natural selection. Furthermore, technological advancements have greatly reduced the need for physical activity for humans and therefore have affected the prevalence of T2DM and AD in older patients.[66] Due to the lack of physical activity; this has greatly affected cognitive function.

Moreover, it can also be concluded that since society is based on a fragile physical state, T2DM has been more prevalent in the recent years. There also stands a direct correlation between the physical systems and the mental and neural systems of the body. Therefore, because of the repressed neural function, AD has become a major negative factor in the lives of millions of adults. The neuropathological aspects of AD have been discovered in patients with T2DM, and these aspects display a disruption in insulin production and consequently results in chronic intermittent hyperglycemia.[66] Conclusively, these two diseases have been connected through generations due to the fact that there exists an evolutionary relationship between the insulin levels in the brain and the skeletal and muscular systems. However, the evolutionary evidence simply scratches the surface of the relationship because AD and T2DM are indeed connected on a genetic and physical level as well. As a result, the parallel evolution of the two diseases can

further be explained.

Although at first glance it may not seem that there is much correlation between AD and T2DM, physical commonalities and genetic mechanisms disprove the thought. AD and T2DM have similar risk factors that allow them to be categorized. Some of the factors are outlined in Table 12 below.

Table 12: Table Outlining Several Risk Factors for AD and T2DM[17]

Factors	Alzheimer's disease	Parkinson's disease	Diabetes mellitus type II	Depression
Gender	Female	Male	Both	Female
Age	Ageing	Ageing	Ageing (nowadays also adolescent)	Midlife (also adolescent)
Hereditary risk factors	Familial (APP, presenilin-1, presenilin-2)	Familial (LRRK2, PARK2, PARK7, PINK1, SNCA)	Familial (HNF4A, GCK, INSR)	Familial (especially at younger age)
	APO E ε4	APO E ε4	ABCC8	? APO E ε4
			CAPN10	
			GCGR	
			PPARγ	
			APO E ε4	
Environmental risk factors	High dietary saturated fat and cholesterol	High dietary saturated fat and cholesterol	High dietary saturated fat and cholesterol	Low level of social activity
	Low level of social activity	Pesticide	Low level of social activity	? Smoking
	Smoking	Smoking (protective)	Smoking	? Alcohol
	Alcohol	Alcohol	Alcohol	Drug abuse
				Early-life events
Mental risk factors	Depression	Depression	Depression	Anxiety
	Anxiety	Anxiety	Anxiety	Dementia
	Chronic stress	Dementia	Dementia	Chronic stress
Metabolic risk factors	Hypertension	? Hypertension	Hypertension	Adiposity
	Diabetes mellitus	? Diabetes mellitus	Adiposity	Coronary artery disease
	Coronary artery disease	? Coronary artery disease	Insulin resistance	
	Hyperhomocysteinemia	Hyperhomocysteinemia	Coronary artery disease	
	Stroke	? High cholesterol	High cholesterol	
	High cholesterol			
Others	Inflammation	Inflammation		
	Head trauma	Head trauma		

As it can be noted, several of the risk factors are similar in both AD and T2DM. For example, both of the disorders have familial hereditary risk factors and are caused by high dietary saturated fat and high cholesterol. Additionally, lack of physical activity and negative mental factors such as depression and dementia can contribute to the two diseases. Depression is a major risk factor since T2DM patients have anxiety, which in

turn increases the body mass index. As noted, depression occurs in 20-40% of AD patients, which is a very high ratio in the patients.[17] Since depression paves a pathway to T2DM, it can also be deduced that diabetes is very prevalent in AD patients as well. The table above clearly describes the similarities very efficiently as major factors of each of the categories are covered.

T2DM has several complications, which are affected by neurodegenerative symptoms that in turn worsen the degeneration in AD. The hypothalamus controls the glucose and the insulin levels in the brain. If there is neuron damage in the brain, the levels fluctuate, causing diabetic symptoms.[17] Thus, diabetic symptoms occur as a result of damage in the brain and it becomes almost a cycle to which the patient adheres.

There are several commonalities, which have also been found in T2DM and AD patients. One of the similarities is the mechanisms of degradation and allowance of Aβ and amylin. These fibrils are degraded by insulin-degrading enzyme (IDE) and amylin is a substrate of the enzyme.[67] Therefore, the degradation of Aβ causes AD and mutations in IDE lead to T2DM. Also, the production level of amylin also increases leading to apoptotic cell death. This in turn leads to AD since the cells in the brain have been destroyed. Conclusively, since the two metabolic factors are related to both AD and T2DM, mutations can lead to one or both disease in severe cases.

Besides the enzymatic mutations, there are other factors which can be comparable to AD and T2DM. As mentioned above, hypertension and obesity are risky factors for dementia. Recent studies show that genetically the relationship between AD and abnormal insulin metabolism can be greatly affected by APOE genotype.[67] If the patient has the APOE allele, diabetes increases the risk of AD in the patient. The reason being is

that insulin resistance causes an increase in the risk of AD due to it being triggered by a low level of glucose breakdown in the brain. Consequently, it results in T2DM and causes sporadic AD.[67]

While AD and T2DM are widespread throughout the world, there have been several treatment strategies proposed which halt the occurrence or treat the disease. These strategies are common to both diseases and predominantly consist of insulin manipulation. Since human evaluation cannot be replicated in a laboratory settings, these studies are conducted on mice with AD and T2DM. This is done because the genome of the mouse is closely related to the human genome. Most recently, it was shown that intrahippocampal injection of insulin has a minor dose-dependent effect on the memory and cognitive function of rodents.[17] The dose-dependent effect proves that insulin may play a role in AD patients and may be a major breakthrough in the treatment. The drawback of using insulin, as a treatment is that insulin, when administered into blood may cause hyperglycemia. One possible way to overcome this problem is to somewhat bypass the blood-brain barrier and the circulatory system altogether. For instance, intranasal injection of insulin goes directly into the brain and the patient has a much lower risk of high levels of insulin in the blood.[17] This treatment may also be faster and more reliable in terms of safety; however further testing may be necessary in order to ensure no life-threatening complications can occur. Another way is treatment with insulin-sensitizing drugs such as thiazolidinedione (TZDs).

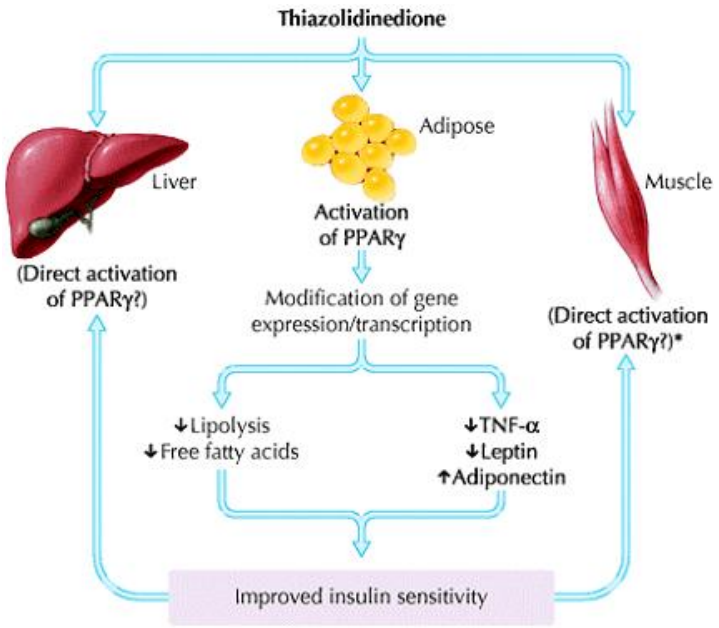


Figure 6: Pathway to show TZD Mechanism[68]

Figure 6 demonstrates how TZDs work in the human body. TZDs function as a specific "agnostics of the peroxisome proliferator-activated receptor (PPAR)."[17] PPAR are transcription factors and their diagnostics may prevent neurodegenerative disorders. In the above figure, TZD allows for direct activation of the PPAR in the liver and the muscle, which improves the insulin sensitivity. If there was a higher sensitivity in the body to insulin, the levels may be regulated more efficiently and thus preventing AD and T2DM. Since insulin is a major factor in the treatment of such disorders, biomarkers allow for regulations within the body in order to prevent the diseases from occurring in the first place.

For future treatments, attempting to improve metabolic regulation may be the best option. Additionally, identifying deregulated genes and proteins can provide a pathway to prevent AD and T2DM from occurring. In order to reach a conclusive treatment strategy, it may be beneficial to consider the different physical, mental, environmental, and metabolic risk factors. By preventing people from partaking in debilitating activities that

are harmful to their physical being, it may be possible to slow down AD and T2DM. However, since the identification and the complete prevention from the risk factors is very tedious, AD and T2DM continue to be very dangerous, epidemic-like diseases. It seems that the two diseases have continued to elude treatment and perfect detection.

11.2 Down's Syndrome

For a long time T2DM seemed irrelevant to AD in terms of medicinal diagnostics, another neurodegenerative disorder that correlates to AD is Trisomy 21, commonly known as Down's syndrome (DS).

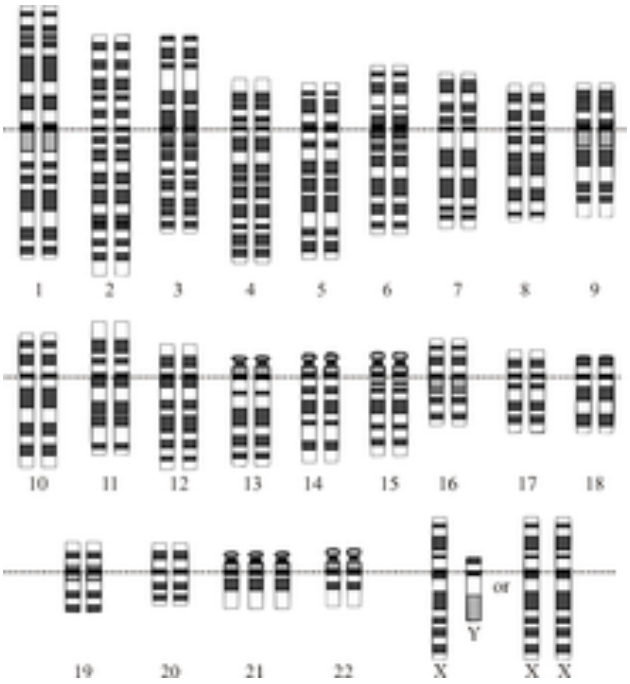


Figure 7: Trisomy 21 or Down's Syndrome[69]

Down's syndrome is the most prevalent disorder related to mental retardation in the United States. Figure 7 displays the extra chromosome at number 21, which causes dementia and mental retardation. Both of the diseases amplify with aging in the human population and are caused by familial and genetic malfunctions in the brain. For the most part, genetic diseases are caused by chromosomal abnormalities or aneuploidies. These

genetic deficiencies are unable to exactly determine the location of the gene, and AD and Down's syndrome show parallelism to each other.

Chromosome 21 is the major cause of DS; and studies have shown AD causing genes on it as well. Therefore, there are similar characteristics between each of the disorders, the main one being neurodegeneration. Studies have recently been able to determine the similarities between the two diseases. The similarities arise from genetic, pathological, and chromosomal aneuploidy tendencies of AD and DS.

In terms of genetic similarities, the main component related to the senile plaques is β-amyloid.[16] The accumulation of the protein causes the faulty programming of the brain. Similarities in brain autopsies of DS patients to AD patients imply that the two diseases are pathologically related. Statistical evidence comparing a control group and a DS group shows that DS is more prevalent among AD patients.[16] Furthermore, it can also be deduced that mothers with DS have a higher chance of passing AD to their offspring which suggests that AD and DS are genetically passed together from the mother to offspring. The risk increases with age as the severity of DS increases in older patients. The risk is similar to that of AD, which only occurs in older patients. Another clinical study has shown that fingerprints of AD and DS patients are very similar in terms of creases and loops on the fingertips. This suggests that even something small as dermatoglyphic similarity can provide information towards the relationship between AD and DS.[16]

Chromosomal aneuploidy is another aspect in which AD and DS can be found to coexist. Studies showed that DS patients who have survived beyond 40 years of age have brain pathology almost the same as AD patients.[70] The brain cells are more prone to

apoptosis, which causes neurodegeneration. One clinical study shows that if a mother with DS has a higher chance of passing AD to her offspring then mothers with DS can give birth to children that are at a higher risk of AD. This suggests that AD degeneration causes the offspring to have DS since the genes are related and due to an extra chromosome. The aneuploidy is also caused by defects in meiosis in AD patients that leads to DS. This leads to the trisomy mosaicism model that explains that the genes are randomly distributed due to mutations.

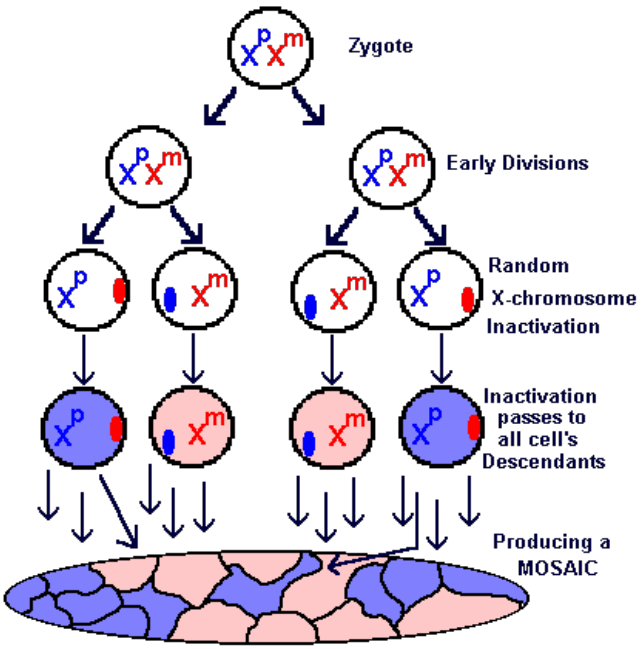


Figure 8: Trisomy 21 Mosaicism Model[71]

The above figure is a clear representation of the model where random X-chromosome inactivation is passed to all of the cells, which ultimately results in a mosaic-like shape. These defects lead to a lack of chromosome segregation and consequently to trisomy 21 and cell apoptosis in AD patients. The trisomy 21 causes DS and occurs as a result of the defect in AD patients as well. The most evident example of the defects in AD has been observed in the metaphase where the chromosome pattern is

similar to spontaneous centromere division that makes cells more susceptible to cell mosaicism.[70] The above mentioned structure is able to disrupt the proper formation of genetic code in AD patients, resulting in the patient having DS, which is in turn passed on to the offspring. AD and DS seem to be interrelated and can have a cause-effect relationship with each other.

Besides aneuploidy, another major factor which supports the similarities between AD and DS is the oxidative stress (OS) in the pathogenesis. As evidence supports, DS patients with dementia have AD present in 75% of the cases with age above 60 years.[72] This statistic shows that there may be a common pathway between the two diseases. One hypothesis that explains the phenomena is the "two-hit" hypothesis, which states that either OS or disruptions in mitosis can initiate AD-like symptoms but both are needed for the prevalence of AD.[70] Although AD can be caused by several other factors, OS and mitosis failure increases the risk of both AD and DS. This relates to DS as it causes neurodegeneration and as previously noted they have similar pathological characteristics. OS is known as the misbalance between the generation and expulsion of OS that can lead to lack of free radicals in the brain, ultimately causing AD and DS. The genetic and the OS pathway have become the major cornerstones in determining the relationship between AD and DS due to their mutations and lack of mechanisms which allow for proper function of the brain.

There are proposed solutions to ameliorate the risk factors and the mutations and these treatments have become a major factor in the control of AD and DS. In terms of early detection and diagnostics, since AD and DS have similar properties and characteristics, it may be easier to diagnose AD or DS with the aid of certain tests.[70] The

diagnostic tests can also contain chromosomal analysis to determine the severity of trisomy 21 in a patient and then use the same results in determining either of the diseases. In terms of finding a cure of AD and DS, drugs which are able to prevent disruptions or problems in mitosis could stop the defect. Furthermore, it may be possible to stop the mosaic-like chromosomal segregation by eliminating the environmental toxins. Since these toxins cause cell apoptosis, drugs could be developed to prevent the toxins from causing damage. A more implausible approach might be to remove the extra chromosome 21 from the body with the use of gene regulation.[70] Although the idea for these techniques are far from being introduced to the general public, they are still possible. Perhaps in the future, it may pave way to a cure for Alzheimer's disease, Down's syndrome, and other neurodegenerative disorders.

In summary, it may seem that several diseases are unrelated to each other; however, genetic and pathological similarities may prove otherwise. While only T2DS and DS were discussed, there are several other types of diseases related to Alzheimer's such as dementia, Parkinson's disease, and other metabolic disorders. Each of the diseases is specifically related to AD whether in terms of genetic pathways or regeneration failures. T2DM and DS are very prevalent in AD patients and because they share similar characteristics, it also allows for a possible future cure for all of the disorders. By having parallel pathways, the disorders can be detected with the use of many more techniques and one cure might be the answer to all. Finally, neurodegeneration and physical degeneration have survived due to genetic misregulation, and the above-mentioned disorders will continue to elude medicine and society until a cure is discovered.

Chapter 12: Socioeconomic Impact of Alzheimer's Disease

Society has been crippled by neurodegenerative disorders such as Alzheimer's disease for ages. Whether it is socially or economically, AD has been a major part of the lives of millions of people around the world causing damaging results in everyday life. Several of the factors not only affect the patient, but also the family and the people around the patient. There have been advancements, which allow them to become more social and improve their cognitive function. The improvement in the cognitive function also enhances the economic impact and the cost-effectiveness of early detection increases. Many economic factors also weigh into the lives of AD patients and there must be several steps that need to be considered in order to prevent the patients from living a life of solitude and disappointment.

12.1 Social Impacts

Several of the impacts that AD patients suffer through also prevent them from associating with the society. One of the major aspects of the disease is the caretakers and the family members of the patients. Since AD is a neurological disorder, it prevents the patients from being able to perform daily tasks needed for survival such as eating and getting dressed. Furthermore, they are also unable to recall everything and need a caretaker by their side at all times. Many of the patients are forced to live in community homes and face the problem of effective housing and care by volunteers or caretakers. Studies have shown that group homes are able to provide better care and quality of life for patients than special care units assembled by medical authorities. Many times the families are "unable to provide the necessary care" [73] and are compelled to place the patients under professional care. The reason for the inability of care could be economical,

emotional, or social.

Aging also plays a factor in neurodegenerative disorders since adults are more difficult to take care of as compared to taking care of children and are thus placed under special care. The quality of life of the patients is also decreased due to "insufficient staff, lack of money and financing, disabilities, lack of communication skills and accessibility issues." [73] This prevents them from living what everyone would consider the "normal" life. Furthermore, the issues are also very serious since Alzheimer's requires a great sum of money for care and detection.

Table 13: Demographic Distribution of Caregivers for AD Patients[74]

Table 1. Sample Description (N = 1,715)

Group	Value
Caregivers	
Age, years, mean ± SD	59.73 ±12.66
Gender	
Male, %	23.1
Female, %	76.9
Race/ethnicity	
African-American, %	6.0
White, %	90.7
Other, %	3.3
Patients' relationship	
Spouse/significant other, %	42.4
Parent, %	42.1
Other, %	15.4
Where patient lives	
Alone, %	7.9
With caregiver, %	74.9
Other, %	17.2
Employment status	
Full-time, %	26.1
Part-time, %	10.9
Homemaker, %	17.4
Retired, %	34.8
Unemployed, %	3.9
Disabled, %	6.9
Health insurance, yes, %	91.0
Patients	
Age, years, mean ± SD	77.86 ± 8.33
Gender	
Male, %	37.9
Female, %	62.1
Race/ethnicity	
African-American, %	6.0
White, %	90.3
Other, %	3.8

SD = standard deviation.

There are also statistical studies done which provide an idea of the demographics of the caregivers. As it is noted, most of the caregivers are women and are part of the family. Psychologically, according the studies, women are more caring and are able to provide better care therefore being easier for the patient to live with the caregiver. Additionally, the caregivers are usually 60 years old and are retired. It is reasonable to conclude that they devote their entire time to the care of the AD patients. The aforementioned study deduced that most of the caregivers are able to aid in the improvement of the quality of life of the patient due to their relationship and background in relation to the patient.

In terms of patients, most of them are in their late 70s and are female. AD also is more prevalent in people of Caucasian race than any other races around the world.

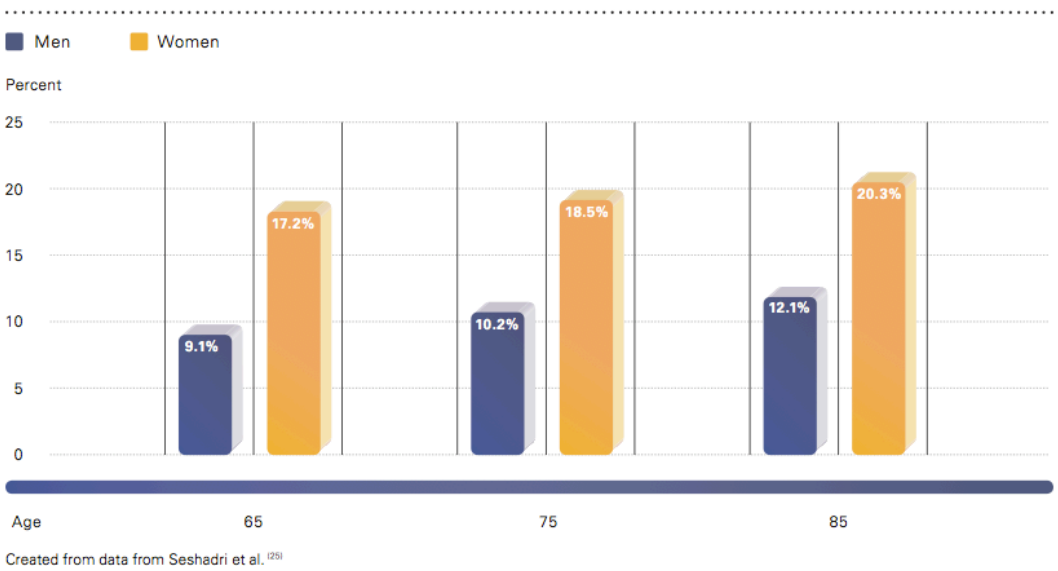


Figure 9: Risk of AD in Males vs. Females[75]

Figure 9 explains the prevalence of AD in males versus females and it proves that females are at a higher risk of getting AD than males. Therefore, female caretakers are able to relate well to patients and communicate better because they are the same gender.

Socially, the staffs of the homes are hired to devote all of their time and effort in order to provide the best quality of life for AD patients, or any other dementia patient for that matter. Moreover, the patients also need to live away from their own families and connect with the patients on a deeper level in order to prevent them from feeling as if they were living in "solitary confinement."

On a personal level, the patients are unable to communicate with anyone including the family members. This ultimately has a devastating effect on the morale of both parties. Due to their disorders and symptoms, AD patients are also unable to participate in everyday activities, which forces them to deviate from the norm of the society and become an outcast. As harsh as that may be, it is the reality and unless a cure is found, the patients will continue to live a life that cannot be replicated under normal conditions.

Any form of dementia, especially AD, is a life altering condition and early detection can aid in the eventual restoration of the lives of the patients. Even though there may not be any short-term benefits for the early detection and diagnosis, there are several positive long-term benefits.[76] As the patient and the family are informed of the early diagnosis, they are shocked and overcome with grief. However, overtime they are able to cope with the condition and accept their respective roles in the treatment and the care for the patient.

The disease progresses severely with age and there are several factors, which contribute to early detection, especially the techniques used, which were discussed in the previous chapters.

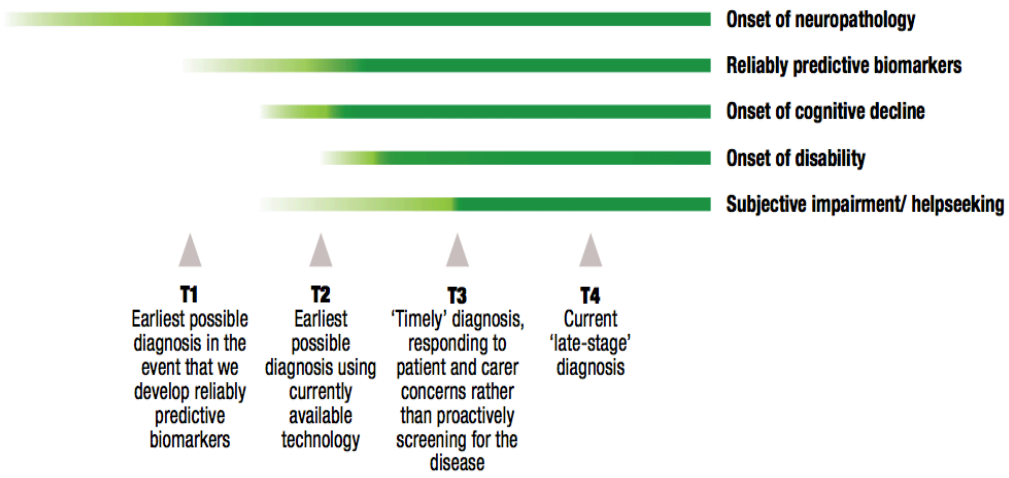


Figure 10: Timeline of the Progression of AD[76]

The above figure taken from the World Alzheimer's Report from 2011 outlines the timeline of the progress of AD from the onset to 'late-stage' diagnosis. As it is noted, the earliest possible diagnosis can only occur when a reliable biomarker is developed and it can be determined only after the primary onset of AD. Furthermore, the technology that is available presently is only able to detect AD after cognitive decline and the patient slowly starts to lose function.

Early detection holds a high importance in the lives of AD patients. Preparing for early detection can be attained through educational programs in primary care and the introduction of care services into the society.[76] Diagnosing the condition earlier can allow the patient to receive proper and better care and treatment. Additionally, the family and friends are able to cope with it much easily and allow their loved ones to live the best quality of life possible for them. Early diagnosis also benefits people with AD and their caregivers in terms of symptomatic decline and quality of life.[76] Through early detection the patient goes through slower cognitive decline and has a better quality of life and improved social abilities. As a result, the caregivers also have a better quality of life and

have reduced strain on their physical and mental state. This ultimately leads to reduced economic and social impact on healthcare and society. With the knowledge of the diagnosis, the family and the healthcare industry are able to better arm themselves with the tools necessary for a more effective and beneficial future. The patient and the family are also able to "think about safety and security issues, including living arrangements, driving, cooking, and managing medication".[76] The basic needs can be more easily taken care of and the patient is able to better manage his/her life with the help of the family.

There are other social impacts of AD as well, including the media. In the pop culture media, there have been several movies about future AD cures and treating the disorder such as *Rise of the Planet of the Apes*. The movie sought to find a cure for AD and improve cognitive function exponentially and has been affected by the idea and principles of the disease. The pop culture media alters the mindset of the society and still is able to accurately display that there is not a certain cure for Alzheimer's presently.

Overall, Alzheimer's has a major impact on the social life of the patient and the family. The disruption of daily life and withdrawal from social activities compels the patient to live a life of confinement within his/her own life. In addition, there are several economic impacts of AD, which have been detrimental to the society as well.

12.2 Economic Impacts

Besides having social impacts, AD also causes severe economic impacts. There are several costs associated with AD patients including care costs, treatment costs, and cost of research. AD has become one of the most expensive and deadliest disorders to deal with in the world. Furthermore, as AD becomes increasingly prevalent throughout the world, the price associated with it increases. Most of the cost is associated with the

treatment and the research; however, care and living expenses also cost a fortune and compel the patient to live a life of misery.

AD has caused a great economic imbalance in the society due to the high-maintenance care and lack of answers to problems relating to the disease. Most of the time, the patients' health and condition do not allow them to live at home with their families, and they are compelled to live in costly care centers, nursing homes, and assisted living homes. For example, the average cost of services at an assisted living facility is approximately $53,220 per year. The cost is even higher at a nursing home, being about $75,920 per year.[75] It can be concluded that the cost is even higher than the average person's salary within the United States, which becomes very problematic for the patient as most of the time they are unable to afford such expensive care and are forced to live in subpar conditions. Additionally, the cost becomes a great burden very quickly as the lack of cure causes a dent in the economic conditions of the society.

There are several direct and indirect costs for AD as well. Direct costs are the resources used by the patient and the indirect costs are the care and the research abilities. The following figure illustrates the division of the costs of AD in 2004:

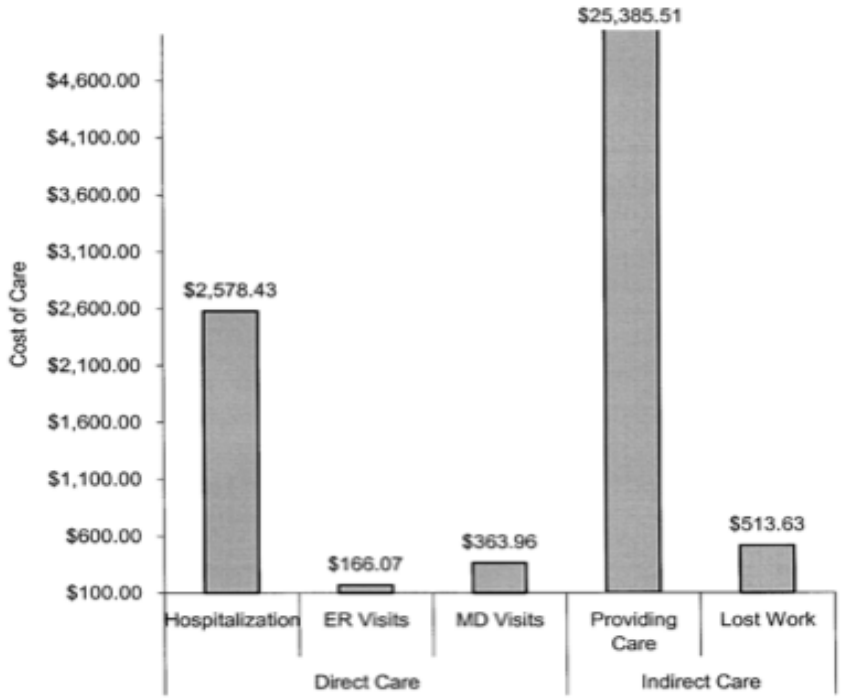


Figure 11: Cost Comparison of the Direct and Indirect Care of AD[74]

As noted, most of the cost is associated with providing care for the patients and the salaries of the caregivers. Additionally, the other majority of the cost goes towards the hospitalization of the patient. This becomes very important as the lack of resources in the hospitals increase the cost of hospital care. Since most of the patients are put into care homes afterwards, they have to incur those costs as well. The money for the care mostly comes from the family of the patient. However, Medicare and health insurance and other governmental allocations also aid them in the process. It is shown that AD uses up about $3 billion in costs every year for the US, including care and research.[75] This number is higher than any other major cause of death in the US including cancer, coronary heart disease, and diabetes. It proves that dementia has become an enormous problem for the US in terms of costs and research and action needs to be taken as soon as possible.

Even though AD has a generalized cost, there are certain factors that fluctuate the

cost of AD. One such factor is early detection in terms of the time period when the patient is diagnosed with a neurodegenerative condition such as AD.

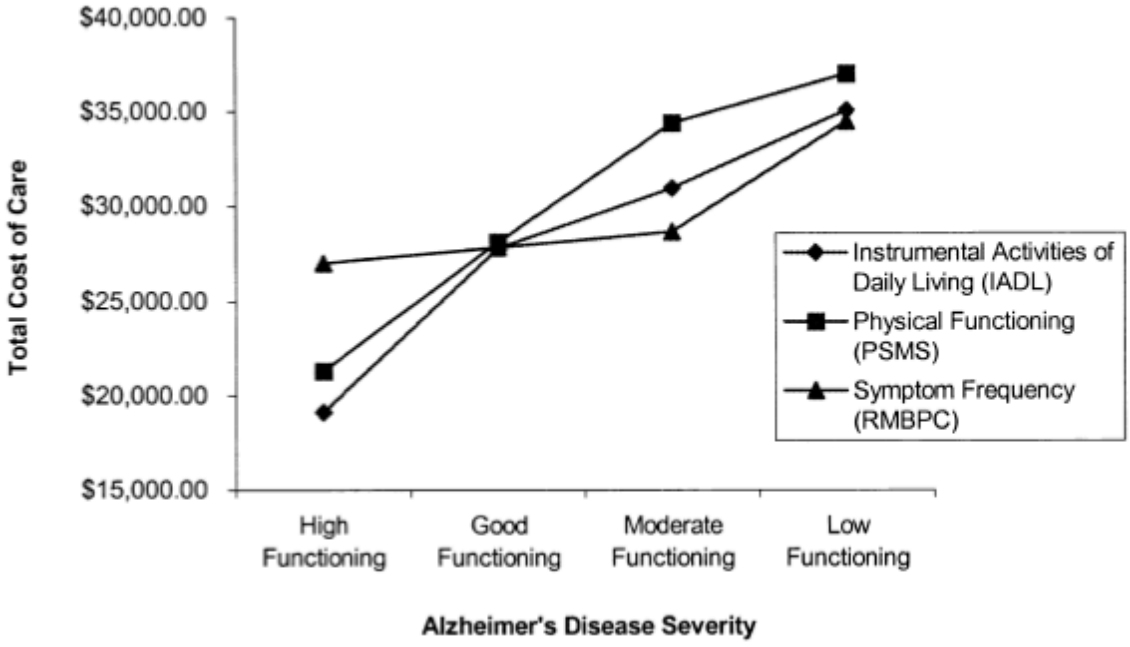


Figure 12: Cost vs. AD Severity in Early Detection[74]

The above graph depicts the cost-effectiveness of AD in response to the time period of the diagnosis. In the very early stages, the symptoms are just arising and the cost is about $27,500. This is lower than the higher pervasiveness of the symptoms in the later stages of the diagnosis, which is about $35,000. Similarly, the daily activities and the physical functioning also have a similar trend. In such aspects, when the patient is diagnosed early, the cost is about $20,000 as opposed to when the patient is diagnosed very late in the disease, which is about $ 36,000 on average. Therefore, it can be concluded that early detection of Alzheimer's becomes very beneficial financially for the patient, as there is almost a $10,000 difference within early and late diagnoses. Furthermore, the high cost of late detection also provides an incentive for providing

future techniques that can detect the disorder in its earliest stages so as to provide the patient with enough time and financial support to be able to live life to the fullest.

Alzheimer's disease has plagued the world in societal and economical aspects. As the disease progresses in a certain patient, the cost of treatment and accommodations also increases. Social and economic reasons have always been intertwined with each other since each of the factors affects the other greatly. For example, familial and local support is necessary in order to cover the expenses of diagnosing and properly treating the condition. Early detection techniques in the present have aided several patients into achieving somewhat of a promising future socially and financially. This not only applies to patients and their families a micro scale but also to the government and the nation on a macro scale. The disorder requires a certain percentage of the whole economy of a nation and that is very demanding. As a result, the future of early treatment needs to succeed in creating detection techniques that will be able to diagnose the disorder as early as possible, which would relieve social detriments and isolation and mitigating financial distresses.

Chapter 13: Future Prevention

AD is one of the most prevalent dementias in the world today. As is the case with every disease and disorder, medicine and science have been trying to find a cure to either prevent the onset or detect it early enough to stop it from occurring in a patient. There are several initiatives, which are being taken to develop a pathway for accelerated AD treatment using the best combinations. Several of the techniques have already been mentioned before using specific genetic relationships, and in the past there have been early basic remedies as well. Furthermore, presently there are also several techniques to early detection and prevention of AD that have been described. However, the future needs to be promising since there is no cure or a foolproof prevention technique that has been developed to prevent AD. There are environmental factors and ideas that may prevent AD to an extent, but medically there is not enough evidence to support a certain cure. Two of the several organizations are also examining the AD symptoms and trying to determine the most promising treatments in order to control AD. These are Alzheimer's Disease Neuroimaging Initiative (ADNI) following the Alzheimer's Prevention Initiative (API). Lastly, research needs to be done in order to improve certain techniques and to increase the success rate of detecting and treating AD onset. Currently, there are measurements being taken; however, AD has become somewhat of an epidemic for which medicinal science needs to find better detection and ultimately, prevention techniques.

13.1 Therapeutic Techniques and Biomarkers

There have been several approaches taken by hospitals in order to properly diagnose and figure out therapeutic methods by using biomarkers, to attempt in finding diagnostic

criteria, and to care for AD patients. As proposed, AD is a major cause of dementia in people of ages over 60 and is more expensive in terms of care than cancer or any other cardiovascular disease. [77] Therefore it is imperative to find a way to control and detect AD in patients to avoid further delays. Presently, most of the research has focused on biomarkers and their effect on AD during the early onset. The biomarkers should be able to aid in the diagnosis and treatment of AD and allow the patient to be monitored.[77] Even though this may seem effective; there are complications such as Aβ positive patients, which may provide faulty results. Therefore, further research needs to be performed on biochemical pathways and AD pathology, which will aid and amplify the effect of detection techniques in AD. Additionally, more research towards the environmental factors and neuroimaging could aid in the detection and prevention process as well. As a result, more patients will be able to adapt to a certain plan that can aid them with AD within the next 10 years.

There are other techniques that could be amplified or altered to attain better results in prevention of AD. One of the current therapies is the use of cholinesterase inhibitors. The bodies of AD patients are found to be lacking acetylcholine (Ach) due to reduced choline intake. Additionally, by increasing the cholinergic balance due to the inhibition of Ach breakdown would slow the progression of AD and ameliorate the symptoms.[78] The cholinesterase inhibitor drug was used in AD therapy, however there was a very short follow up period. For that reason, patients were unable to attain long-term effects for the drug even though the drug did alleviate AD symptoms. Another type of therapy is the anti-amyloid therapy, which states that AD can be prevented by decreasing the production of Aβ and preventing Aβ from forming into amyloid plaques.[78]

Aβ is the major cause of AD in patients and if there are techniques that are able to halt the overproduction of Aβ, AD can be prevented. Additionally, decreasing Aβ generation can aid in the understanding of the disease but that has yet to be achieved. In conclusion, there is research being conducted, however, there must be a "disease-modifying intervention [to] reduce AD." [78] Specifically, in order to attain such a result, there must be a complete understanding of the disease and finally testing of new drugs to prevent the disease from prevailing.

13.2 Organizations for Cure

Besides the research being performed, there are several organizations that are aiming at curing AD, specifically, Alzheimer's Disease Neuroimaging Initiative (ADNI) following the Alzheimer's Prevention Initiative (API). The API examines the most promising pre-symptomatic AD treatments and drugs and aids in the production of a mechanism for their use into the public market. On the other hand, ADNI is a study "aiming to elucidate biomarker trajectories in AD."[79] ADNI seeks to find a cure in terms of biomarkers and their effectiveness in curing AD. ADNI has been able to and is trying to improve the early detection techniques for the future including, "Structural MRI, FDG-PET, amyloid PET, LPs, genetic analysis, and blood studies."[79] The aforementioned techniques are the most common AD detection methods that in the future will be able to diagnose AD more accurately. As a result, if AD is detected very early, the drugs in the future may be able to prevent the onset altogether and successfully find a way to end the epidemic.

As a result, the future holds a hazy view of the cure for AD due to uncertainties in the detection techniques and the lack of firm understanding of the disease itself. As mentioned, the techniques and the drugs in process today are effective; however they

need to be improved in order to completely undermine the effectiveness of AD. Other organizations and laboratories besides ADNI are also working on better detection techniques so that maybe someday in the future AD can be diagnosed in the infant as opposed to in an adult. This would require genetic analysis and manipulation in order to diagnose the disease that early in life, if at all possible. Furthermore, the third-world countries need the most care due to lack of hospital and medicinal training, and since AD is found worldwide, it is taking a higher toll on those nations. In conclusion, the level of detection and prevention of AD needs to be raised in the future in order to successfully combat and possibly defeat Alzheimer's disease and its unpleasant effect on the society.

Conclusion

There are two types of AD, familial and sporadic, which differ depending on the onset of the disease and whether there is an involvement of gene mutations. Gene mutations are more involved in familial AD while the possible risk factors are more associated with sporadic AD. The characteristics as well as the pathological mechanisms of the disease occur in the same way in both types of AD. What happens in AD is that the increased expression and production of APP and Aβ42 results in high accumulation of senile plaques that can interfere with synapses, leading to destructive inflammatory processes and the activation of apoptosis. The deterioration of the microtubules leads to the production of free cytosolic tau proteins that aggregate together to form the neurofibrillary tangles. The presence of the tangles in the cytoplasm as well as the absence of microtubules prevents the trafficking and transportation of proteins and nutrients, which are necessary for the neurons to survive.

Along with the presence of high concentration of amyloid plaques around the neurons and neurofibrillary tangles in the neurons, the cells also exhibit another newly characteristic of AD: disruption of the calcium homeostasis. The high concentration of intracellular calcium levels causes overfilling of the ER and the mitochondria resulting in the deterioration of the mitochondria. The content of the mitochondria such as caspases, cytochrome c oxidase, and apoptosis-inducing factors are released into the cytoplasm where they cleave many of the proteins in the cell, leading to apoptosis of the neuron. The entire process along with plaque and tangle formation and disruption of calcium homeostasis causes an increase in production of ROSs, which results in higher oxidative stress and further promotion of neurodegeneration. The region of the brain that is most

affected by the neurodegeneration and ultimately by AD are the regions that functions in learning and memory formation particularly the hippocampus, frontal and temporal lobes. However, in order to effectively gain an understanding of AD, researchers have developed biomarkers to learn about the neurodegeneration.

Several neurochemical and MRI-based markers are currently being analyzed in order to determine their sensitivity and specificity. Moreover, these biomarkers are being studied in order to elevate our understanding of the pathology of Alzheimer's disease. The combination of CSF tau and amyloid beta proteins would be an accurate diagnostic tool. In CSF, haptoglobin has been reported to show a consistent increase as well. Furthermore, oxidative stress markers 3-nitrotyrosine, DNA oxidation product 8-hydroxyl-2'-deoxyguanosine, and isoprostanes have increased concentrations in CSF. In serum, 24S-hydroxycholesterol and GFAP antibody concentrations are the most promising markers to date in screening a large group as 24S-hydroxycholesterol has been shown to be an early marker and GFAP autoantibodies as late markers. The α-ACT concentration in serum is currently the most convincing inflammation marker, whereas haptoglobin and IL-6 need to be further investigated. In addition, oxidative stress markers such as vitamin A, C, and E need further investigation because dietary supplementation is a variable factor. Homocysteine must be combined with other sets of markers because although it is not specific to brain-related processes, it has high levels of concentration in both AD and normal cognitive aging patients.

As for neuroimaging markers, hippocampal volumetry is the best established structural biomarker for AD. However, since it is laborious, it is only used for clinical studies and as an end point for treatment effects. VBM, DBM, and measurement of

cortical thickness are currently being investigated as well. PET is the gold standard in *in vivo* diagnosis and gives a better distinction between controls and AD. Furthermore, a combination of FDG-PET and PIB-PET shows improvement in the diagnostic discrimination between MCI and AD and between MCI and control. Other markers such as SPECT, entorhinal cortex volumes, and functional magnetic resonance imaging need to be further developed for accuracy in the early diagnosis of AD. Even though there are techniques present for detecting the symptoms, they are not enough to prevent the onset completely.

Although the state of current Alzheimer's medicine is important in treating some cognitive function, it is not sufficient for those patients suffering from AD. These current treatments, acetylcholinesterase inhibitors and memantine, do not actually modify the disease, so they cannot prevent or completely stop the process of AD onset in the patients. Research is now focused towards finding a disease-modifying treatment, and there is much hope in this field of study. Much of the press and priority has been given to Aβ modulating and alteration as a means of therapy, both of which prevent Aβ formation and aggregation. However, lesser-known research that is emerging is just as important to learn about and understand Alzheimer's therapy.

Newer areas of research are interesting, important, and hopeful. As researchers learn more about the nature of AD and Aβ aggregation, they can begin to develop more reliable treatments and therapies for AD patients. Treatments to inhibit the action of trace metals are growing, and this field of study is extremely promising. Therapies devoted to immunotherapy are being explored as well, where vaccines are used to defend against toxic Aβ. There are even more fields of investigations devoted to searching for an AD

therapy on other known aspects of the disease, such as APOE and tau. However, there are still some issues with these newer fields of research. Many of the mechanisms involved with these parts of the disease are not fully understood, and although many upcoming treatments in development can be labeled as "hopeful" it is very rare that any of them advance deep into clinical trials. This is due to issues with treatment in humans, such as the blood brain barrier. With such a broad field of research and with so many possible treatments that could be explored, this is an area of study that can be very overwhelming. Yet it is a very important topic to look into and try to paint a broad picture of, as many of these treatments have great potential for the future of AD therapy. One of the major factors in determining treatments for AD is the relationship of the disease to other disorders.

As the paper displays, there are several factors associated with Alzheimer's disease. The relationship between Alzheimer's and other diseases provides a more urgent need for a solution. The similarities may make it easier to detect the disease, which provides a basis for future prevention techniques. Using genetic modifications and evolutionary explanations, early detection techniques can be developed to cure not only AD, but also other types of dementia and type II diabetes. There are several socioeconomic impacts of such a disorder, relating to the society and the economic health of the patient and their family. There are very harsh impacts on the patient due to the inability to socialize with the world, and the families are forced to live away from the affected patient and pay for their care. The large scale impact of the disease is that it has affected the economy in several ways, thereby becoming one of the most researched diseases on the planet. However, all of the money and the society's efforts may go to

waste if there are no earlier prevention and diagnosis techniques developed. There are several ways to achieve this as mentioned earlier, both socially and medicinally. Nevertheless, the future research needs to be more extensive if the world is to find a cure someday. One may never know, the monetary and the societal efforts may provide a solution to curing the disorder. In the end, the harsh truth about Alzheimer's is that it may erase the memory of a person, but it will not erase the true façade of the society; and the future of medicine will determine whether Alzheimer's is or isn't.

References

1. Munoz, D. and Feldman, H. 2000. Causes of Alzheimer's disease. *Canadian Medical Association* **162**: 65-72.
2. Luchsinger, J., and Mayeux R. 2004. Dietary factors and Alzheimer's disease. *The Lancet Neurology* **3**: 579-587.
3. Kawashima, M. and Ihara, Y. 2002. Alzheimer's Disease: β-Amyloid Protein and Tau. *Journal of Neuroscience Research* **70**: 392-401.
4. Small, D. 2009. Dysregulation of Calcium Homeostasis in Alzheimer's Disease. *Neurochemistry Research* **34**: 1824-1829.
5. Mattson, M. 2004. Pathways towards and away from Alzheimer's disease. *Nature* **430**: 631-639.
6. Garcia-Osta, A. and Alberini, C. 2009. Amyloid beta mediates memory formation. *Learning and Memory* **4**: 267-272.
7. Korol, T., Korol, S., Kostyuk, E., and Kostyuk, P. 2008. Disruption of Calcium Homeostasis in Alzheimer's Disease. *Neurophysiology* **40**: 385-382.
8. Steiner, H., Capell, A., Leimer, U., and Haass, C. 1999. Genes and mechanisms involved in β-amyloid generation and Alzheimer's disease. *European Archives of Psychiatry and Clinical Neuroscience* **249**: 266-270.
9. Forloni, G., Terreni, L., Bertani, I., Fogliarino, S., Invernizzi, R., Assini, A., Ribizzi, G., Negro, A., Calabrese, E., Volonte, M., Mariani, C., Franceschi, M., Tabaton, M., and Bertoli, A. 2002. Protein misfolding in Alzheimer's and Parkinson's disease: genetics and molecular mechanisms. *Neurobiology of Aging* **23**: 957-976.
10. Selkoe, D. 2004. Cell biology of protein misfolding: The examples of Alzheimer's and Parkinson's diseases. *Nature Cell Biology* **6**: 1054-1061.
11. 2008. Tau and cytoskeleton formation. *National Institutes of Health.* http://neurologicalcorrelates.com/wordpress/2008/01/16/genetics-200-family-members-having-dementia-mutation-studied-for-13-years/tau-and-cytoskeletal-formation/.
12. Berridge, M. 2011. Calcium Signalling and Alzheimer's Disease. *Neurochemistry Research* **36**: 1149-1156.
13. Gorwood, P., Corruble, E., Falissard, B., and Goodwin, G. 2008. Toxic Effects of Depression on Brain Function: Impairment of Delayed Recall and the Cumulative Length of Depressive Disorder in a Large Sample of Depressed Outpatients. *The American Journal of Psychiatry* **165**: 731-739.
14. Castellani, R., Hirai, K., Aliev, G., Drew, K., Nunomura, A., Takeda, A., Cash, A., Obrenovich, M., Perry, G., and Smith, M. 2002. Role of Mitochondrial Dysfunction in Alzheimer's Disease. *Journal of Neuroscience Research* **70**: 357-360.
15. Green, K., Demuro, A., Akbari, Y., Hitt, B., Smith, I., Parker, I., and LaFerla, F. 2008. SERCA pump activity is physiologically regulated by presenilin and regulates amyloid β production. *Journal of Cell Biology* **181**: 1107-1116.
16. Petronis, A. 1999. Alzheimer's Disease and Down Syndrome: From Meiosis to Dementia. *Experimental Neurology* **158**: 403-413.
17. Riederer, P., Bartl, J., Laux, G., and Grunblatt, E. 2010. Diabetes Type II: A Risk Factor for Depression–Parkinson–Alzheimer? *Neurotoxicity Research* **19**: 253-265.
18. Wachter, K. 2007. Stroke and Alzheimer's pathology raise risk of dementia. *Clinical Psychiatry News*: 33.

19. Honig, L., Tang, M., Albert, S., Costa, R., Luchsinger, J., Manly, J., Stern, Y, and Mayeux, R. 2003. Stroke and the Risk of Alzheimer Disease. *Archives of Neurology* **60**: 1707-1712.
20. Fleminger, S., Oliver, D., Lovestone, S., Rabe-Hesketh, S., and Giora, A. 2003. Head injury as a risk factor for Alzheimer's disease: the evidence 10 years on; a partial replication. *Journal of Neurology, Neurosurgery, and Psychiatry with Practical* Neurology **74**: 857-862.
21. Lyketsos, C., and Olin, J. 2002. Depression in Alzheimer's Disease: Overview and Treatment. *Society of Biological Psychiatry* **52**: 243-253.
22. Andersen, K., Lolk, A., Kragh-Sorensen, P., Petersen, N., and Green A. 2005. Depression and the Risk of Alzheimer Disease. *Epidemiology* **16**: 233-238.
23. Lee, C., Dai, W., Becker, J., Kuller, L., and Gach, H. 2007. High Blood Pressure May Heighten Effects of Alzheimer's Disease. Cardiovascular Business Week: 32.
24. Schneider, J. 2009. High Blood Pressure and Microinfarcts: A Link Between Vascular Risk Factors, Dementia, and Clinical Alzheimer's Disease. *Journal of the American Geriatrics Society* **57**: 2146-2147.
25. Sakurai, H., Hanyu, H., Sato, T., Kanetaka, H., Shimizu, S., Hirao, K., Kikukawa, M., and Iwamoto, T. 2011. Vascular risk factors and progression in Alzheimer's disease. *Geriatrics and Gerontology International* **11**: 211-214.
26. Brewer, G. 2007. Iron and Copper Toxicity in Diseases of Aging, Particularly Atherosclerosis and Alzheimer's Disease. *Experimental Biology and Medicine* **232**: 323-335.
27. Curtain, C., Ali, F., Volitakis, I., Cherny, R., Norton, R., Beyreuther, K., Barrow, C., Masters, C., Bush, A., and Barnham, K. 2001. Alzheimer's Disease Amyloid-β Binds Copper and Zinc to Generate an Allosterically Ordered Membrane-penetrating Structure Containing Superoxide Dismutase-like Subunits. *The Journal of Biological Chemistry* **276**: 20466-20473.
28. Nation, D., Hong, S., Jak, A., Delano-Wood, L., Mills, P., Bondi, M., and Dimsdale, J. 2011. Stress, exercise, and Alzheimer's disease: A neurovascular pathway. *Medical Hypotheses* **76**: 847-854.
29. Perez, C. and Carral, J. 2008. Benefits of Physical Exercise for Older Adults With Alzheimer's Disease. *Geriatric Nursing* **29**: 384-391.
30. Sando, S., Melquist, S., Cannon, A., Hutton, M., Sletvold, O., Saltvedt, I., White, L., Lydersen, S., and Aasly, J. 2008. Risk-reducing effect of education in Alzheimer's disease. *International Journal of Geriatric Psychiatry* **23**: 1156-1162.
31. Hampel, H., Bürger, K., Teipel, S.J., Bokde, A.L.W., Zetterberg, H., andBlennow, K. 2008. Core candidate neurochemical and imaging biomarkers of Alzheimer's disease. *Alzheimer's & Dementia* **4:** 38-48.
32. Teunissen, C.E, de Vente, J., Steinbusch, H.W.M., and De Brujin, C. 2002. Biochemical markers related to Alzheimer's dementia in serum and cerebrospinal fluid. *Neurobiology of Aging* **23**: 485-508.
33. Irizarry, M. C. 2004. Biomarkers of Alzheimer's Disease in plasma. *NeuroRx: The Journal of the American Society for Experimental NeuroTherapeutic* **1**: 226-234.
34. Mueller, S.G., Weiner, M.W., Thal, L.J., Petersen, R.C., Jack, C.R., Jagust, W., Trojanowski, J.Q., Toga, A.W., and Beckett, L. 2005. Ways toward an early diagnosis in

Alzheimer's disease: The Alzheimer's Disease Neuroimaging Initiative (ADNI). *Alzheimers Dementia* **1**: 55-66.
35. Goldstein, L., Muffat, J., Cherny, R., Moir, R., Ericsson, M., Huang, X., Mavros, C., Coccia, J., Faget, K., Fitch, K., Masters, C., Tanzi, R., Chylack Jr, L., and Bush, A. 2003. Cytosolic β-amyloid deposition and supranuclear cataracts in lenses from people with Alzheimer's disease. *Lancet* **361:** 1258-1265.
36. D'Aniello, A., Fisher, G., Migliaccio N., Cammisa, G., D'Aniello, E., and Spinelli, P. 2005. Amino acids and transaminases acitivity in ventricular CSF and in brain of normal and Alzheimer patients. *Neuroscience Letters* **388**: 49-53.
37. Zhang, Y., Londos, E., Minthon, L., Wattmo, C., Liu, Huaijun; A.P., and Wahlund, L.O. 2008. Usefulness of computed tomography linear measurements in diagnosing Alzheimer's disease. *Acta radiologica* **49**: 91-97.
38. Craig-Schapiro, R., Fagan, A.M. and Holtzman, D.M. 2009. Biomarkers of Alzheimer's disease. *Neurobiology of Disease* **35**: 128-140.
39. Vemuri, P. and Jack Jr, C.R. Role of Structural MRI in Alzheimer's disease. 2010. *Alzheimer's Research & Therapy* **2**:23.
40. Borthakur, A., Sochor, M., Davatzikos, C., Trojanowsji, J.Q., and Clark, C.M. 2008. T-1-rho MRI of Alzheimer's disease. *NeuroImage* **41**: 1199-1205.
41. Jack, C.R, Shiung, M.M., Gunter, J.L., O'Brien, P.C., Weigand, S.D., Knopman, D.S., Boeve, B.F., Ivnik, R.J., Smith, G.E., Cha, R.H., Tangalos, E.G., and Petersen, R.C. 2004. Comparison of Different MRI Brain Atrophy Rate Measures with Clinical Disease Progression in AD. *Neurology* **62**: 591-600.
42. Prvulovic, D., Bokde, A.L.W., Faltraco, F., and Hampel, H. 2011. Functional magnetic resonance imaging as a dynamic candidate biomarker in Alzheimer's disease. *Progress in Neurobiology* **95**: 557-569.
43. Zhou, J., Greicius, M.D., Gennatas, E.D., Growdon, M.E., Jang, J.Y., Rabinovici, G.D., Kramer, J.H., Weiner, M., Miller, B.L., and Seeley, W.W. 2010. Divergent network connectivity changes in behavioural variant frontotemporal dementia and Alzheimer's disease. *Brain: A Journal of Neurology* **133**: 1352-1367.
44. McNeill, R., Sare, G.M., Manoharan, M., Testa, H.J., Mann, D.M.A., Snowden, J.S., and Varma, A.R. 2007. Accuracy of single-photon emission computed tomography in differentiating frontotemporal dementia from Alzheimer's disease. *Journal of Neurology, Neurosurgery & Psychiatry* **78**: 350-355.
45. Mentis, M.J. 2000. Positron emission tomography and single photon emission computed tomography in Alzheimer's disease. *Neurologist* **6**: 28-43.
46. Yuan, Y., Gu, Z-X., and Wei, W-S. 2009. Fluorodeoxyglucose-Positron-Emission Tomography, Single-Photon Emission Tomography, and Structural MR Imaging for Prediction of Rapid Conversion to Alzheimer Disease in Patient with Mild Cognitive Impairment: A Meta-Analysis. *American Journal of Neuroradiology* **30**: 404-410.
47. Chen, G., Chen, P., Tan, H., Ma, D., Dou, F., Feng, J., and Yan, Z. 2008. Regulation of the NMDA receptor-mediated synaptic response by acetylcholinesterase inhibitors and its impairment in an animal model of Alzheimer's disease. *Neurobiology of Aging* **29:** 1795-1804.
48. Melnikova, I. 2007. Therapies for Alzheimer's disease. *Nature Reviews Drug Discovery* **6:** 341-342.

49. Takada-Takatori, Y., Kume, T., Sugimoto, M., Katsuki, H., Sugimoto, H., and Akaike, A. 2006. Acetylcholinesterase inhibitors used in treatment of Alzheimer's disease prevent glutamate neurotoxicity via nicotinic acetylcholine receptors and phosphatidylinositol 3-kinase cascade. *Neuropharmacology* **51**: 474-486.
50. Reisberg, B., Doody, R., Stoffler, A., Schmitt, F., Ferris, S., and Mobius, H. 2003. Memantine in Moderate-to-Severe Alzheimer's Disease. *New England Journal of Medicine* **348:** 1333-1341.
51. Hynd, M., Scott, H., and Dodd, P. 2004. Glutamate-mediated excitotoxicity and neurodegeneration in Alzheimer's disease. *Neurochemistry International* **45:** pages 583-595.
52. Citron, M. 2010. Alzheimer's Disease: Strategies for Disease Modification. *Nature Reviews and Drug Discovery* **9:** 387-398.
53. Weggen, S., Eriksen, J., Das, P., Sagi, S., Wang, R., Pietrzik, C., Findlay, K., Smith, T., Murphy, M., Butler, T., Kang, D., Marquez-Sterling, N., Golde, T., and Koo, E. 2001. A subset of NSAIDs lower amyloidogenic Aβ42 independently of cyclooxygenase activity. *Nature* **414:** 212-216.
54. Weggen, S., Rogers, M., and Eriksen, J. 2007. NSAIDs: small molecules for prevention of Alzheimer's disease or precursors for future drug development? *Trends in Pharmacological Sciences* **28**: 536-543.
55. Citron, M. 2004. β-Secretase inhibition for the treatment of Alzheimer's disease – promise and challenge. *Trends in Pharmacological Sciences* **25:** 92-97.
56. Crouch, P., Savva, M., Hung, L. et al. 2011. The Alzheimer's therapeutic PBT2 promotes amyloid-β degradation and GSK3 phosphorylation via a metal chaperone activity. *Journal of Neurochemistry* **119:** 220-230.
57. Frederickson, C., Koh, J., and Bush, A. 2005. The neurobiology of zinc in health and disease. *Nature Reviews Neuroscience* **6:** 449-462.
58. Bush, A., and Tanzi, R. 2008. Therapeutics for Alzheimer's Disease Based on the Metal Hypothesis. *Neurotherapeutics* **5:** 421-432.
59. Dreifus, C. "CONVERSATION WITH: RUDOLPH TANZI; A Gene Detective on the Trail of Alzheimer's". New York Times [New York City] 5 December 2000. Accessed 20 February 2010. http://www.nytimes.com/2000/12/05/science/a-conversation-with-rudolph-tanzi-a-gene-detective-on-the-trail-of-alzheimer-s.html?pagewanted=all&src=pm
60. Schenk, D., Barbour, R., Dunn, W., et al. 1999. Immunization with amyloid-β attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* **400:** 173-177.
61. Frenkel, D., Dewachter, I., Van Leuven, F., and Solomon, B. 2003. Reduction of β-amyloid plaques in brain of transgenic mouse model of Alzheimer's disease by EFRH-phage immunization. *Vaccine* **21:** 1060-1065
62. Bertram, L., and Tanzi, R. 2008. Thirty years of Alzheimer's disease genetics: the implications of systematic meta-analyses. *Nature Reviews Neuroscience* **9:** 768-778.
63. Corder, E., Saunders, A., Strittmatters, W. et al. 1993. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* **261:** 921-923.

64. Bu, G. 2009. Apolipoprotein E and its receptors in Alzheimer's disease: pathways, pathogenesis and therapy. *Nature Reviews Neuroscience* **10:** 333-344.
65. Mahley, R., Weisgraber, K., Huang, Y. 2006. Apolipoprotein E4: A causative factor and therapeutic target in neuropathology, including Alzheimer's disease. *Proceedings of the National Academy of Sciences of the United States of America* **103:** 5644-5651.
66. Brito, G. 2009. Exercise and cognitive function: a hypothesis for the association of type II diabetes mellitus and Alzheimer's disease from an evolutionary perspective. *Diabetology & Metabolic Syndrome* **1**: 1-6.
67. Gotz, J., Ittner L.M., and Lim Y.A. 2009. Common features between diabetes mellitus and Alzheimer's disease. *Cellular and Molecular Life Sciences* **66**: 1321-1325. Print.
68. Kumar, A. 2008. Mechanism of action of thaizolidinediones. *Pharmainfo.net*. http://www.pharmainfo.net/files/images/stories/article_images/Mechanism%20of%20action%20of%20thiazolidinediones.jpg.
69. 2006. Down Syndrome Karyotype. *Genome.gov.* http://upload.wikimedia.org/wikipedia/commons/thumb/1/11/Down_Syndrome_Karyotype.png/220px-Down_Syndrome_Karyotype.png.
70. Potter, H. 2008. Down's syndrome and Alzheimer's disease: two sides of the same coin. *Future Neurology* **3**: 29-37.
71. Love, J. 2010. Mosaicism. *Synapses.co.uk*. http://www.synapses.co.uk/genetics/mosaic1.gif
72. Zana, M., Zoltán, J., and János, K. 2007. Oxidative stress: A bridge between Down's syndrome and Alzheimer's disease. *Neurobiology of Aging* **1**: 648-676.
73. Chaput, J. L. 2003. Adults with Down Syndrome and Alzheimer's Disease. *Journal of Gerontological Social Work* **38**: 197-211.
74. Small, G. W., McDonnell D. D., et al. 2002. The Impact of Symptom Severity on the Cost of Alzheimer's Disease. *American Geriatrics Society* **50**: 321-327.
75. 2010. 2010 Alzheimer's Disease Facts and Figures. *Alzheimer's Association* **6**: 1-28.
76. Prince, M., Renata B., and Cleusa F. 2011. World Alzheimer Report 2011: The benefits of early diagnosis and intervention. *Alzheimer's Disease International* **15**: 5-65.
77. Hampel, H., Prvulovic, D., et al. 2011. The future of Alzheimer's disease: The next 10 years. *Progress in Neurobiology* **95**: 718-728. Print.
78. Van Marum, R. J. 2008. Current and future therapy in Alzheimer's disease. *Blackwell Publishing Limited* **22**: 265-274.
79. Aisen, P. S. 2011. Q&A: The Alzheimer's disease neuroimaging initiative. *BMC Medicine 2011* **9**: 1-2.

Glossary

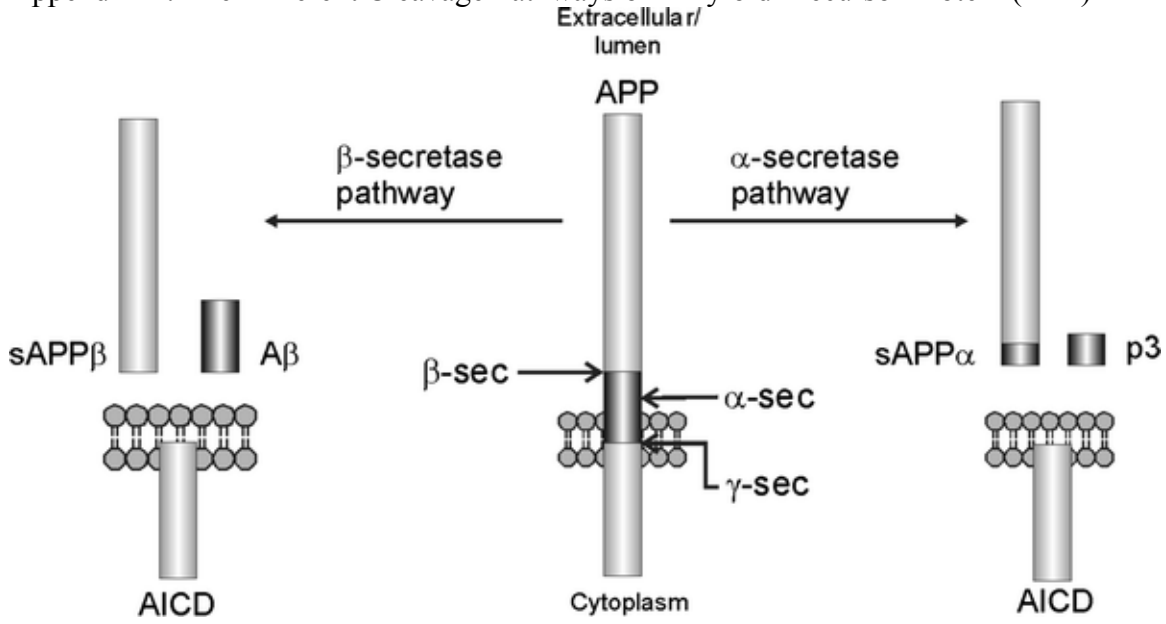
24S-hydroxycholesterol - oxysterol that is produced through enzymatic oxidation for the removal of cholesterol from the brain
α-antichymotrypsin - glycoprotein that inhibits the activity of certain enzymes
α-secretase - cleaving enzyme that cleaves the middle section of Aβ
Amyloid Beta (Aβ) - a protein that aggregates together to form senile plaques which are considered to be one of the main causes of AD
Acetylcholine - neurotransmitter in central nervous system (CNS) and peripheral nervous system (PNS)
Acetylcholinesterase (AChE) - an enzyme which specifically breaks down acetylcholine
Acute-phase protein - protein that increases or decreases its concentration in plasma in response to inflammation
Amyloidogenic - ability of a protein to aggregate together
Amyloid precursor protein (APP) - transmembrane glycoprotein that extends the lipid bilayer of a cell and contains an Aβ section within the membrane
APP intracellular doman (AICD) - transcription factor that regulates the production of ryanodine receptors and Ca2+ buffer calbindin; peptide that is located inside the cell when APP is cleaved by γ-secretase
Apolipoprotein E (APOE) - susceptibility genes involved in synaptic repair in response to tissue injury as well as maintenance of neuron structutres and cholinergic function
Aneuploidy - having a chromosome number that is not an exact multiple of the haploid counterpart
Aneurysm - swelling of the artery walls
Apoptotic - a cell that is genetically regulated to process for death
Atrophy - decrease in size
Automation protocol - uses control systems and information technology to reduce human work in the measurements of MRI methods
Autouimmune - referring to a failure in the immune system whereupon the the immune system no longer recognizes the body's own cells, and the body produces an immune response against one's own cells or tissues
β-site APP cleaving enzyme or β-secretase (BACE1) - cleaves the Aβ section that is located outside the cell; one of the key enzymes responsible for the pathology of amyloid cleavage of the amyloid precursor protein (APP)
Biomarker - any detectable biological feature that provides information about its source
Blood-brain barrier - a layer of endothelial cells which separate the circulating blood from the brain's extracellular fluid. This barrier restricts diffusion of both small and large molecules into the cerebral spinal fluid
Boundary shift integral (BSI) - tracks stuctural changes in the brain over time in order to monitor the progression of the disease
C terminus - one end of a protein that contains the free carboxylic acid (-COOH) group
Caspase - a type of protease that facilitaes apoptosis
Central nervous system (CNS) - the part of the nervous system that consists of the brain, the spinal cord, and the meninges
Cerebral amyloid angiopathy - form of angiopathy in which amyloid deposits form in the walls of blood vessels of the CNS
Cerebral infract - localized cell death

Chelator - a chemical compound which removes a heavy metal from the bloodstream or cells by combining with the metal compound to form a ring. Chelation is used medically to treat heavy metal poisoning by this process
Cholinergic System - a system of nerve cells that uses the neurotransmitter acetylcholine to transmit signals
Computed tomography (CT) - medical imaging method that uses tomography created by computer processing
Cortex - the outer layer of the brain
Cross-sectional study - a descriptive study that observes and provides data on an entire population that consists of different subgroups
Dementia - an illness that affects an individual's brain resulting in memory and behavioral issues
ELISA assay - biochemistry assay that detects the presence of a substance
Endoplasmic reticulum (ER) - a structure in the cell that functions to help control protein synthesis and cellular organization
Epitope - the part of an antigen that is recognized by the immune system
Excitotoxicity - a process by which nerve cells are damaged or destroyed by excessive stimulation by neurotransmitters; typically results due to overstimulated glutamate
Flow cytometry - technique for counting and examining molecules
Fluorescent ligand scanning (FLS) - noninvasive quantitative measurement
Functional magnetic resonance imaging (fMRI) - measurement of brain activity during cognitive tasks at high resolution without radiation exposure
γ-secretase - cleaving enzyme that cleaves the section of Aβ that is inside the plasma membrane
Gel electrophoresis - method that seprates protein by charge or size
Glial fibrillary acidic protein (GFAP) - intermediate filament cytoskeletal protein expressed mainly by astrocytes
Glycoprotein - a protein that has a carbohydrate
Glycosaminoglycan - complex sugar polymer that aggregates with Aβ42 to form senile plaques
Golgi apparatus - a structure in the cell that functions to package cell products and coordinate their transport to the outside of the cell
Gray matter - a component of the central nervous system that consists of neurons, dendrites, unmyelinated axons, and glial cells
Haptoglobin (Hp) - iron transporting protein involved in the process of oxidative stress
Hemorrhage - the loss of blood from the circulatory system, generally due to leaks from blood vessels in the body. Bleeding can occur internally or through orifaces
Hippocampus - a region in the brain that functions in learning and memory storage
Homocysteine - precursor in making methionine and cysteine
Homozygocity - referring to identical alleles of a certain gene on both homologous chromosomes
Hypercortisolemia - refers to a high amount of cortisol levels in the body. Cortisol is a steroid hormone secreted by the adrenal gland.
Hypertension - elevation of diastolic blood pressure
Hypothalamus - a region that lies between the thalamus and the midbrain and functions as the control center for the autonomous nervous system, also partakes in producing

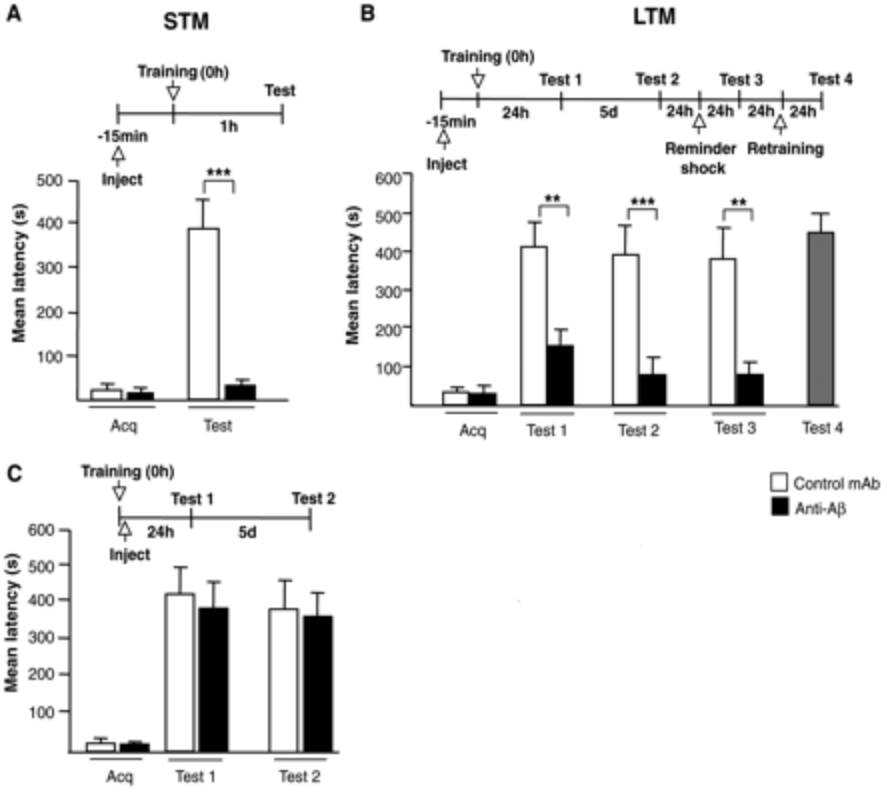
hormones
Intermittent hyperglycemia - a prediabetic condition in which an excess amount of blood sugar circulates in the body.
Intrahippocampal - within the hippocampus
Kinase - an enzyme that activates other enzymes or proteins by attaching a phosphate group
Lewy bodies - abnormal aggregates of protein that develop inside nerve cells
Lipid bilayer - a layer that surrounds a cell that is composed of two layers of lipids, it is what the cell membrane is made of
Longitudinal study - studies that can be repeated over long periods of time with the same variables
Mild cognitive impairment (MCI) - transitional region between normal aging and Alzheimer's disease
Mitochondria - a structure in the cell that functions to produce energy
Morphometry - volume of an object measured by drawing regions of interests on the image of the object and calculating the volume of the object from those regions of interests
Neurodegenerative - relating to a progressive loss of neurological functions
Neurons - the type of cells in the brain
Neurotoxic - poisonous to neurons
Nicotinic acetylcholine receptor (nAchr) - transmembrane protein that mediates rapid transduction to initiate memory development
Nitrosamine - carcinogen that helps form cancerous cells
N-metyl-D-aspartate (NMDA) - a subtype of a specific glutamate receptor in the brain which mediates influxes of synaptic calcium
Notch-1 - cell receptor that gets cleaved by γ-secretase when activated by a ligand
phosphorylate - to add phosphate groups to a molecule
N terminus - one end of a protein that contains the free animo acid (-NH2) group
Oxidative stress - a condition of increased oxidant production in animal cells characterized by the release of free radicals and resulting in cellular degeneration.
Pathology - the study of the causes of diseases and how they affect people
Peptides - polymers of linked amino acids joined together with peptide bonds, also known as a protein
Peripheral nervous system (PNS) - the part of the nervous system that lyes outside of the brain, the spinal cord, and the meninges
Plasma membrane - a membrane that surrounds a cell to allow for the movement of materials
Platelet - irregularly shaped cell fragments
Positron emission tomography (PET) - imaging method used to study cortical metabolism
Post-mortem autopsy - a medical examination after death
Presenilin - intramembran aspartly protease that is present on the membranes of multiple organelles including the endoplasmic reticulum, Golgi apparatus, and endosome; involved in multiple function including Notch signaling
Presynaptic terminals - an area in between neurons where neurotransmitters are released

Protease - an enzyme that cleaves peptide bonds within a protein
Quasi-elastic light scattering (QLS) - noninvasisve quantitative measurement
Relaxation time - time it takes for the protons to emit their signal
Single photon emission computed tomography (SPECT) - imaging measurement of cortical metabolism similar to PET
Tau - soluble microtubule-binding protein that plays a major role in stabilizing microtubules in axons
Temporal lobe - a region in the brain that functions in smell and hearing as well as memory and learning and where choice is made of thoughts to express
Transaminase - removes the amino group from the amino acid leaving behind an α-keto acid and converting it into an amino acid
Transgenic - an organism that contains foreign DNA from another organism which can be from different species
Ubiquitin - small protein that acts as a signal for degradation
Unfolded protein respone (UPR) - signaling pathways that initiates the proper folding of misfolded or unfolded proteins.
Voxel - volumetric pixel representing a value in 3D space
White matter - part of the central nervous system that consists mostly of glial cells and myelinated axons

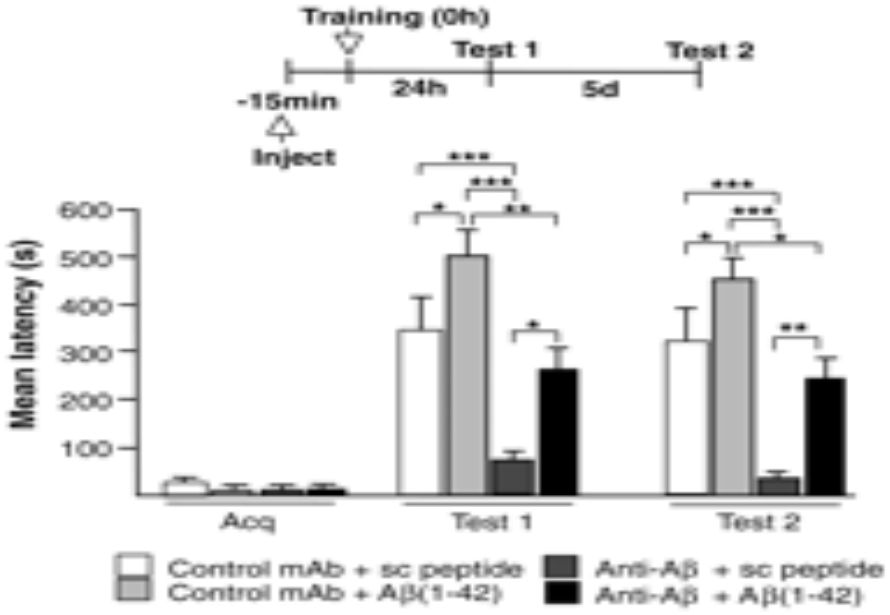
Appendix A: The Different Cleavage Pathways of Amyloid Precursor Protein (APP)



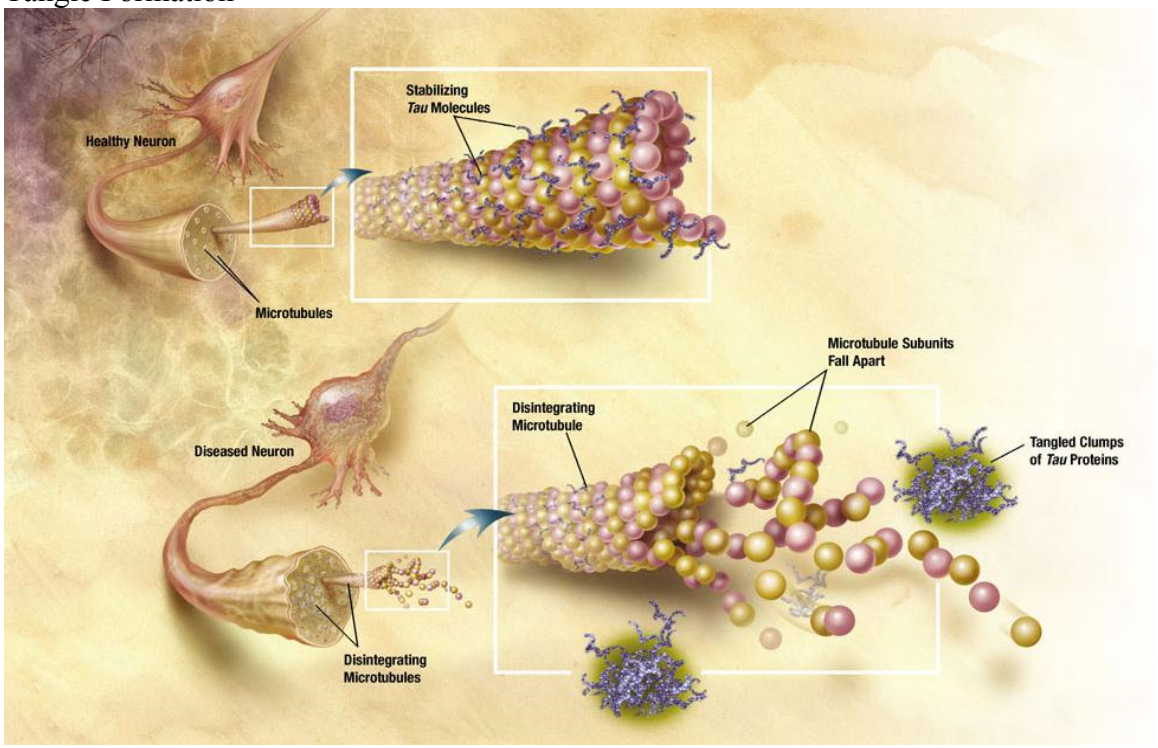
Appendix B: Avoidance Tests Conducted on Rats with Anti-Aβ Antibodies (anti-Aβ) Before or After a Training for Short-Term and Long-Term



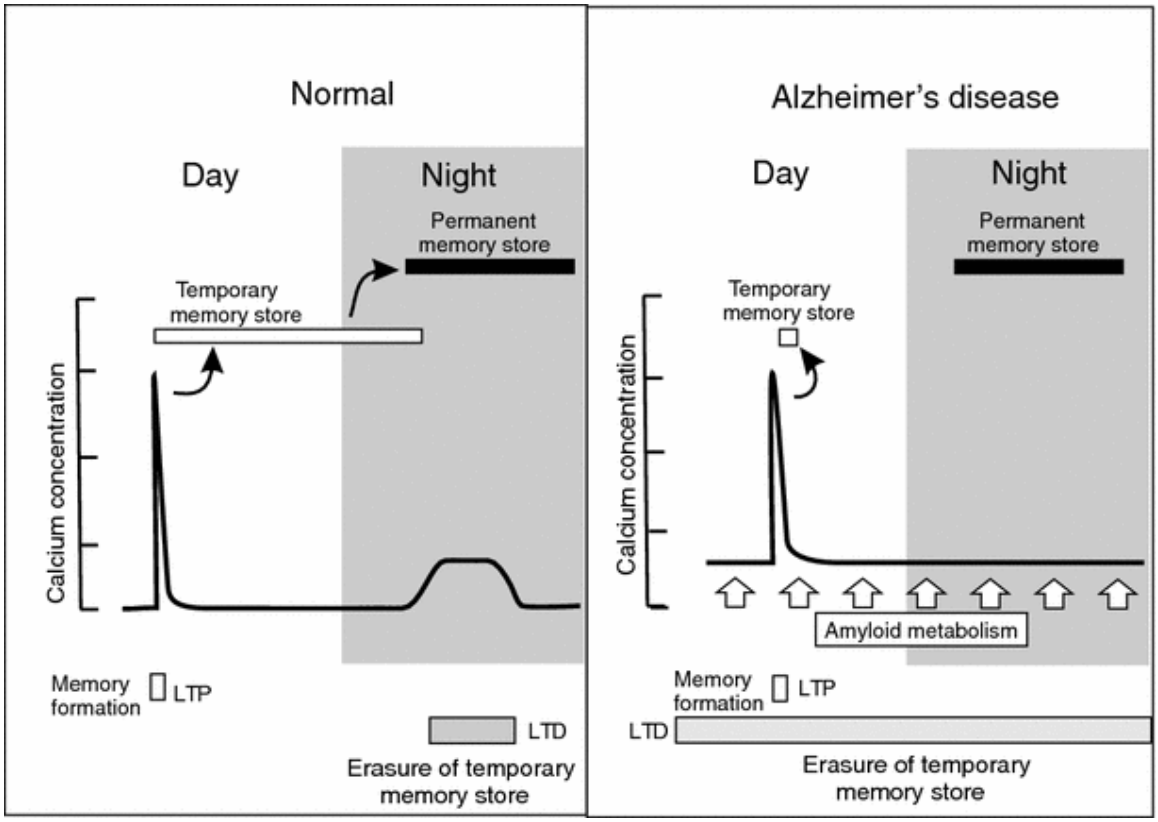
Appendix C: Memory Tests Conducted on Rats using Control Monoclonal Antibody (mAb), Scramble (sc) Peptide, Anti-Aβ Antibodies (anti-Aβ), and β-Amyloid42 (Aβ42)



Appendix D: The Proper Function of Tau Protein and the Process of Neurofibrillary Tangle Formation



Appendix E: The Process of Storing and Erasing Memories in Relation to Calcium Concentrations in Normal and AD Brains



Appendix F: Possible Risk Factors for Alzheimer's Disease

Possible Risk Factors	Reasoning
Down's Syndrome	Extra copy of chromosome 21 results in an increase production or mutation of APP leading to a higher concentration of Aβ[16]
Type II Diabetes Mellitus	Insulin resistance promotes increase secretion of Aβ and hyperphosphorylation of tau proteins as well as inhibiting Aβ degrade[17]
Stroke	Lack of blood flow damages the brain[18,25]
History of Head Trauma	Damages the brain with it being more serious when loss of consciousness accompanies the head injury[20]
Depression	Causes neuronal death in regions of the brain like the hippocampus[13]
High Blood Pressure	Decrease blood flow to the brain resulting in hypoxia injuries and later cognitive decline[23,24,25]
Dietary (high consumption of calories, cholesterol, saturated fats, and red meat as well as low consumption of fruits and vegetables)	High levels of cholesterol, calories, and saturated fats contributes to heart disease like high blood pressure[1], promotes the production of Aβ42[5], aid in insulin resistance[1], and neuronal degeneration[5]; high levels of red meat increases the level of iron[26]; low levels of fruits and vegetables provide low levels of antioxidants[1]
Vitamins (low levels of vitamin B, A, C, and E)	Low levels of vitamin B increases the level of homocysteine[1,5]; low levels of vitamin A, C and E gives low levels of antioxidants[1]
Metals (high levels of iron, copper, and zinc)	High levels of iron, copper, and zinc leads to oxidative stress, production of reactive oxygen species and later neuronal death[26,27]
Sedentary Lifestyles	Decreases neurogenesis, hippocampal synaptic plasticity, and long-term potentiation[28,29]
Chronic Stress	Increases the production of APP and apoptotic enzymes along with enhancing senile plaques accumulation and tau phosphorylation[28]
High Alcohol Consumption	Promotes oxidative damage in the brain leading to brain atrophy and causes mitochondrial dysfunction and deterioration[1]
Low Education Levels	Less network of synapses and inefficient use of neural networks as well as a less ability to recruit networks[30]

Appendix G: Summary of concentration of pathological alterations markers in serum and CSF of AD patients

	Marker	AD vs. Normal
CSF	Aβ	- (9)
	Aβ autoantibodies	= (2)
	Tau	+ (14)
	Hyperphosphorylated tau	+ (1)
	Combination tau	+ (5)
	Combination tau with Aβ42/Aβ40 ratio	- (1)
	β-secretase (BACE1)	+ (1)
	GFAP protein	+ (4)
	GFAP autoantibodies	+ (2)
Serum/plasma	Aβ	+ (3) = (2)
	Aβ40	+ (with age and initial stages)(2)
	Aβ42	+ (with age and initial stages)(1) - (1)
	GFAP autoantibodies	+ (4)
	Aβ autoantibodies	= (2)
	Platelet APP isoforms	- (2)

+: Increased levels observed in AD patients compared to controls
=: No difference observed in levels of AD patients compared to controls
-: Decreased levels observed in AD patients compared to controls
#: Number of studies conducted

Appendix H: Summary of concentrations of cholesterol homeostasis markers in serum and CSF of AD patients

	Marker	AD vs. Normal
CSF	Apolipoprotein ε4	+ (1) = (1) - (1)
	24S-hydroxycholesterol	+ (2)
Serum/plasma	Apolipoprotein ε4	= (3) - (2)
	24S-hydroxycholesterol	+ (1) - (2)
	Cholesterol	+ (4) = (1) -(2)
	Lp(a)	+ (1) =(1)
	Homocysteine	+ (6) = (1)

+: Increased levels observed in AD patients compared to controls
=: No difference observed in levels of AD patients compared to controls
-: Decreased levels observed in AD patients compared to controls
#: Number of studies conducted

Appendix I: Summary of concentrations of oxidative stress markers in serum and CSF of AD patients

	Marker	AD vs. Normal
CSF	8-hydroxy-2'-deoxguanosine	+ (1)
	F2-isoprostanes	+ (6)
	F4-isoprostanes	+ (1)
	3-nitrotyrosine	+ (3)
	8,12-isoprostane-F2α-VI	+ (1)
Serum/plasma	8,12-isoprostane-F2α-VI	+ (1)
	F2-isoprostanes	= (3)
	4-hydroxynonenal	= (1)

+: Increased levels observed in AD patients compared to controls
=: No difference observed in levels of AD patients compared to controls
-: Decreased levels observed in AD patients compared to controls
#: Number of studies conducted

Appendix J: Summary of concentrations of vitamins in serum and CSF of AD patients

	Marker	AD vs. Normal
CSF	Vitamin A	= (1)
	Vitamin C	= (1)
	Vitamin E	- (1) = (1)
Serum/plasma	Vitamin B6	- (1)
	Vitamin B12	- (1) = (1)
	Folic acid	- (2)
	Homocysteine	- (1)
	Vitamin A	- (3)
	Vitamin C	- (4) = (1)
	Vitamin E	- (6) = (1)

+: Increased levels observed in AD patients compared to controls
=: No difference observed in levels of AD patients compared to controls
-: Decreased levels observed in AD patients compared to controls
#: Number of studies conducted

Appendix K: Summary of inflammatory proteins in serum and CSF of AD patients

	Marker	AD vs. Normal
CSF	IL-6	+ (2) = (8) - (1)
	IL-6 receptor	= (1) - (2)
	Glycoprotein 130	= (1) - (1)
	IL-1β	+ (2) = (6)
	IL-1β receptor II	+ (1)
	TNF-α	+ (1) = (3)
	TNF-α receptors	= (3)
	α-ACT	+ (3) = (2)
	Haptoglobin	= (1)
	Haptoglobin fragments	+ (2)
	Lithostathine	+ (1)
	Proteinase kallikrein	+ (1)
	Mannan-binding lectin	- (1)
	Cleavage products of kininogen	- (1)
Serum/plasma	IL-6	+ (5) = (4)
	IL-6 receptor	- (1)
	IL-1β	+ (1) = (5)
	TNF-α	+ (3) = (4) - (1)
	α-ACT	+ (5) = (2)
	Haptoglobin	= (2)
	Haptoglobin 2-1	+ (1) = (4)
	Proteinase kallikrein	+ (1)
	Histamine	+ (1)
	Anti-glial fibrillary acidic protein	+ (1)
	Anti-nerve growth factor	+ (1)
	Anti-histone	+ (1)
	Anti-double stranded DNA	+ (1)
	Mannan-binding lectin	= (1)

+: Increased levels observed in AD patients compared to controls
=: No difference observed in levels of AD patients compared to controls
-: Decreased levels observed in AD patients compared to controls
#: Number of studies conducted

Appendix L: Overview of studies that assessed the diagnostic value of resting state fMRI (rs-fMRI) candidate biomarkers

Study	fMRI candidate biomarker	Patients and subjects	Diagnostic value
Li et al. (2002)	rs-fMRI (COSLOF-Index)	AD (*N* = 14) vs. HC (*N* = 13)	Sensitivity: 80% Specificity: 90%
Greicius et al. (2004)	rs-fMRI- ICA components of the DMN	AD (*N* = 13) vs. HC (*N* = 13)	Sensitivity: 85% Specificity: 77%
Koch et al. (2010b)	rs-fMRI- Combined functional connectivity (ROI based) and coactivation magnitude (ICA-based)	AD (*N* = 15) vs. HC (*N* = 21)	Sensitivity: 100% Specificity: 95%
Chen et al. (2011)	rs-fMRI- ROI-based pairwise product moment coefficients	AD (*N* = 20) vs. Non-AD group (=pooled aMCI and HC (*N* = 35))	Sensitivity: 85% Specificity: 80%
Supekar et al. (2008)	rs-fMRI- Small world network properties (clustering coefficient)	AD (*N* = 21) vs. HC (*N* = 18)	Sensitivity: 72% Specificity: 78%

Appendix M: Summary of combined MRI and CSF studies in AD

Study	Subjects	Diagnostic measures	Associations
Schönknecht *et al.*, 2003	88 AD, 17 CN		In AD, CSF tau was not correlated to MRI.
Wahlund and Blennow, 2003	23 MCI, 24 AD		At baseline, CSF $A\beta_{1-42}$ was correlated with MRI. During the follow-up period, increases in tau and p-tau correlated with ventricular increase.
de Leon *et al.*, 2004	32 stable CN, 13 CN progressed to MCI	Accuracy for prediction of CN progression to MCI: Baseline: MRI: 78%; CSF: 78% to 89%.	Hippocampal volume decrease correlates with P-tau_{231} increase and $A\beta_{1-42}$ decrease.
Hampel *et al.*, 2005	22 AD		CSF p-tau_{231} correlated with baseline hippocampus and rates of hippocampal atrophy.
Schoonenboom *et al.*, 2005	39 MCI		CSF $A\beta_{1-42}$ was correlated with MRI and not tau.
de Leon *et al.*, 2006	9 CN, 7 MCI	Accuracy for separation of CN and MCI: Baseline: MRI: 94%, CSF: 63% to 88%; MRI + CSF: 94% Longitudinal: MRI: 88%; CSF: 73% to 88%; MRI + CSF: 94%	In MCI, longitudinal hippocampal volume decrease correlated with P-tau_{231} increase and $A\beta_{1-42}$ decrease.
Herukka *et al.*, 2008	21 MCI, of whom 8 progressed to AD		In all MCI, increases in tau and p-tau correlated with a decrease in hippocampal volumes.
Schoonenboom *et al.*, 2008	32 CN, 61 AD	Odds ratio between AD and CN: MRI: 28; CSF: 57	There were no correlations between visual assessment of MRI and CSF biomarkers within CN and AD.
Sluimer *et al.*, 2008	23 CN, 9 MCI, 47 AD		In AD, CSF p-tau_{181} had mild association with whole-brain atrophy rate. Only MRI was associated with change in cognitive measures.
Brys *et al.*, 2009	21 CN, 16 stable MCI, 8 MCI progressed to AD	Accuracy for prediction of MCI progression to AD: MRI: 74%; CSF: 70%; MRI + CSF: 84%	There were no longitudinal correlations between MRI and CSF.
Chou *et al.*, 2009	80 CN, 80 MCI, 80 AD (ADNI)		CSF $A\beta_{1-42}$ was correlated with ventricular expansion.
Fagan *et al.*, 2009	69 CN, 29 mild AD		In CN, decrease in CSF $A\beta_{1-42}$ correlated with brain atrophy. In mild AD, increases in CSF t-tau and p-tau_{181} correlated with brain atrophy.
Henneman *et al.*, 2009	19 CN, 25 MCI, 31 AD		Baseline CSF p-tau_{181} was independently associated with subsequent disease progression, measured by hippocampal atrophy rate.
Leow *et al.*, 2009	40 CN, 40 MCI, 20 AD (ADNI)		Baseline CSF correlated with temporal atrophy rates over the course of 12 months.
Schuff *et al.*, 2009	112 CN, 226 MCI, 96 AD (ADNI)		In MCI, an increase in rates of hippocampal atrophy correlated with lower CSF $A\beta_{1-42}$.
Thomann *et al.*, 2009	15 CN, 23 MCI (AACD), 16 AD		Increases in CSF t-tau and p-tau_{181} correlated with cortical atrophy in temporal, parietal, and frontal regions.
Vemuri *et al.*, 2009	109 CN, 192 aMCI, 98 AD (ADNI)	AUROC separating CN, aMCI, and AD: MRI: 0.77; CSF: 0.68 to 0.73; MRI + CSF: 0.81	Within each clinical group, only MRI correlated with cognition in aMCI and AD groups.
Vemuri *et al.*, 2009	109 CN, 192 aMCI, 98 AD (ADNI)	Proportional hazards for predicting time to conversion from aMCI to AD: MRI: 2.6;	Baseline MRI was a better predictor of subsequent cognitive and functional decline than baseline CSF

		CSF: 1.7 to 2.0	was.
Vemuri *et al*., 2010	92 CN, 149 MCI, 71 AD (ADNI)	Sample size required to detect treatment effects in AD: MRI: 100; CSF >10^5.	Longitudinal annual changes were observed only in MRI and not in CSF. Change in MRI was associated with change in cognitive measures.
Walhovd *et al*., 2010	42 CN, 73 MCI, 38 AD (ADNI)	Accuracy for baseline separation of CN and AD: MRI: 85%; CSF: 81.2%; CSF + MRI: 88.8%	In MCIs, only baseline MRI and FDG were correlated to (or predictive of) future clinical decline during 2 years.
Fjell *et al*., 2010	71 CN		Below a certain threshold, baseline CSF $A\beta_{1-42}$correlated with ventricular increase and volumetric brain decrease over the course of 1 year.
Fjell *et al*., 2010	Baseline: 105 CN, 175 MCI, 90 AD (ADNI)		In MCI and AD, baseline CSF measures were not related to baseline MRI but were related to longitudinal atrophy. Baseline MRI predicted change in cognition better than CSF did.

Appendix N: Summary of combined MRI and FDG studies in AD

Study	Subjects	Diagnostic measures	Associations
Yamaguchi *et al.*, 1997	13 AD, 13 CN		Hippocampal volume and mean cortical cerebral glucose metabolic rates of the temporal lobe, temporo-parieto-occipital, and frontal regions were correlated.
De Santi *et al.*, 2001	11 CN, 15 MCI, 12 AD	Accuracy for separation of MCI and CN: MRI: 73%; FDG: 73% to 85% AD and CN: MRI: 83%; FDG: 100%	FDG and MRI measures in hippocampal formation best characterize MCI, and additional neocortical damage best characterizes AD.
Ishii *et al.*, 2005	30 CN, 30 very mild AD		VBM: decrease in MRI in medial temporal lobes and decrease in FDG in posterior cingulate and parietal lobule
Kawachi *et al.*, 2006	60 CN, 30 very mild AD, 32 mild AD	Accuracy for separating very mild AD and CN: FDG: 89%; MRI: 83%; MRI + FDG: 94%	VBM: decrease in MRI in bilateral amygdala/hippocampus complex and decrease in FDG in bilateral posterior cingulate and parietotemporal area
Mosconi *et al.*, 2006	7 CN, 7 asymptomatic at-risk FAD	Accuracy for separation of both groups: MRI: 43% to 86%; FDG: 50% to 100%	FDG showed significant decrease but little sMRI change in asymptomatic subjects.
Ishii *et al.*, 2007	20 very mild AD, 20 DLB, 20 CN	Accuracy for separation of DLB and AD: MRI: 62% to 80%; FDG: 66% to 87%	Both MRI and FDG had a hippocampal decrease due to AD.
Matsunari *et al.*, 2007	Group 1: 40 CN, 27 AD Group 2 (early- and late-onset): 50 CN, 34 AD	Accuracy for different comparisons: MRI: 74% to 92%; FDG: 92% to 100%	VBM: decrease in MRI in hippocampal complex and decrease in FDG in posterior cingulate and parietotemporal area
Samuraki *et al.*, 2007	73 CN, 39 AD		VBM: FDG uptake was preserved in the medial temporal lobe before as well as after correction with MRI.
Chetelat *et al.*, 2008	15 CN, 18 mild AD		FDG hypometabolism exceeds MRI atrophy in the posterior cingulate-precuneus, orbitofrontal, inferior temporo-parietal, parahippocampal, angular, and fusiform areas. Similar degrees of atrophy and hypometabolism were observed in the hippocampus.
Hinrichs *et al.*, 2009	CN and AD subjects from ADNI: MRI: 183, FDG: 149	AUROC for discrimination of AD and CN: MRI: 0.88; FDG: 0.87	
Walhovd *et al.*, 2009	22 CN, 44 MCI		MRI predicted diagnostic groups for most regions of interest, but PET did not, except a trend for the precuneus metabolism.
Yuan *et al.*, 2009	Meta-analysis of 24 MCI studies (1112 subjects)	Odds ratio of predicting MCI conversion to AD: MRI: 10.6; FDG: 40.1	FDG was better than MRI in predicting conversion of MCI to AD.
Morbelli *et al.*, 2010	12 CN, 11 stable MCI, 9 MCI who progressed to AD		MCI converters showed MRI changes in left parahippocampus and both thalami, whereas FDG showed MRI changes in left PCC, precuneus, superior parietal lobule.
Walhovd *et al.*, 2010	42 CN, 73 MCI, 38 AD (ADNI)	Accuracy for baseline separation of AD and CN: MRI: 85%; FDG: 82.5%	MRI and FDG were largely overlapping in value for discrimination.

Appendix O: Summary of combined MRI and PIB studies in AD

Study	Subjects	Associations
Archer *et al.*, 2006	9 AD	Positive correlation between rates of whole-brain atrophy and regional PIB uptake
Jack *et al.*, 2008	20 CN, 17 MCI, 8 AD	[a]Proportional odds to separate all groups: PIB: 0.75; MRI: 0.84; MRI + PIB: 0.86. Global PIB and MRI were correlated with each other as well as with clinical measures.
Jack *et al.*, 2009	21 CN, 32 MCI, 8 AD	Longitudinal annual change was observed only in MRI and not in PIB. Change in MRI was associated with change in cognitive measures.
Mormino *et al.*, 2009	37 CN, 39 PIB + MCI	PIB and MRI were correlated with each other as well as with episodic memory.
Scheinin *et al.*, 2009	13 CN, 14 AD	During 2 years, only longitudinal MRI change was observed but not in PIB.
Strorandt *et al.*, 2009	135 CN	PIB was associated with cross-sectional brain atrophy and longitudinal cognitive decline.
Bourgeat *et al.*, 2010	92 CN, 32 MCI, 35 AD	In CN, PIB retention in the inferior temporal region and hippocampal volume were strongly correlated.
Chetalat *et al.*, 2010	94 CN (49 subjective cognitive impairment), 34 MCI, 35 AD	Global atrophy and regional atrophy were strongly related to PIB load in CN subjects with subjective cognitive impairment but not MCI and AD.
Driscoll *et al.*, 2010	57 CN	In CN, current PIB load was not related to longitudinal MRI changes in the preceding years.

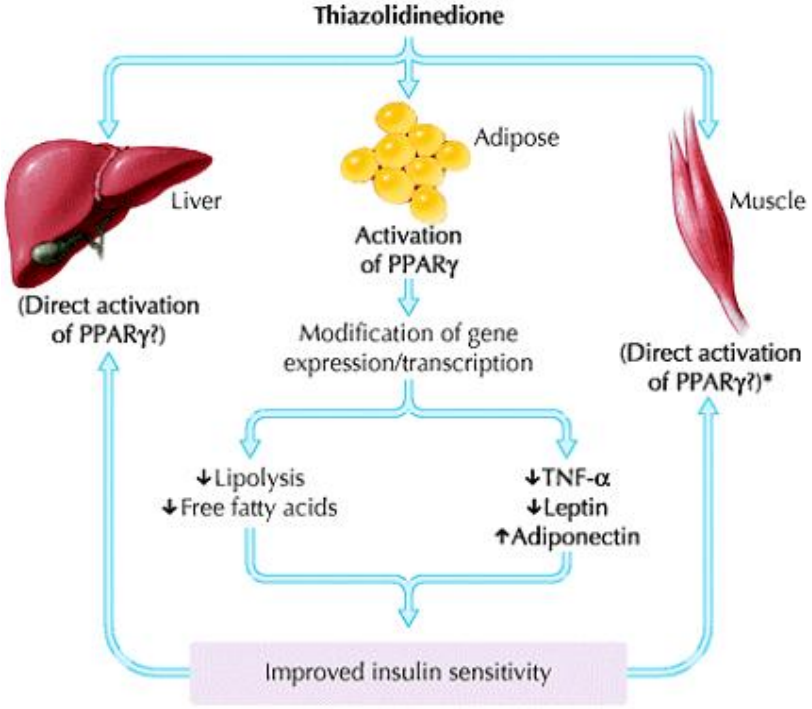
Appendix P: Weighted summary of sensitivity and specificity for each modality

Modality	Sensitivity	Specificity
FDG-PET	88.8%	84.9%
SPECT	83.8%	70.4%
MRI	72.8%	81.0%

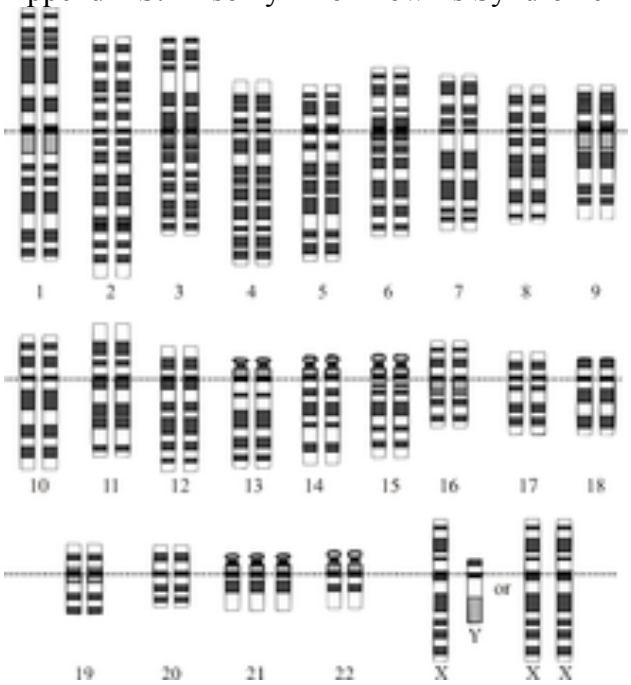
Appendix Q: Table Outlining Several Risk Factors for AD and T2DM

Factors	Alzheimer's disease	Parkinson's disease	Diabetes mellitus type II	Depression
Gender	Female	Male	Both	Female
Age	Ageing	Ageing	Ageing (nowadays also adolescent)	Midlife (also adolescent)
Hereditary risk factors	Familial (APP, presenilin-1, presenilin-2)	Familial (LRRK2, PARK2, PARK7, PINK1, SNCA)	Familial (HNF4A, GCK, INSR)	Familial (especially at younger age)
	APO E ε4	APO E ε4	ABCC8	? APO E ε4
			CAPN10	
			GCGR	
			PPARγ	
			APO E ε4	
Environmental risk factors	High dietary saturated fat and cholesterol	High dietary saturated fat and cholesterol	High dietary saturated fat and cholesterol	Low level of social activity
	Low level of social activity	Pesticide	Low level of social activity	? Smoking
	Smoking	Smoking (protective)	Smoking	? Alcohol
	Alcohol	Alcohol	Alcohol	Drug abuse
				Early-life events
Mental risk factors	Depression	Depression	Depression	Anxiety
	Anxiety	Anxiety	Anxiety	Dementia
	Chronic stress	Dementia	Dementia	Chronic stress
Metabolic risk factors	Hypertension	? Hypertension	Hypertension	Adiposity
	Diabetes mellitus	? Diabetes mellitus	Adiposity	Coronary artery disease
	Coronary artery disease	? Coronary artery disease	Insulin resistance	
	Hyperhomocysteinemia	Hyperhomocysteinemia	Coronary artery disease	
	Stroke	? High cholesterol	High cholesterol	
	High cholesterol			
Others	Inflammation	Inflammation		
	Head trauma	Head trauma		

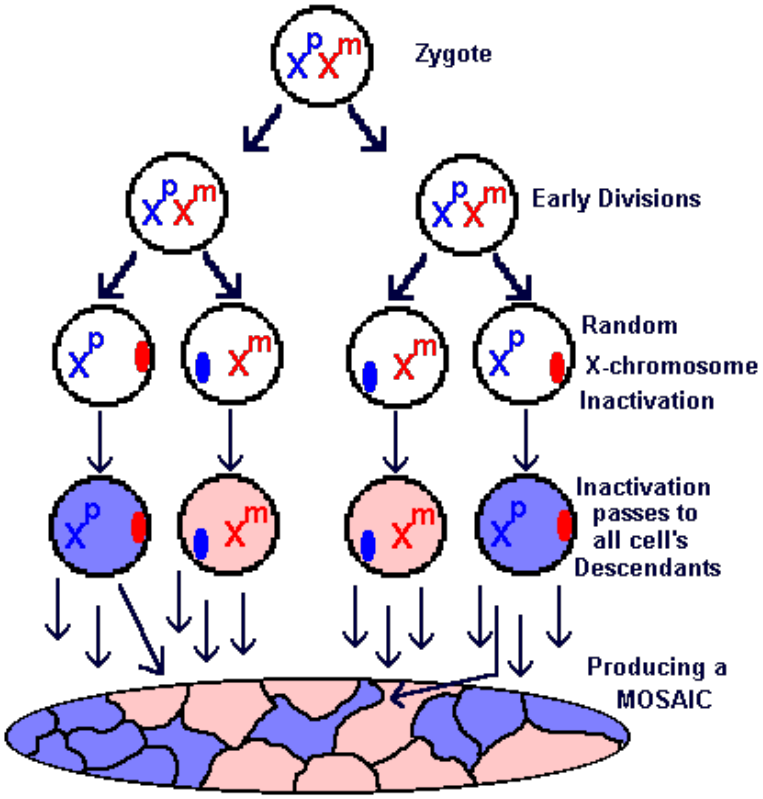
Appendix R: Pathway to Show TZD Mechanism



Appendix S: Trisomy 21 or Down's Syndrome



Appendix T: Trisomy 21 Mosaicism Model



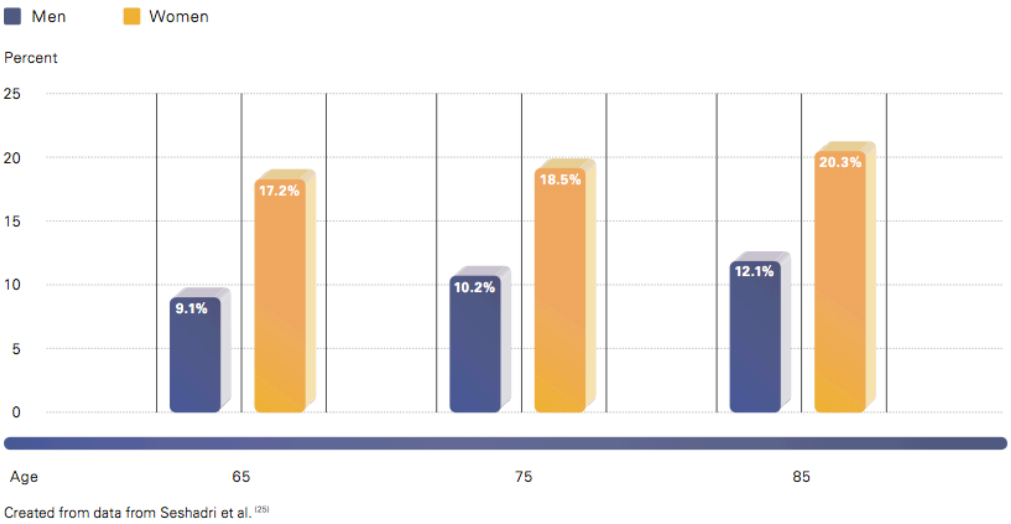
Appendix U: Demographic Distribution of Caregivers for AD Patients

Table 1. Sample Description (N = 1,715)

Group	Value
Caregivers	
Age, years, mean ± SD	59.73 ±12.66
Gender	
Male, %	23.1
Female, %	76.9
Race/ethnicity	
African-American, %	6.0
White, %	90.7
Other, %	3.3
Patients' relationship	
Spouse/significant other, %	42.4
Parent, %	42.1
Other, %	15.4
Where patient lives	
Alone, %	7.9
With caregiver, %	74.9
Other, %	17.2
Employment status	
Full-time, %	26.1
Part-time, %	10.9
Homemaker, %	17.4
Retired, %	34.8
Unemployed, %	3.9
Disabled, %	6.9
Health insurance, yes, %	91.0
Patients	
Age, years, mean ± SD	77.86 ± 8.33
Gender	
Male, %	37.9
Female, %	62.1
Race/ethnicity	
African-American, %	6.0
White, %	90.3
Other, %	3.8

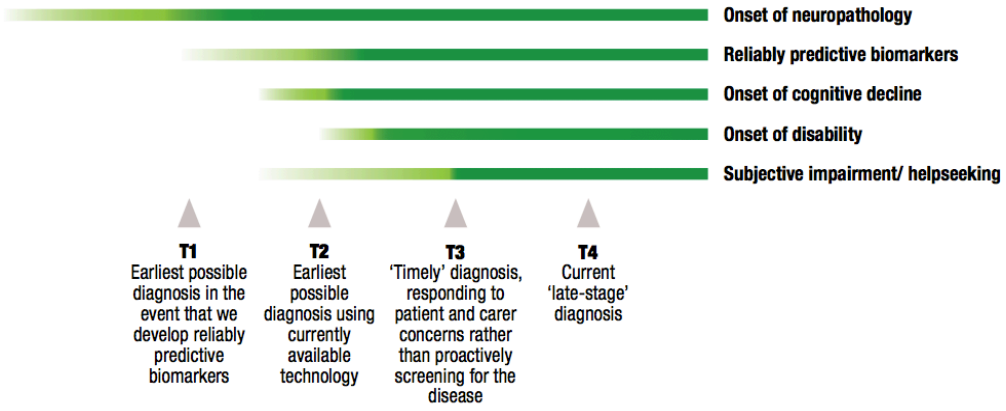
SD = standard deviation.

Appendix V: Risk of AD in Males vs. Females

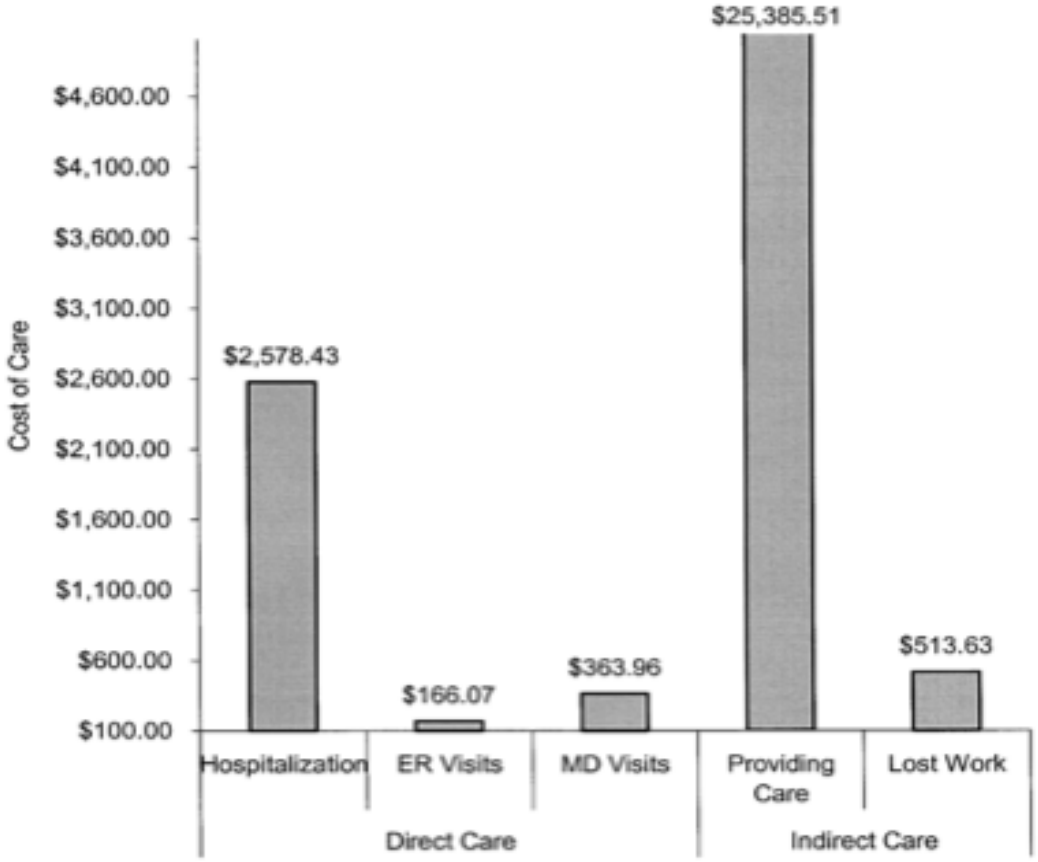


Created from data from Seshadri et al. [25]

Appendix W: Timeline of the Progression of AD



Appendix X: Cost Comparison of the Direct and Indirect Care of AD



Appendix Y: Cost vs. AD Severity in Early Detection