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Analysis of Diagnostic, Preventive, and Disease-Modifying Therapeutic Measures of Alzheimer's Disease

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HALMOS COLLEGE OF NATURAL SCIENCES AND OCEANOGRAPHY

ANALYSIS OF DIAGNOSTIC, PREVENTIVE, AND DISEASE-MODIFYING THERAPEUTIC MEASURES OF ALZHEIMER'S DISEASE

By

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Contents

Abstract

Alzheimer's disease (AD) is the most common late-onset neurodegenerative disorder and cause of dementia, characterized by the formation of neurofibrillary tangles and senile plaque deposits. The heterogeneous nature of the disease (both genetically and environmentally) makes it difficult to prevent or cure. Without prevention, the prevalence of AD is expected to triple by 2050. However, because the diagnosis of AD is usually preceded by years of cognitive impairment, early detection may aid in reducing prevalence. Thus, there is a need for validated diagnostic measures for early and improved diagnosis and prevention. In this review, current and ongoing classifiers of early detection and tools for monitoring disease progression are discussed. In this present analyses, the diagnostic value of the following tools were statistically analyzed between three cognitive levels (cognitively normal, MCI, and AD) within (Alzheimer's Disease Neuroimaging Initiative) ADNI databases: hippocampus volume, the Mini-Mental State Evaluation (MMSE) test, the Alzheimer's Disease Assessment Scale (ADAS-Cog), APOE-ε4 genotype screening, and CSF biomarkers (including AB-42, P-tau, and T-tau). Hippocampal volumes were significantly different between cognitive groups over a 24-month period. These volumetric differences correlated to cognitive test scores, with ADAS-Cog 13 being more sensitive to time changes than the MMSE. APOE-ε4 genotype had only significant effects on hippocampal volume within MCI subjects, suggesting that the possession of the APOE-ε4 allele may have an effect on disease conversion. Of the biomarkers, $A\beta_{42}$ yielded the highest sensitivity (84.09%) and negative predictive value (88.14%). $A\beta_{42}$/P-tau demonstrated the highest specificity (95.45%) and positive predictive value (91.18%). The combination of the several validated diagnostic tools (including hippocampal atrophy, cognitive screening tests, genotype, and CSF biomarkers) may increase the diagnostic accuracy of AD, possibly leading to improved diagnosis and reduction of AD prevalence.

Keywords: Alzheimer's Disease, Alzheimer's Disease Neuroimaging Initiative, Hippocampal Atrophy, Neuropsychological Test, APOE-ε4, Cerebrospinal Fluid (CSF) Biomarkers

I. Introduction

Alzheimer's disease (AD), the most common late-onset neurodegenerative disorder and cause of dementia, is a devastating and incurable condition affecting an estimated 5.4 million Americans (Alzheimer's Association, 2016). AD is characterized by increased formation of plaques and neurofibrillary tangles, causing selective damage of brain regions for cognition and memory. This damage leads to progressive memory loss, impairment of cognitive functions, and changes in behavior and personality. People in the late stages of AD require assistance with all aspects of personal care and are often placed in nursing homes (Sloane et al., 2002). Memory loss is generally followed by death within 7-10 years after diagnosis (Hu et al., 2007).

Alzheimer's disease starts in the hippocampus, deep within the temporal lobe of the brain, responsible for forming memories and for spatial navigation. From the hippocampus, the disease then spreads to the temporal lobes, impairing language encoding and visual input, before it continues to spread through the brain. The cerebral cortex begins to thin as the brain gradually shrinks, and older memories are lost (Alzheimer's Association, 2016).

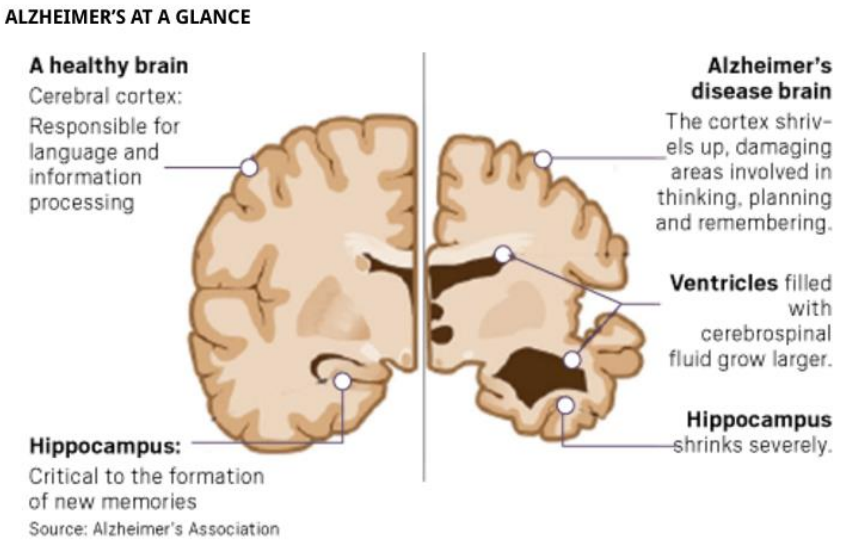

Figure 1. **Alzheimer's at a glance (Alzheimer's Association, 2016).** Image of a crosswise section comparing brain characteristics of Alzheimer's disease brain to a healthy brain.

Despite this disease being the most well studied cause of dementia, there is no current cure. In the absence of prevention, the number of individuals with AD is predicted to triple by 2050 due to aging of the population. In fact, since 2000, the prevalence of AD has risen by 89% (Alzheimer's Association, 2016). Additionally, the cost of caring for AD patients in the United States is roughly $259 billion annually and continues to rise with the increasing number of diagnosed persons, causing major strains on the national economy (Atri et al., 2008).

Decreasing AD prevalence necessitates early diagnosis strategies, exclusion of misdiagnosed patients, targeted treatment of symptoms, and increased awareness of preventative approaches. Current screening methods include neuropsychological tests, neuroimaging tools, and biomarkers. Presently, the treatment of AD includes to provision of a supportive environment, symptomatic drugs, and nonpharmacological therapies. Although there is evidence that these approaches may retard disease progression and ameliorate concurrent symptoms of AD, the prevalence of AD remains on the rise. Thus, methods of prevention, early detection, and neuroprotection/reversal are of grave necessity.

This literary review will discuss the pathology, genetic and environmental risk factors, diagnosis methods, and treatment advances of AD with the aim to examine the scope of AD prevention. This review also incorporates a statistical analysis of the diagnostic value of current neuroimaging, genotype, biomarkers, and cognitive testing data within the Alzheimer's Disease Neuroimaging Initiative (ADNI) database. ADNI is a longitudinal study conducted by many researchers aiming to reduce the prevalence of AD via effective early detection methods and the development of targeted treatments. Statistical data from three ADNI cohorts, comprised of a total of 819 subjects of various levels of cognitive impairment, has been analyzed to determine whether current imaging, biomarker, and cognitive testing methods effectively measure AD progression.

II. Alzheimer's Disease Background

Pathogenesis and Disease Models

Pathologically, AD is characterized with dysfunction and degeneration of mainly cholinergic neural circuits, leading to progressive loss of memory, resulting in dementia and eventually death (Ribotta, 2001). The progression of the disease is described in two stages: mild cognitive impairment (MCI) and AD. MCI represents the preclinical stage of AD. Although clinical diagnosis of MCI is not a necessary precursor to dementia, it serves as a risk factor of dementia progression (Health Quality Ontario, 2014). Patients with MCI have an increased risk of developing dementia. About one-third of patients diagnosed with MCI develop dementia within 5 years. Studies have shown that around 80% of progressed MCI patients convert to AD after 6 years of follow-up (Lopez, 2013).

There are three phases of AD, with each stage of the disease progressively worsening as the dementia spreads throughout the brain (Figure 2). The medial temporal lobes (primarily hippocampus and entorhinal cortex) are typically the first location to demonstrate atrophy, and remain the most severely affected region as illness progresses. This atrophy continues to spread to the temporal and parietal association cortices, with very little left of the primary sensory and motor cortices by the late course of the disease (McGinnis, 2012). Early stage AD may present itself with loss of memory or recent events and impaired judgement. These changes become more marked in moderate stage AD, in which one may experience delusions and lose orientation of place or time. Within a decade of initial onset, late state AD presents itself with pronounced memory loss of familiar people and even basic abilities, including how to self-feed, speak, or urinate.

People in the late stage of AD require assistance with all aspects of personal care and are often placed in nursing homes (Sloane et al., 2002).

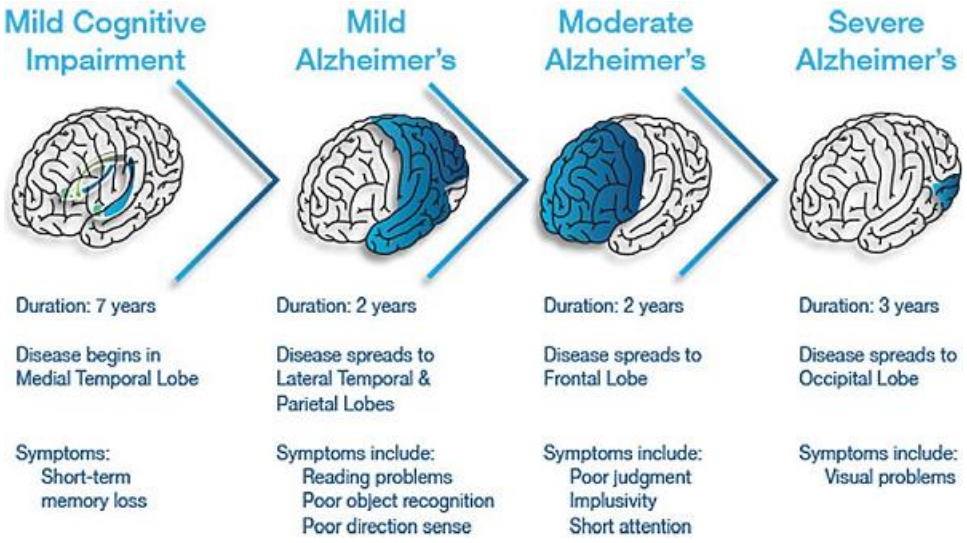

***Figure 2*. Stages of Alzheimer's Disease (Seniordirectory.com).** Stages of Alzheimer's Disease include mild cognitive impairment and Alzheimer's. Alzheimer's disease progresses in 3 stages: mild, moderate, and severe.

AD may be broken down into subcategories by age of onset and genetic heritability, as shown in Figure 3. Accounting for between 1-5% of all AD cases, EOAD occurs in individuals younger than 65 years of age. The remainder of AD cases affect individuals older than 65 years, known as late-onset AD (LOAD) (McGinnis, 2012). Furthermore, there are two genetic manifestations of AD: familial and sporadic. Though the progression of dementia in these subgroups are the outwardly the same, familial AD is caused by heritable genetic mutations while sporadic AD is due to an accumulation of age-related processes. The underlying cause of AD remains incompletely understood. However, a number of hypotheses aim to explain its origins.

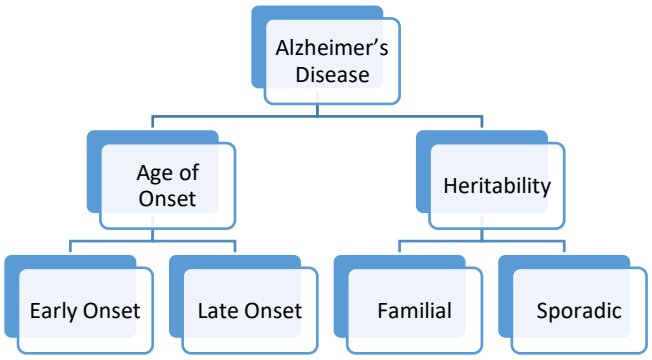

***Figure 3*. Subtypes of Alzheimer's Disease (AD).** Types of AD include early onset, late onset, familial, and sporadic. The disease is divided into categories based on age of onset and heritability.

The Amyloid Cascade Hypothesis

The most dominant explanation of AD pathogenesis is explained by the Amyloid Cascade Hypothesis, proposed by John Hardy and David Allsop in 1991. This hypothesis explains that neuronal death and cognitive impairment is attributed to build-up of β-amyloid (Aβ) plaques and neurofibrillary tangles (NFTs) (Figure 4). (Folch et al., 2016).

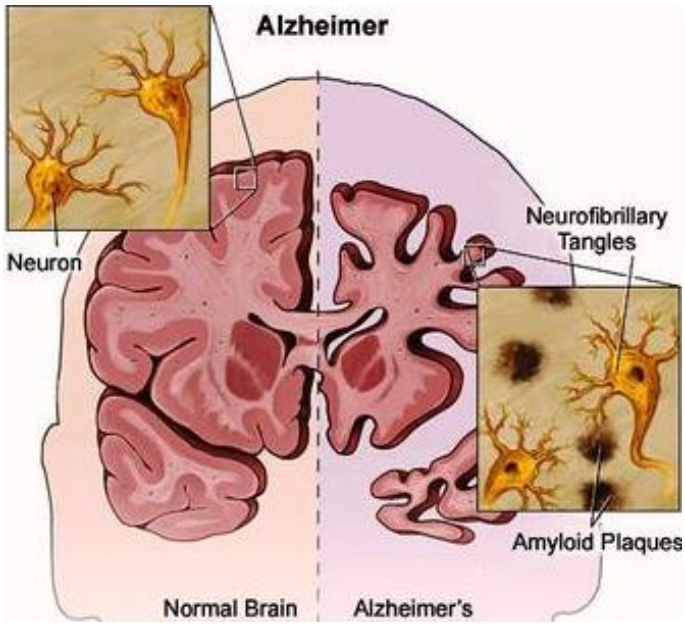

Figure 4. **Comparison of a normal brain and a brain affected by Alzheimer's Disease (Pescosolido et al, 2014).** Amyloid plaques (abnormal clusters of protein fragments) build up between nerve cells, whereas neurofibrillary tangles made up of Tau protein grow inside neurons.

Aβ is a 39-42 amino acid peptide that comes from neural cell membranes. Aβ exists in two isoforms (Aβ40 and Aβ42), derived through the cleavage of an amyloid precursor protein (APP) by β and γ secretases (Hu et al., 2007). The Aβ deposits, specifically $A\beta_{42}$, primarily cause neuronal dysfunction in the basal forebrain, cortex, hippocampus and amygdala (Ballard et al., 2011); (Winner et al., 2011). Small oligomers of $A\beta_{42}$ may be more toxic than mature fibrils because they can block cell-to-cell signaling. The processing of APP to Aβ amyloid plaques, resulting in neuronal death is depicted in Figure 5.

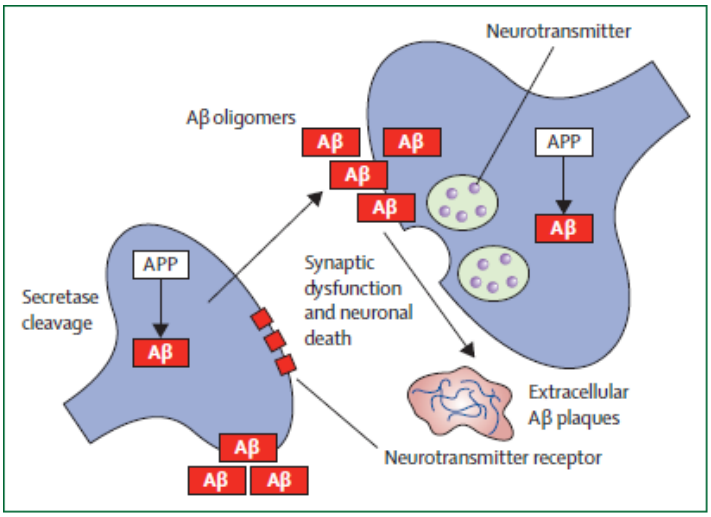

***Figure 5*. Amyloid Hypothesis (Ballard et al., 2011).** Aβ=amyloid β. APP=amyloid precursor protein. APP is processed into Aβ, which accumulates inside neuronal cells and extracellularly, where it aggregates into plaques.

Aβ deposits are noted to induce neuronal cell death, but the pathway of neurodegeneration is unclear. In normal metabolism, Aβ levels are regulated by proteases including neprilysin, insulin degrading enzyme, and angiotestin converting enzyme I (Folch et al., 2016). However, these Aβ-degrading enzymes may be imbalanced in subjects with AD, thereby reducing clearance from the brain. This imbalance may be due to genetic mutations or age-related oxidative stress of Aβ-degrading enzymes. Additionally, Aβ may accumulate in AD brains due to endosomal enlargement (Cataldo et al., 2014).

The Amyloid Cascade Hypothesis holds that toxic levels of Aβ may contribute to the formation of neurofibrillary tangles (NFTs) (Ballard et al., 2011). These NFTs are composed of hyperphosphorylated protein tau. Tau is a microtubule associated protein that stimulates tubulin to assemble microtubules in the brain, promoting neurite extension and stabilization. Tau normally exists in 6 different isomers, each created by alternative splicing of *tau* mRNA. All six isoforms exist in an adult human brain. However, altered proportions of tau isoforms are observed in patients with neurodegenerative diseases, including AD (Gong and Iqbal, 2008).

The overexpression of tau is due to hyperphosphorylation by various kinases, including CDK5, GSK3β, Fryn, stress-activated protein kinases JNK and p38, and mitogen-

activated kinases ERK1 and ERK2 (Folch et al., 2016). Comparisons of control and AD autopsies reveal phosphorylated tau 3-4 times more than in normal brains. Although tau is a soluble protein, abnormally hyperphosphorylated tau promotes its polymerization, causing insoluble aggregates. The abnormal tau monomers combine to form oligomers, which then combine into insoluble paired helical filaments which form insoluble neurofibrillary tangle (NFT) aggregates (Folch et al., 2016). These NFTs can then disrupt the structure and function of a neuron and spread to other parts of the brain (Ballard et al., 2011). Figure 6 depicts the disintegration of microtubules via tau hyperphosphorylation as result of AD.

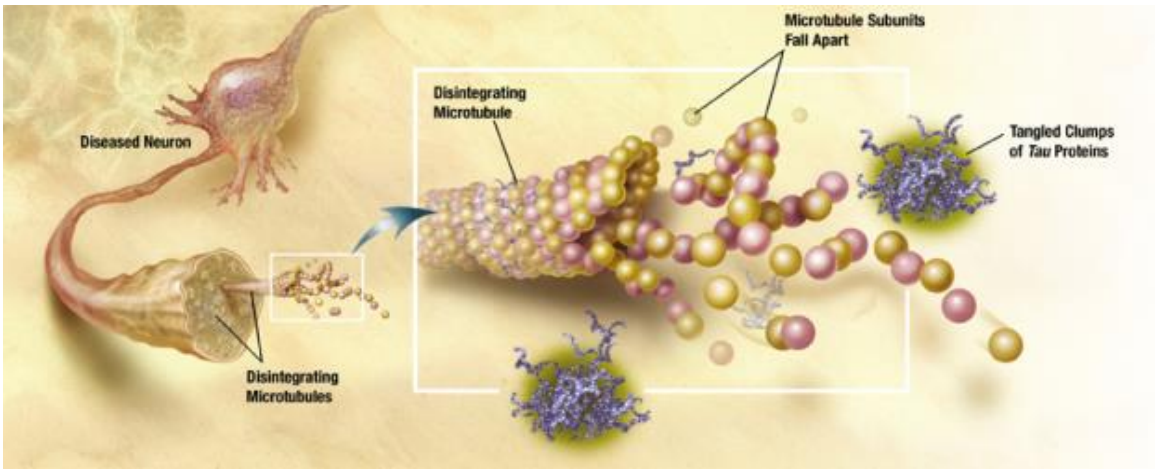

Figure 6. **Illustration of neurofibrillary tangles in Alzheimer's Disease (Cruchaga et al., 2010).** Tau hyperphosphorylation causes microtubule disintegration within neurons, leading to accumulation of neurofibrillary tangles (NFTs). When NFTs form, brain cells die and release tau.

Though the triggers for increase Aβ differ between familial and sporadic AD, increased levels contribute to both forms of the disease. Familial AD is generally caused by mutations in the APP processing genes. On the other hand, sporadic AD seems to act independently from APP gene mutations. Instead, sporadic AD appears to be caused by

the imbalance of Aβ production and clearance (Pohlkamp et al., 2017). Figure 7 below illustrates the multiple factors described by the Amyloid Cascade Hypothesis.

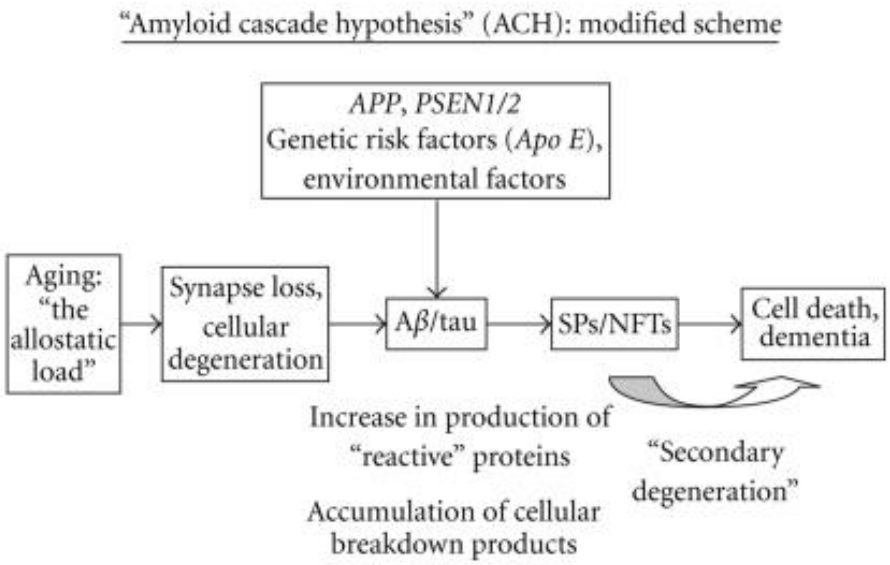

***Figure 7*. The Amyloid Cascade Hypothesis - Modified (Armstrong, 2011).** Updated version of the Amyloid Cascade Hypothesis where Aβ: β-amyloid, APOE: apolipoprotein E, APP: amyloid precursor protein, PSEN1/2: presenilin genes 1 and 2, NFTs: neurofibrillary tangles, and SPs: senile plaques.

The Neuroinflammatory Hypothesis

In addition to the Amyloid Cascade Hypothesis, recent data from preclinical and clinical studies suggest that the immune system plays a role in the pathogenesis of AD. The Neuroinflammatory Hypothesis is based on the premise that damage to the central nervous system (CNS) induces a chronic inflammatory reaction that leads to cell damage and neurodegenerative disease (Morales et al., 2014). Damage or injury to the CNS causes an acute inflammatory response in which glial cells, i.e., microglia and astrocytes, are activated. Although glial cells play a role in the removal of Aβ plaques, overactivation from constant damage signals may lead to neuroinflammation and neural death. In fact, marked astrogliosis (abnormal increase in astrocytes) has been found in post-mortem samples of AD affected brain samples.

The presence of the persisting damage may trigger an inflammatory condition in which feedback loops between neurons and glial cells lead to neurodegenerative disease. In individuals with AD, the presence of Aβ and NFTs produce high expression of pro-inflammatory cytokines, prostaglandins, and nitric oxide synthase, which contribute to a

state of perpetual stress and eventually neuronal death (Folch et al., 2016). Figure 8 below depicts the mechanism of the Neuroinflammatory Hypothesis.

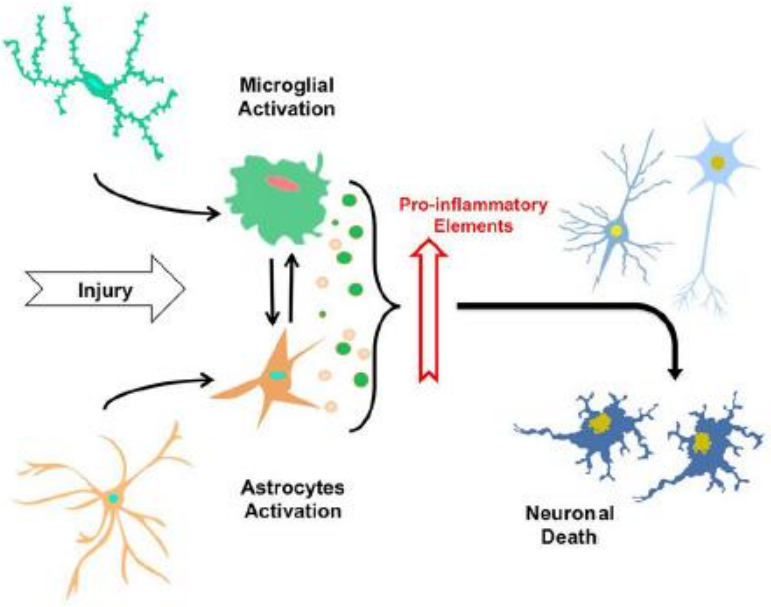

***Figure 8*. The Neuroinflammatory Hypothesis (Morales et al., 2014).** By sensing signals of damage or injury, astrocytes and microglia suffer a gradual activation process, leading to morphological changes and secretion of pro-inflammatory elements (i.e., cytokines, cytotoxic elements, ROS). The constant exposure of astrocytes and microglia eventually trigger neuronal death.

Several neurodegenerative disorders, including AD, have been associated with modification of glial cell activation in the brain, inducing a neuroinflammatory response and thus neuronal cell death. Tau aggregates have been shown to activate microglia, which release pro-inflammatory cytokines, causing a circuit of constant neuroinflammation. Table 1 below indicates the role of the specific cytokines involved in neurodegeneration and AD.

***Table 1*. Pro-inflammatory Elements in Neuroinflammation (Morales et al., 2014).**

Pro-Inflammatory elements	Effect
Chemokines	Dysfunction, apoptosis and necrosis of neuron, microglia and astrocytes
IL-1β, IL-6, IL-12 INF-γ, TNFα	Astrocytes and microglia activation, dysfunction, apoptosis and necrosis of neuron, microglia and astrocytes
NO, ROS, O_2^-	Oxidative stress in cells; dysfunction, apoptosis and necrosis of neuron, microglia and astrocytes

Pro-inflammatory elements secreted by astrocytes and microglia during the process of neuroinflammation.

Moreover, epidemiological studies have established a link between non-steroidal anti-inflammatories (NSAIDs) and reduced risk of AD (Morales et al., 2014). Anti-inflammatory treatments will be discussed further in *Treatment*.

Neurotransmitter Systems and AD

There are other pathologies associated with various neurotransmitter systems and the development of AD. AD often coexists with the presence of Lewy bodies (aggregations of protein alpha-synuclein) and cerebral amyloid angiopathy (CAA) (Aβ in the walls of small arterioles) (Ringman et al., 2016). Another component of AD pathology is its association with declines in various neurotransmitter systems. These include a reduction in acetylcholine production and an excess of glutamate activity (Casey et al., 2010). Additionally, the Golgi apparatus and related secretory pathways have also been established to play a role in the neuropathology of AD. It has been found that the size of the Golgi apparatus in the vertical limb of the diagonal band of Broca is more pronounced in patients with AD (Hu et al., 2007). This finding may be helpful for future efforts in the early diagnosis and prevention of AD.

The Blood Brain Barrier

In both aging and AD, the body's capacity to eliminate toxic compounds greatly decreases. The barriers of the brain, including the blood brain barrier (BBB) and blood cerebrospinal fluid barrier, are targets of the disease. In normal aging, permeability of the brain barriers increase, reducing the brain's ability to be rid of toxic metabolites. The BBB is constituted of various extracellular matrix proteins, forming the neurovascular unit (NVU), the functional unit of the BBB. The NVU's role is to control the homeostasis and microenvironment of the brain, complemented by transport systems such as ion channels, pumps, receptors, and transporters. The dysfunction of the NVU has been proposed to be causative in AD development, due to reduced Aβ clearance from the brain (ElAli and Rivest, 2013). The accumulation of Aβ peptides in the brain can result from

their increased production as well as a decrease in excretion through the brain barriers (Figure 9).

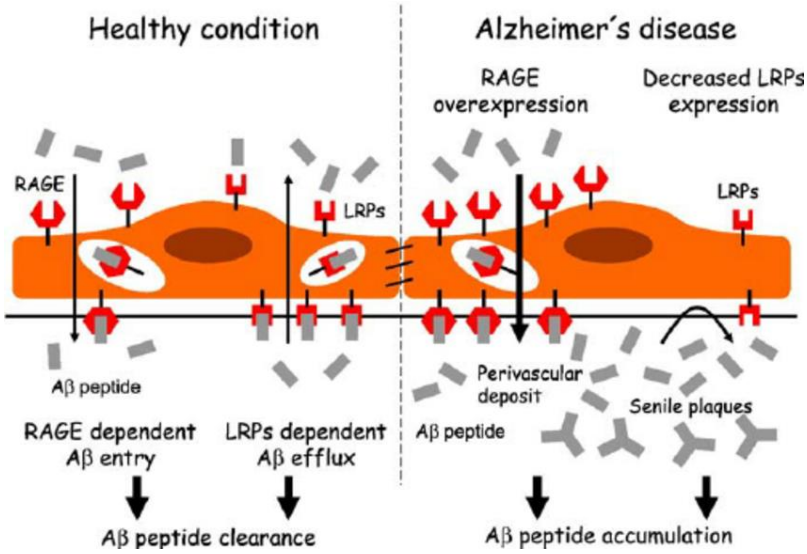

***Figure 9*. The blood-brain-barrier in Alzheimer disease (Weiss et al., 2009).** In healthy condition (right), Aβ amyloid peptide is transported to brain by the receptor for advanced glycosylation products (RAGE) and cleared from the brain to the blood by LDL-Receptor-Proteins (LRPs). In Alzheimer's disease (left), these transport systems are impaired. RAGE is overexpressed and the expression of LRPs is decreased, leading to the accumulation of Aβ in the brain.

Various transporter systems are responsible for the flux of A*β* through the BBB. Animal studies reveal that the receptor for advanced glycation end products (RAGE) seems to contribute to Aβ accumulation in aging. Additionally, the low-density lipoprotein receptor-related protein 2 (LRP-2) has been found to decrease with age, supporting decreased A*β* clearance from the brain (Marques et al., 2013).

Epidemiology

Modifiable Risk Factors

AD prevalence varies among a wide range of risk factors. There is sufficient evidence to suggest that some modifiable risk factors contribute to dementia in late life. Some of these risk factors are potentially modifiable, including cardiovascular risk factors, psychosocial risk factors, and health behaviors. A 2010 systematic review published by the US National Institute of Health highlighted risk factors most highly associated with increased risk of cognitive decline or AD. These factors included diabetes mellitus, present smoking, depression, cognitive inactivity, physical inactivity, and poor diet (Barnes and Yaffe, 2011). Smoking, hypertension, and diabetes between the ages of 40-

44 years is associated with a 20-40% increased risk of dementia in old age (Kotze and van Rensburg, 2012). Patients with Aβ plaques have been found to have higher lipid measurements, including cholesterol and triglycerides, suggesting the association of abnormal lipid metabolism and AD pathology. Interestingly, possession of the APOE-ε4 gene, a risk factor for developing LOAD, increases the risk of coronary heart disease by 40% (Kotze and van Rensburg, 2012). Thus, there is evidence of overlap between modifiable risk factors and the occurrence of AD.

In 2011, Barnes and Yaffe published a review aiming to summarize the evidence related to each of these risk factors and the projected effect of risk factor reduction on the prevalence of AD. Using systematic reviews and meta-analysis, population attributable risk (PAR) was calculated for each risk factor, both worldwide and within the US. PAR is the proportion of people in a population the disease that can be attributed to a given risk factor. PAR is calculated using the Levin formula:

$$PAR = \frac{P_{RF} \times (RR - 1)}{1 + P_{RF} \times (RR - 1)}$$

Where P_{RF} is the population prevalence for the risk factor and RR is the relative risk. Their findings suggest that half of AD cases worldwide were potentially attributable to modifiable risk factors. However, the Levin formula does have limitations. One limitation is that PAR estimates are calculated by assuming there *is* a causal relationship between each risk factor and the disease prevalence. It is important to remember that each of the risk factors summarized are potential contributors of the disease.

According to the statistical analysis conducted by Barnes and Yaffe, low education contributed to the largest proportion of AD worldwide. Several retrospective studies have established declining dementia prevalence in specific cohorts since 1970 due to improvements in educational attainment (Baumgart et al., 2015). This was followed by smoking. The third largest risk factor was physical inactivity. Depression is the fourth most significant risk factor. Lastly midlife cardiovascular risk factors, including obesity, hypertension, and diabetes, contribute to substantial AD cases worldwide, based on the Levin formula (Barnes and Yaffe, 2011).

Follow-up studies by other researchers have shown that treatment of hypertension may decrease the risk of AD by 50%. Moreover, a recent meta-analysis demonstrated that individuals with MCI and diabetes were more likely to progress to AD than those without diabetes (Baumgart et al., 2015). Figure 10 below summarizes the strength of risk factor evidence on AD, according to the National Institute of Health.

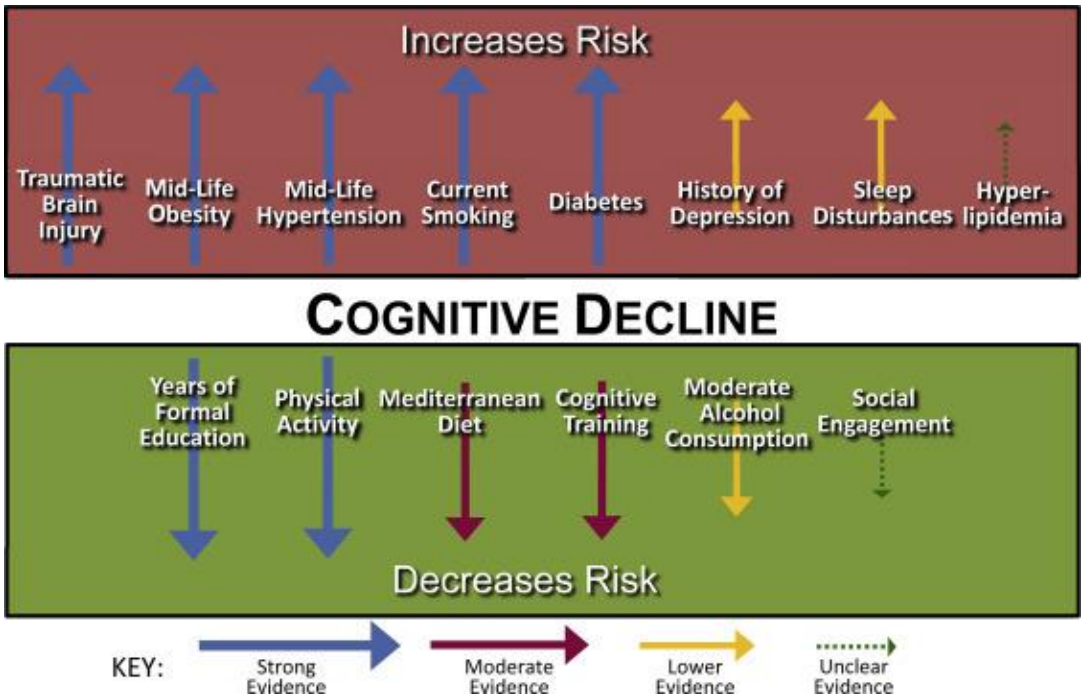

***Figure 10*. (Baumgart et al., 2015).** Strength of evidence on risk factors for cognitive decline calculated using the Levin formula**.**

Thus targeting modifiable risk factors may aid in the prevention of AD. According to the PAR study, a 10-25% reduction in all seven risk factors could prevent up to 3 million cases worldwide every year. The most effective strategy to reduce the risk of developing AD may require addressing multiple risk factors simultaneously. This multivariate approach was tested by the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER), which resulted in overall improvement of cognitive performance and executive functions (Baumgart et al., 2015). Likewise, the Alzheimer's Association believes there is sufficient evidence to conclude that regular physical activity and management of cardiovascular risk factors, as well as a healthy diet and cognitive training, may reduce the risk of dementia and AD in late life (Alzheimer's Association, 2016).

Genetic Epidemiology

Although environmental factors play a role in the pathophysiology of the disease, about 70% of AD risk is attributed to genetics (Ballard et al., 2011). The heritability, or proportion of phenotypic variance contributed by genetic factors, of AD is remarkably heterogeneous and complex. Although familial AD is characterized by classic Mendelian inheritance, loci for sporadic late-onset AD are difficult to identify due a complex pattern of inheritance (Figure 11).

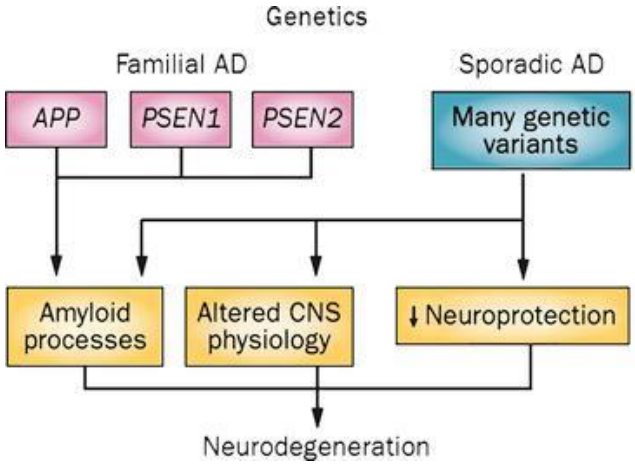

Figure 11. **Genetic components of Alzheimer's Disease (AD) (Simone et al., 2015).** Familial AD is caused by mutations of APP (amyloid processing gene), PSEN1 (presenilin 1) and PSEN2 (presenilin 2). Sporadic AD is caused by many genetic variants.

The rare, familial form of AD is well understood, known to be inherited as rare autosomal-dominant mutations in three genes related to amyloid processing: *APP*, *PSEN1*, and *PSEN2* (Lord et al., 2014). Specific point mutations of *APP* are noted in rare examples of autosomal dominant early-onset familial AD (Schmechel et al., 1993). While these genes are located on three different chromosomes, they share a common biochemical pathway; the altered production of Aβ leading to an overabundance of Aβ_{42}, eventually leading to neuronal cell death and dementia (Betram and Tanzi, 2005). More than 160 mutations occur in these three genes have been reported to cause early onset AD. Although these autosomal dominant chromosomal mutations account for less than 5% of total AD patients, inheritance of any of these mutations results in complete penetrance (Ballard et al., 2011); (Sloane et al., 2002). Moreover, those inheriting a mutation of the *PSEN2* have a 95% chance of developing the disease. To date, autosomal dominant AD mutations can be seen in Table 2 below.

Table 2. **Autosomal dominant AD mutations (Ringman et al., 2016).**

PSEN1 **(n = 46)**		
A79V (5 cases)	I143T (2 cases)	M233L (2 cases)
Y115C (2 cases)	M146L (2 cases)	L235V
Y115H	Y156insFI	T245P (2 cases)
E120D	H163R (4 cases)	V261F
N135D	S170F	P267A[a]
N135S (2 cases)	G206A (6 cases)	A431E (6 cases)
M139I	G209V	L435F
M139V	L226R	
APP **(n = 10)**		
E693G	V717I (3 cases)	
V717F (4 cases)	V717L (2 cases)	
PSEN2 **(n = 4)**		
N141I (4 cases)		

[a]Novel *PSEN1* mutation.

52 cases from the National Alzheimer's Coordinating Center were included for whom autosomal dominant AD mutations were verified.

However, the majority of patients (99%) acquire sporadic late-onset AD (LOAD), at age 65 or older, for which the genetic components much more complex and less understood. To identify these variants, a variety of methods have been used. These include genome-wide linkage studies (GWLS), gene association studies, meta-analysis of linkage and gene association studies, GWAS, and whole genome/exome sequencing (Naj et al., 2016). Hundreds of genes have been tested for LOAD association. Out of these variants, few have been replicated and only one has gained wide acceptance, the ε4 allele of the apolipoprotein E gene (*APOE-ε4*), with Bayes Factor >50.

The *APOE* gene is associated with the reduced ability to clear Aβ from the brain by binding to Aβ. *APOE* contains three allelic variants (*ε*2, *ε*3, and *ε*4), encoding for different isoforms (*ApoE2, ApoE3, and ApoE4*) (Cauwenberghe et al., 2016). In comparison to individuals with *APOE* genotype ε3/ ε3, those with a single copy of the ε4 allele have 5-fold risk of developing LOAD. Moreover, those with two copies have an estimated 20 fold increased risk (Simic et al., 2016). However, although *APOE-ε*4 is a risk factor for AD, it is not necessary or sufficient to cause the disease. Roughly only 27% of individuals with LOAD have this genotype. Up to 50% of individuals with AD do not carry the *APOE-ε*4 allele (Cauwenberghe et al., 2016). Thus, a large portion of AD heritability remains unexplained and remains a driving force for many years of research (Lord et al., 2014). Although *APOE-ε4* has been found to be the most well established

risk factor for late-onset AD, other genes have been found to be associated with the late-onset AD phenotype. GWAS studies have laid the foundation for meta-analysis studies that have revealed up to 20 LOAD variants.

Currently, the top nine most highly-associated susceptibility genes for developing LOAD, after *APOE*, are listed on Alzgene.org. This can be viewed in Table 3 below. This ranking is based on meta-analysis results using *P*-value and Bayes factor (BF) to determine association with LOAD development. Ranked from highly associated to least associated LOAD susceptibility factor is *BIN1* (BF = 23.4) that encodes several isoforms of nuclear proteins, *CLU* (BF = 20.1), encoding for apolipoprotein J, *ABAC7* (BF = 18.8), *CR1* (BF = 18.1), *PICALM* (BF = 17.3), *MS4A6A* (BF = 8.*7*), *CD33* (BF = 7.7), *MS4A4E* (BF = 6.9), and *CD2AP* (BF = 6.6). Illustrated below is the ranking of polymorphisms from Algene.org, a database that reviews published genome-wide association studies and meta-analyses relating to Alzheimer's Disease.

Table 3. **Polymorphism ranking based on genetic association studies with late onset AD.**

#	Gene	Polymorphism	Ethnicity	OR (95% CI)	P-value
1	APOE e2/3/4	APOE_e2/3/4	All	3.685 (3.30-4.12)	<1E-50
2	BIN1	rs744373	All	1.166 (1.13-1.20)	1.59E-26
3	CLU	rs11136000	Caucasian	0.879 (0.86-0.90)	3.37E-23
4	ABCA7	rs3764650	All	1.229 (1.18-1.28)	8.17E-22
5	CR1	rs3818361	Caucasian	1.174 (1.14-1.21)	4.72E-21
6	PICALM	rs3851179	Caucasian	0.879 (0.86-0.9)	2.85E-20
7	MS4A6A	rs610932	All	0.904 (0.88-0.93)	1.81E-11
8	CD33	rs3865444	All	0.893 (0.86-0.93)	2.04E-10
9	MS4A4E	rs670139	All	1.079 (1.05-1.11)	9.51E-10
10	CD2AP	rs9349407	All	1.117 (1.08-1.16)	2.75E-09

This data was received from 320 meta-analysis performed using 1,395 studies examining 695 genes and 2973 polymorphisms. Only meta-analysis results with *P*-values <0.00001 are displayed in this table. Accessed 3/13/2017 on Alzgene.org.

In the effort to identify a greater portion of genetic risk factors contributing to LOAD, a two-staged meta-analysis by Lambert et al. performed on four GWAS data sets, revealed 11 new loci for LOAD susceptibility. Prior to this meta-analysis, 9 genetic susceptibility factors for LOAD risk had been identified, including *ABCA7*, *BIN1*, *CD33*, *CLU*, *CR1*, *CD2AP*, *EPHA1*, *MS4A6A–MS4A4E* and *PICALM*. The GWAS examined were

conducted by four consortia: Alzheimer's Disease Consortium (ADGC), CHARGE, EADI, and GERAD. In addition to the *APOE* locus, 19 other genomic regions reached genome-wide significance in the combined stage 1 and 2 analysis. Of these 19 genomic regions, 11 were found to be associated with AD pathology (Lambert et al., 2013).

These variants were verified in future large scale GWAS and by the International Genomics of Alzheimer's Project. An additional variant found included the *SLC4A4/RIN3* genes, the first involved in brain expression and the second, a known interactor of *BIN1* gene product (Cauwenberghe et al, 2016). Table 4 below shows at least 20 genetic risk loci for LOAD development reaching genome-wide significance.

Table 4. **Overview of AD susceptibility loci defined by GWAS and meta-analysis (Cauwenberghe et al., 2016)**.

Gene	Location	SNP	Risk allele frequency controls	OR (95% CI)	Population-attributable fraction (%)	Potential functional variant
APOE (apolipoprotein E)	19q13.32	ε4	0.16	3.78 (2.60–5.48)	30.8[a]	ε4
SORL1 (sortilin-related receptor-1)	11q24.1	rs11218343-T	0.96	1.30 (1.22–1.39)	0.91[b]	Common and rare pathogenic variants[24,25]
BIN1 (bridging integrator 1)	2q14.3	rs6733839-T	0.41	1.22 (1.18–1.25)	8.2[a]	rs59335482, 3 bp insertion[40]
CR1 (complement component (3b/4b) receptor 1)	1q32.2	rs6656401-A	0.20	1.18 (1.14–1.22)	3.5[a]	Intragenic CNV resulting in different CR1 isoforms[41]
CLU (clusterin)	8p21.1	rs9331896-T	0.62	1.16 (1.12–1.19)	5.1[a]	Rare coding and common regulatory variants[26,21]
PICALM (phosphatidylinositol-binding clathrin assembly protein)	11q14.2	rs10792832-G	0.64	1.15 (1.12–1.18)	4.5[a]	—
ABCA7 (ATP-binding cassette transporter A)	19p13.3	rs4147929-A	0.19	1.15 (1.11–1.19)	2.8[a]	Loss-of-function variants[27,28]
FERMT2 (fermitin family member 2)	14q22.1	rs17125944-C	0.09	1.14 (1.09–1.19)	1.2[a]	—
CASS4 (Cas scaffolding protein family member 4)	20q13.31	rs7274581-T	0.92	1.14 (1.09–1.19)	1.0[b]	—
MS4A6A locus (membrane-spanning 4-domains, subfamily A)	11q12.2	rs983392-A	0.60	1.11 (1.09–1.15)	3.8[b]	—
EPHA1 (EPH receptor A1)	7q35	rs11771145-G	0.66	1.11 (1.08–1.14)	3.3[b]	—
HLA-DRB5, HLA-DRB1 locus (major histocompatibility complex, class II, DR beta 5/beta 1)	6p21.32	rs9271192-C	0.28	1.11 (1.08–1.18)	3.0[a]	—
PTK2B (protein tyrosine kinase 2 beta)	8p21.2	rs28834970-C	0.37	1.10 (1.08–1.13)	3.6[a]	—
CD2AP (CD2-associated protein)	6p12.3	rs10948363-G	0.27	1.10 (1.07–1.13)	2.6[a]	—
ZCWPW1 locus (zinc finger, CW type with PWWP domain 1)	7q22.1	rs1476679-T	0.71	1.10 (1.06–1.12)	2.5[b]	—
SLC24A4/RIN3 locus (solute carrier family 24/Ras and Rab interactor 3)	14q32.12	rs10498633-G	0.78	1.10 (1.06–1.14)	1.9[b]	—
INPP5D (inositol polyphosphate-5-phosphatase)	2q37.1	rs35349669-T	0.49	1.08 (1.05–1.11)	3.8[a]	—
MEF2C (myocyte enhancer factor 2C)	5q14.3	rs190982-A	0.59	1.08 (1.05–1.11)	2.8[b]	—
NME8 locus (NME/NM23 family member 8)	7p14.1	rs2718058-A	0.63	1.08 (1.05–1.11)	2.5[b]	—
CELF1 locus (CUGBP, Elav-like family member 1)	11p11.2	rs10838725-C	0.32	1.08 (1.05–1.11)	2.5[a]	—
CD33 (CD33 molecule)	19q13.41	rs3865444-C	0.69	1.06 (1.04–1.1)	1.8[b]	rs12459419 located in a putative SRSF2 splice site of exon 2, leading to alternative splicing of the IgV domain[44]

This summary includes 20 genetic variants, its chromosomal location, position and closest gene, described OR, meta *P-value*, and *P-value* data for each LOAD susceptibility loci.

The merit of such risk factor studies is the elucidation of the pathways involved in the disease. Although a number of these variants are related with Aβ or tau, many of these GWAS-identified genes are associated with cholesterol/lipid metabolism, immune system/inflammatory response, or endosomal vesicle cycling (Cauwenberghe et al., 2016). Table 5 below depicts and overview of the GWAS AD-susceptibility genes sorted by function and properties.

***Table 5*. Overview of the single-locus AD-susceptibility genes identified by GWAS and meta-analysis: function and characteristics (Cauwenberghe et al., 2016).**

Gene	Pathway	Function	Effect on APP pathway	Effect on tau pathway
SORL1	Endosomal vesicle cycling	Vesicle trafficking	Aβ generation and clearance	—
BIN1	Endosomal vesicle cycling	Clathrin-mediated endocytosis	—	tau toxicity
CR1	Immune response	Regulation of complement activation	Aβ clearance	—
CLU	Cholesterol and lipid metabolism	Chaperone function; regulation of cell proliferation	Aβ aggregation and clearance	—
PICALM	Endosomal vesicle cycling	Trafficking of synaptic vesicle proteins	APP trafficking and Aβ clearance	Co-localization in NFTs
ABCA7	Lipid metabolism and immune response	Efflux of phospholipids and phagocytosis	Aβ clearance	—
FERMT2	Cytoskeletal function and axonal transport	Actin assembly and cell shape modulation	—	Tau toxicity
CASS4	Cytoskeletal function and axonal transport	Scaffolding protein of unknown function (in Drosophila ortholog binds to CD2AP ortholog)	—	—
EPHA1	Endosomal vesicle cycling and immune system	Brain development, modulating cell migration, axon guidance, and synapse development and plasticity	—	—
PTK2B	Cell migration and synaptic function	Ion signaling and induction of long-term potentiation in the hippocampal CA1 neurons	—	—
CD2AP	Endosomal vesicle cycling	Cytoskeletal reorganization and vesicle movement	Aβ clearance	Protection against tau toxicity
INPP5D	Immune response	Regulation of gene expression and posttranslational modification of proteins, microglial and myeloid function	—	—
MEF2C	Immune response, neural development, synaptic function	Synaptic plasticity	—	—
CD33	Immune system and inflammatory response	Cell-cell interactions and cell functions in the innate and adaptive immune systems	Aβ clearance	—

For each gene, the pathway, gene function, and effect on the APP or tau pathway are described.

APP, amyloid precursor protein; Aβ, amyloid β; NFT, neurofibrillary tangles.

Diagnosis and Detection

The definitive diagnosis of AD can only be made post-mortem via microscopic analysis of brain tissue. However, probable diagnosis of AD is presently possible. The most used clinical tool for diagnosis of AD utilize the NINCDS-ADRDA criteria. Proposed by the National Institute of Neurological and Communicative Disorders (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA), these criteria require the presence of cognitive impairment confirmed by neuropsychological testing. However,

there may not be any other comorbid diseases capable of contributing to dementia if a diagnosis of probable AD is to be made. Patients can be diagnosed with MCI if they exhibit cognitive decline, evidenced by neuropsychological tests. Subjects with MCI are at risk for developing AD (Alzheimer's Association, 2016).

The ability to distinguish between AD and other dementias has limited accuracy, between 23% and 88%, and remains a difficulty (Ballard et al., 2011). For example, small and large vessel cerebrovascular disease can cause dementia syndromes that often are difficult to distinguish from, and can occur concurrently with AD (Sloane et al., 2002). However, early diagnosis of AD may improve the medical care that affected individuals receive. Currently, the best classifiers for early detection include MRI, f-fluorodeoxyglucose-PET, CSF biomarkers, and clinical tests (Weiner et al., 2015). Criteria for probable AD diagnosis include presence of significant episodic memory impairment, neuropsychological tests, supportive neuroimaging, and biomarker evidence.

Medical History

To begin the process of making a clinical diagnosis of AD, a detailed history must be obtained from either the patient or the caregiver. This includes family medical history, coexisting medical issues, progression of cognitive symptoms, and current mental state. Ruling out other sources of dementia (e.g. medication side-effects, vitamin deficiencies, thyroid problems) or types of dementia (e.g. diffuse Lewy body dementia, frontotemporal dementia, and vascular dementia) is imperative. The inclusion of falsely diagnosed patients may hinder the effectiveness of clinical therapy (Olsson et al., 2016). Ruling out other possibilities of dementia may be conducted via obtaining medical history, neuroimaging, urine samples, and blood work.

Cognitive Tests

After a patient has been identified with having memory impairment via health and family history, they are given cognitive assessments. Such tests are usually used by medical doctors due to their inexpensiveness and global availability, in comparison to more technological diagnostic tests, such as PET scans or biomarkers. Cognitive tests usually involve a series of simple tasks and questions to assess orientation, problem-solving skills, memory, attention, and language (Matias de Lemos, 2012).

The most widely used and globally studied cognitive test within clinical screening is the Mini-Mental State Exam (MMSE). The MMSE utilizes a series of questions to assess the subject's overall mental status out of 30 points. These questions are divided into five parts: orientation, registration, attention, recall, and language. A score of 20 to 24 suggests mild dementia, 13 to 20 suggests moderate dementia, and less than 12 indicates severe dementia. On average, the MMSE score of a person with AD declines about two to four points each year. Despite its common usage, the MMSE does have several limitations. First, this test is sometimes misunderstood as a diagnostic test, whereas is actually a screening test. Second, MMSE scoring has been evaluated to be biased by social variables, such as age and education. Lastly, it has been found that the MMSE is relatively insensitive to change and disease progression due to floor and ceiling effects (Sheehan, 2012).

The MMSE is often used in combination with the Alzheimer's Disease Assessment Scale – Cognitive sub-scale (ADAS–Cog) in clinical trials, which is based on eleven (original ADAS-Cog) or thirteen domains (modified ADAS-Cog). The ADAS-Cog is generally used to complement the MMSE because it is more sensitive to cognitive changes over time (Sheehan, 2012). The score is based on the weighted score of multiple cognitive domains. The original eleven-item scale is scored from 0-70, where higher numbers indicate increased cognitive impairment. Average scores for patients diagnosed with cognitive impairment is around 31.2. The ADAS-Cog is the preferred scoring method if cognition is of particular interest, despite its lengthiness (Carnero-Pardo et al., 2013) (Rockwood et al., 2007). Though the ADAS is widely used for diagnostic purposes, the ADAS-Cog exhibits a ceiling effect in MCI and mild AD patients. Clinical trials have shown that changes in earlier stages of AD are difficult to detect using this test (Silverberg et al., 2011). In addition, the ADAS-Cog has been labelled by some as having low sensitivity for measuring disease progression in clinical trials (Verma et al., 2015). The reason for its low sensitivity can be attributed to its inability to capture all domains of cognition. For example, in an open study of 100 AD patients, 43% of patients had declining ADAS-Cog scores but increased performance in at least two clinical measures (Rockwood et al., 2007). The modified 13-item scale includes two additional domains,

word recall and number cancellation, to broaden the range of symptoms consistent with MCI (Skinner et al., 2012). This modified version is scored out of 85.

Other highly selected cognitive tests aiding clinicians to diagnose AD include the Wechsler Memory Scale, the General Practitioner Assessment of Cognition (GPCOG), Mini-Cog, the Memory Impairment Screen (MIS), and the Clock Drawing Test (CDT). Each of these tests are developed for primary care settings and takes five minutes or less to administer. In comparison to the MMSE, these tests are relatively free from educational, cultural, and language biases and do not require payment for copyrights (Cordell et al., 2013). However, these tests have less ability to detect MCI patients and have been found to have little diagnostic utility in clinical settings (Carnero-Pardo et al., 2013).

Neurological Imaging

Neurological imaging has played a variety of roles in the diagnosis and treatment of AD. Initially, neurological imaging was used for exclusionary purposes. Computed tomography (CT) and magnetic resonance imaging (MRI) were the most widely used imaging techniques to assist in ruling out other causes of dementia. However, over the last four decades, the role of neurological imaging has become more centrally used as a tool for diagnosing AD and MCI, as well as drug discovery. We are now capable of detecting morphological changes in the brain over time and visualizing the molecular pathology of the disease. Neurological imaging can be broken down into three major categories: structural, functional, and molecular (Johnson et al., 2012).

The first widely used method of detecting AD involves structural imaging of the brain. Structural neuroimaging aims to visualize anatomical features of the brain. MRI and CT scans, the most widely used mode of structural imaging, are most useful in diagnosing AD in cases of uncertainty (Health Quality Ontario, 2014). MRI has become the most used tool in the diagnosis of AD, allowing for visualization of medial temporal lobe atrophy, ventricular enlargement and decreased total brain volume (Ferreira and Busatto, 2011). Although not as rapid or widely available as CT scans, MRIs are safe from carcinogenic effects because they do not involve ionizing radiation exposure, allowing patients to receive multiple scans. Due to its wide availability, safety from carcinogenic

effects, and time effectiveness, MRIs have become the structural imaging modality of choice.

Structural MRIs are used to assess white matter loss, particularly hippocampal atrophy, for the clinical diagnosis and progression of AD. T1-weighted MRI are useful to evaluating topographic distribution due to white matter atrophy. T2-weighted MRI allow for tracking of signal changes on T2-weighted images (Yin et al., 2015). The medial temporal lobes (primarily hippocampus and entorhinal cortex) are typically the first location to demonstrate atrophy, and remain the most severely affected region as illness progresses. Compared to older controls, subjects with AD demonstrate medial temporal atrophy measures ranging from 77% to 92% (Small, 2002). Figure 12 depicts key structural differences between control and AD subjects using structural MRI.

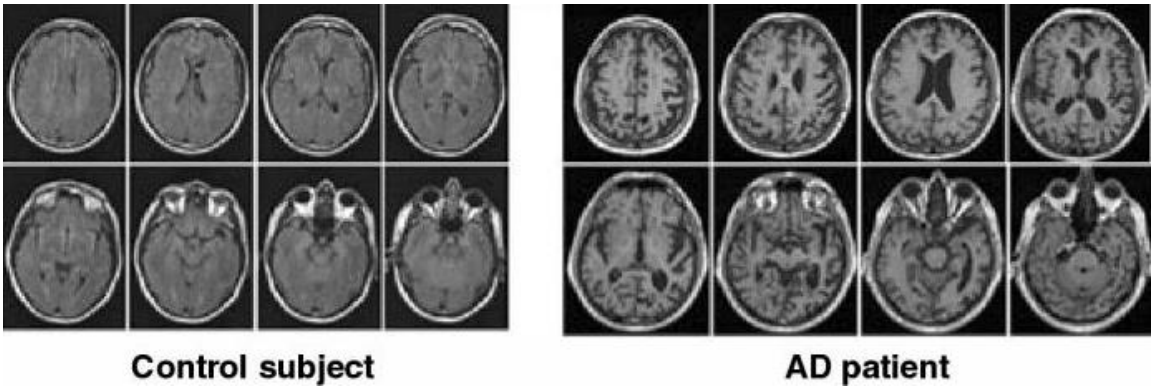

***Figure 12*. Comparable T1-weighted Coronal MRI Imaging of Control and MCI Medial Temporal Structures (Lindesay et al., 2010).** Structural magnetic resonance imaging (MRI) in Alzheimer's disease: whole brain atrophy and ventricular enlargement are key features.

Structural neuroimaging can also be used to predict future AD in patients with MCI. Longitudinal MRI studies have suggested that hippocampal volumes are reduced by 10% before receiving a clinical diagnosis of AD. CT and MRI scans have shown a reduction of entorhinal volumes by 20-30% and hippocampal volumes by 15-25% in subjects with even mildly affected individuals (MMSE of ~24/30) (Johnson et al., 2012). Figure 13 below demonstrates a comparison of hippocampal atrophy between control and MCI subjects via T1-weighted MRI imaging.

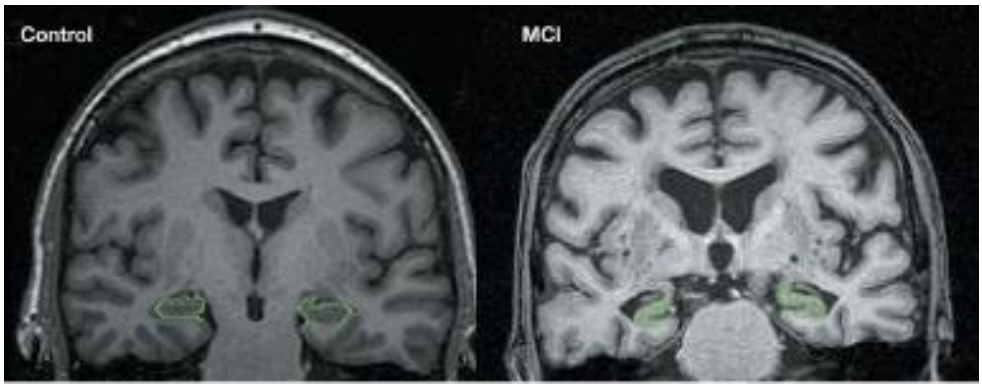

***Figure 13*. Comparable T1-weighted Coronal MRI Imaging of Control and MCI Medial Temporal Structures (Ballard et al., 2011).** Coronal T1-weighted coronal MRI slices perpendicular to the long axis of the hippocampus showing a normal sized hippocampus in a control subject and a smaller hippocampus in an MCI patient.

Another study, by Schuff et al., aimed to evaluate the predictive value of hippocampal atrophy, utilizing a Markov chain approach to derive the rate of volume change over 6-12 months in normal, MCI, and AD subjects. Schuff et al. were able to conclude that hippocampal atrophy rates indeed were more significant in MCI and AD subjects compared to cognitively normal subjects (Schuff et al., 2009). Thus, assessment of medial temporal atrophy via structural neuroimaging does indeed have a predictive role for AD.

Along with the detection of cerebral atrophy, other benefits of structural imaging include its wide availability, time effectiveness, and safety. However, structural imaging does have its limitations. The utility of CT and MRI scans have been found to be highest in cases of ambiguity, such as dementia caused by vascular disease, and low for patients already diagnosed with AD. Furthermore, a meta-analysis concluded that although structural neuroimaging is useful in detecting brain abnormalities in persons with dementia, less than 10% of detected abnormalities lead to a change in the course of treatment and decision making process (Health Quality Ontario, 2014). Additionally, CT scans are not utilized much in clinical diagnosis of AD due to its lower special resolution and lack the ability to differentiate between grey and white matter (Ferreira and Busatto, 2011; Small, 2002). Lastly, structural MRIs lack the feature of molecular specificity. In order to assess the molecular pathology (i.e. amyloid plaques and NFTs) of AD, functional and molecular imaging techniques are more useful (Johnson et al., 2012).

Functional neuroimaging aims to find relationships between brain area activities and specific mental imaging using measurements of brain function. Functional neuroimaging can be used to complement the diagnostic investigation of AD in the case of uncertainty. Widely utilized functional neuroimaging methods in AD include functional MRI (fMRI), single photon emission computed tomography (SPECT), and f-fluorodeoxyglucose-positron emission tomography (FDG-PET). fMRI measures changes in blood oxygen levels to examine correlations in brain function. fMRI can be task-related, or acquired during cognitive tasks to compare how information is encoded. The test can also be acquired during one's resting state to examine connectivity of brain networks. Memory function is carried out by a network of brain regions, known as the "default network," which deactivate during cognitive tasks in normal subjects. However, several studies have found fMRI hyperactivity among MCI and at-risk subjects during memory trials, suggesting a possible compensatory mechanism (Johnson et al., 2012).

Functional neurological imaging can also be combined with tracers to assess the pathology of AD. SPECT is a technique that measures brain perfusion (blood flow) via metabolism-dependent uptake of a radioactive tracer. PET scans with f-fluorodeoxyglucose (FDG-PET) have shown promising results in distinguishing patients with AD from those with non-AD dementias by measuring glucose metabolism (Ballard et al., 2011). Both SPECT and FDG-PET have revealed a pattern of hypometabolism/hypoperfusion in the temporoparietal cortex of AD and MCI patients in comparison to controls (Ferreira and Busatto, 2011). FDG hypometabolism correlates to atrophy of areas responsible for cognitive function, such as the "default network", which are highly vulnerable to amyloid depositions (Johnson et al., 2012). Figure 14 below depicts FDG hypometabolism in the AD brain, in comparison to that of a control.

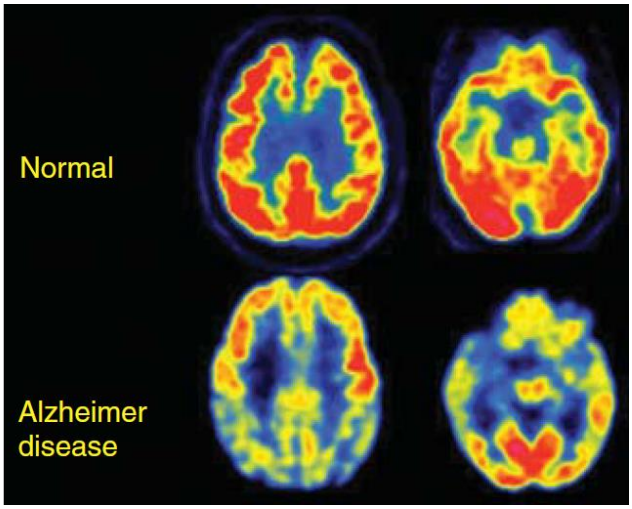

***Figure 14*. FDG-PET images of normal control and a patient with mild AD (Johnson et al., 2012).** Transaxial FDG-PET images of a normal control subject and a patient with mild AD. Note severe hypometabolism (yellow and blue cortical regions) in association and limbic cortex.

FDG-PET can also be used to discriminate between other forms of dementia. For example, when frontotemporal hypofunction is more prominent than temporoparietal hypofunction, a clinical diagnosis of AD may be changed (Johnson et al., 2012). However, significant limitations to FDG-PET is the high cost and exposure to radioactivity. Despite this limitation, use of functional neuroimaging aids in reducing uncertainty of AD as a complement to structural imaging.

In addition to visualizing neurostructural changes and brain activity with relation to AD pathology, it is now possible to view the specific molecular pathology of the disease. Amyloid deposition can be viewed via the use of radiotracers paired with PET imaging. One advance in the study of AD imaging has been the development of the *in vivo* amyloid-labelling tracer, Pittsburgh Compound-B (PIB), which binds to $A\beta_{40}$ and $A\beta_{42}$ cortical plaques. Used with PET scans, measurement of PIB binding retention rates allow for visualization of AD pathology, shown in Figure 15 below. Currently, the most widely used ligand for cerebral Aβ imaging, PIB-PET serves as a research tool for the diagnosis of AD (Clifford et al., 2008).

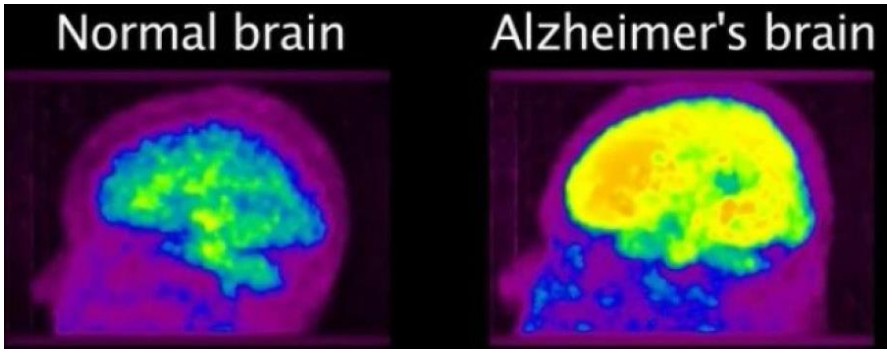

Figure 15. **PiB PET scan comparing brains of people with and without Alzheimer's disease.** Yellow color indicating amyloid positivity. **Photo credit**: The Commonwealth Scientific and Industrial Research Organization.

One publication evaluating the use of amyloid PET on clinically diagnosed AD patients revealed 96% of patients with amyloid positivity. However, the amyloid negative patients remained the same after 5 years, suggesting AD was not the likely cause of dementia. Thus, amyloid PET may be used to rule out other cases of dementia. Amyloid PET scans may also be used as a tool to mark the progression of MCI to AD. For instance, PIB neuroimaging has demonstrated more intense signals of amyloid deposition in MCI patients who convert to AD than non-converters (Ferreira and Busatto, 2011).

Amyloid imaging scans serve to identify the absence or presence of amyloid deposits in the brain. Despite the advances in amyloid imaging, one downfall is that amyloid deposition is not specific to the pathogenesis of AD. For example, amyloid deposition can also be found in cognitively normal controls, as well as subjects with other forms of dementia (such as Lewy body dementia). Whereas structural MRIs and FDG-PETs would better serve to aid in the evaluation of AD progression, amyloid PET scans can rule out misdiagnosed AD subjects (Johnson et al., 2012). Despite limited use in clinical settings, amyloid imaging is being used as a validating biomarker tool to evaluate the success of AD clinical trials (Ferriera and Busatto, 2011).

In summary, neuroimaging studies aid in the detection of preclinical AD, evaluation of disease progression, and clarification of underlying disease mechanisms. The most utilized neuroimaging tools for the diagnosis and detection of AD in clinical settings include MRI, CT, FDG-PET, SPECT, and amyloid PET. Table 6 summarizes the extent of utility for each mode of imaging below. Each modality of imaging has different

strengths and limitations, but may be used to complement each other within the study of AD.

***Table 6*. Neuroimaging modalities in patients with suspect Alzheimer's disease (Ferreira and Busatto, 2011).**

Modality	Information afforded	Clinical utility in dementia	Current availability
MRI	Visualization of gray matter, white matter and cerebrospinal fluid	Important to identify non-Alzheimer's disease etiologies Useful to characterize supportive features for the diagnosis of Alzheimer's disease (medial temporal lobe atrophy)	Available in developed geographic regions. Limited availability in underdeveloped areas
CT	Identification of gross brain abnormalities	Identification of large lesions. Useful if there are contraindications for magnetic resonance imaging	Widely available
FDG-PET	Regional brain glucose metabolism	Useful in cases of diagnostic uncertainty despite a thorough evaluation[12] SPECT/FDG-PET should not be used as the only imaging measure[14]	Limited to specialized centers
SPECT	Regional brain perfusion		
Amyloid imaging with PET	Identification of amyloid deposition in the brain	In the future may be used as a very sensitive (though nonspecific) test for Alzheimer's disease	Only in a very few specialized research centers in the world

The clinical utility of neuroimaging modalities used in patients with suspected AD. CT = computed tomography; FDG-PET = 18F-fluorodeoxyglucose-positron emission tomography; MRI = magnetic resonance imaging; SPECT = single photon emission computed tomography.

Computer-Aided Neuroimaging

Currently structural MRIs have been the most extensively utilized diagnostic tool due to their accurate visualization of brain structures. Computer-based algorithms may be used to complement the diagnostic process. Computer-based diagnostics use algorithms to design classifiers that can categorize groups (e.g. MCI, AD, and cognitively normal) based on atrophy in medial temporal lobe structures (hippocampus, amygdala, entorhinal cortex and parahippocampal gyrus) (Bron et al., 2015). And so, computer-based diagnosis may accompany imaging and biomarker tools to diagnose presymptomatic AD, as well as to predict conversion of MCI to AD.

Neuroimaging can be complemented by the use of quantitative algorithm tools, such as diffusion tensor imaging (DTI). Using a strategy called tract-based spatial statistics (TBSS), DTI is capable of detecting white matter abnormalities within the limbic-diencephalic network, which is affected in the earliest stages of AD, by measuring the diffusion of water molecules in neural tissue (Sexton et al., 2017). One recent study used DTI in conjunction with 3T MRI to show microstructural changes in the hippocampus of MCI participants that were not detected using structural MRIs. Compared to age matched controls, MCI participants demonstrated raised hippocampal mean diffusivity and smaller hippocampal volumes (Rose et al., 2006). Thus, despite its novelty, DTI may serve as a

sensitive complementary clinical tool to MRI for the diagnosis of AD/MCI individuals. However, though DTI-derived maps are less invasive and less expensive than fMRI and PET modalities, they remain a challenge due to their inability to gather anatomical information.

Another computer-based neuroimaging technique is voxel-based morphometry (VBM), a technique using 3-D MRI imaging to detect differences in regional gray matter across the whole brain (Busatto et al., 2008). Unlike MRI and other region-of-interest (ROI) methods, this whole-brain approach is free of hypotheses or *a priori* decisions which reduce statistical power of analysis, allowing for a more comprehensive view of the brain. The VBM technique is conducted by normalizing an MRI scan into the same field, segmenting the image into gray matter white matter and CSF, and comparing each segment of gray matter voxel-by-voxel. Similar to DTI studies, VBM studies also identify volume decreases in the left hippocampus and parahippocampal gyrus, associated with conversion from MCI to AD (Ferreira et al., 2011).

Additional computer-based diagnostic techniques employs the integration of multiple imaging modalities into one framework. This approach, called the Multiple Kernel Learning technique, has been seen to increase discrimination between clinical groups. By combining data from DTI and structural MRI, Ahmed et al. demonstrated increased classification accuracy, with 90.2% between AD and controls, 79.42% between MCI and controls, and 76.63% between AD and MCI (Ahmed et al., 2017). This work supports the idea of using multiple modalities of imaging, including both qualitative and computer-based models, to improve the diagnosis of the disease.

To summarize, computer-based algorithms are currently being developed to complement the diagnosis and prediction of AD. The benefit of such studies is that they are fully automated and capable of detecting quantitative differences across the whole brain. This may reduce human error and increase the speed during the interpretation of a diagnostic image (Bron et al., 2015). In addition to optimizing AD diagnostics, neuroimaging, biomarker, and computer-based algorithms may also be used to predict development of

AD in MCI patients. Taken together, each diagnostic technique mentioned may aid in early detection, thereby facilitating optimal treatment and delaying cognitive decline.

Biomarkers

Research is underway to develop biomarkers to accurately diagnose, monitor progression, and quantify changes *in vivo* with regards to AD. Over the past 25 years, three cerebrospinal fluid (CSF) markers have been identified and tested to increase the diagnostic validity of sporadic AD: total tau (T-tau), phosphorylated tau (P-tau), and $A\beta_{42}$ (Humpel, 2011, Olsson et al., 2016). Each core CSF biomarker is an indication AD pathology. Tau can be abnormally hyperphosphorylated at specific sites (P-tau), leading to the formation of neurofibrillary tangles and axonal dysfunction (Blennow et al., 2015). $A\beta_{42,}$ the least soluble peptide produced from amyloid precursor protein, is a measure of amyloid-beta plaques in the brain. These biomarkers have been found to discriminate AD subjects from controls, as well as other dementia-related diseases, with high sensitivity and specificity using ELISA.

CSF biomarker concentrations can serve as accurate markers of AD progression. Biomarker studies have shown that individuals with AD contain increased CSF concentrations of T-tau (300% higher than controls), due to cortical neuronal loss, and P-tau (200% higher than controls), due to cortical tangle formation (Forlenza et al., 2015). Conversely, the concentration of $A\beta_{42}$ is decreased in AD subjects due to the sequestration of Aβ plaques reducing the amount available for clearance in to the CSF (Leuzy et al., 2016). $A\beta_{42}$ is on average 50% lower than in controls (Forlenza et al., 2015). The comparison of CSF biomarker concentrations in healthy, MCI, and AD patients can be viewed in Table 7.

***Table 7*. Changes of the core CSF biomarkers in MCI and AD (Mao, 2012).**

	Aβ42	T-tau	P-tau	tau/Aβ42
Healthy	—	—	—	—
MCI	↓	↑	↑	↑
AD	↓	↑	↑	↑

—: Normal level; ↓: Decrease; ↑: Increase; Bold arrows: Full decrease/increase.

$A\beta_{42}$ = Beta-amyloid $_{42}$, T-Tau = total tau, P-Tau = phosphorylated tau, tau/$A\beta_{42}$ = the ratio of tau to beta-amyloid $_{42}$.

Cutoff values discriminating AD from controls for each biomarker have been established internationally, and can be found in Table 8. T-tau increases in the CSF with age in healthy controls: <300 pg/mL (21-50 years), <450 pg/mL (51-70 years), and <500 pg/mL (>70 years). However, these concentrations are much larger for AD subjects, with a cutoff value of >600 pg/mL. High levels of T-tau have been found in 90% of MCI patients, suggesting that T-tau may be able to predict conversion to AD (Humpel, 2011). Subjects with AD demonstrate a cutoff value of >60 pg/mL for P-tau, compared to controls. Lastly, individuals with AD show a significant reduction of $A\beta_{42}$, with cutoff values of <500 pg/mL.

***Table 8*. Internationally established biomarkers in CSF used to diagnose AD (Humpel, 2011).**

Biomarker	Controls (pg/ml)	AD (pg/ml)
Aβ(1–42)	794±20	<500*
Total tau	136±89 (21–50 years)	[b]
	243±127 (51–70 years)	>450
	341±171 (>71 years)	>600*
Phospho-tau-181	23±2	>60*

CSF biomarker cut-off values obtained by Innogenics 96-well ELISA kits between age-matched controls and AD subjects, yielding combined sensitivity of 95% and specificity of 85%. P<0.001.

Interestingly, the ratio of $A\beta_{42}$/T-tau and $A\beta_{42}$ /P-Tau also serve as diagnostic signatures and predictors of conversion from MCI to AD, as demonstrated by an ADNI cohort study revealing a sensitivity of 86% and specificity of 85% (Ritchie et al., 2013).

The cut-off values displayed in Table 7 cannot be utilized universally to calculate sensitivity and specificity within clinical studies, due to biomarker concentration variability. Instead, sensitivity (SE) and specificity (SP) must be calculated using cut-off values derived from ROC (Receiving Operating Characteristics) Curves. ROC curves illustrate the diagnostic ability of a binary classifier system by calculating discrimination threshold between "control" and "diseased". This is done by plotting the true positive rate by the false positive rate at various thresholds. One study by Forlenza et al. established cutoff values, sensitivity, and specificity of biomarkers $A\beta_{42}$, T-tau, P-tau, $A\beta_{42}$/T-tau and $A\beta_{42}$/P-tau, using the aforementioned method. Using these cutoff values, it was determined that the best predictor of disease conversion was $A\beta_{42}$ (SE = 83% and SP = 70%) and $A\beta_{42}$/P-tau (SE = 88% and SP = 78%). According to these values, a combination of biomarker signatures may be the most accurate predictor of AD conversion (Forlenza et al., 2015).

Additionally, levels of the three core CSF biomarkers differ in subjects other forms of neurodegenerative disease, such as Parkinson's, Creutzfeldt-Jakob Disease (CJD), and vascular dementia (VaD) (Table 9). For example, whereas cutoff values for T-tau are >600 pg/mL in AD subjects, these levels are dramatically increased in subjects with CJD (>3000 pg/mL). Levels of P-tau can also distinguish AD subjects from frontotemporal lobar degeneration, VaD, and Lewy Body Dementia (Humpel, 2 011). Thus the concentration of these three CSF biomarkers serves as a signature for AD and a molecular tool used to confirm amyloid pathology in clinical trials (Humpel, 2011; Teunissen and Parnetti, 2016).

***Table 9*. Changes in CSF biomarkers in different central nervous system diseases (Humpel, 2011).**

Disease	Aβ(1–42)	Total tau	Phospho-tau-181
Acute stroke	–	↑(↑)	–
Alcohol dementia	–	–	–
AD	↓	↑	↑
CJD	↓↓	↑↑↑	–
Depression	–	–	–
FTLD	↓	↑	–
LBD	↓	↑	↑
Neuroinflammation	↓	–	–
Normal aging	–	–	–
Parkinson's disease	–	–	–
VaD	↓(↓)	↑	–

The clinical utility of neuroimaging modalities used in patients with suspected AD. CT = computed tomography; FDG-PET = 18F-fluorodeoxyglucose-positron emission tomography; MRI = magnetic resonance imaging; SPECT = single photon emission computed tomography.

In addition to the three core CSF biomarkers ($A\beta_{42}$, T-tau, and P-tau), other CSF biomarkers have been established to have high association with AD and are deemed as possible candidates for clinical research. One additional CSF biomarker specific to AD pathology is neurogranin (Ng), a post-synaptic protein and marker of synaptic loss (Mattsson et al., 2016). Ng is highly expressed in the cortex, hippocampus, and amygdala (the regions most affected in AD). Studies reveal higher CSF Ng concentrations in AD patients than in controls and patients with dementia-causing disease, confirming the high specificity of Ng (Figure 16) (Wellington et al., 2016).

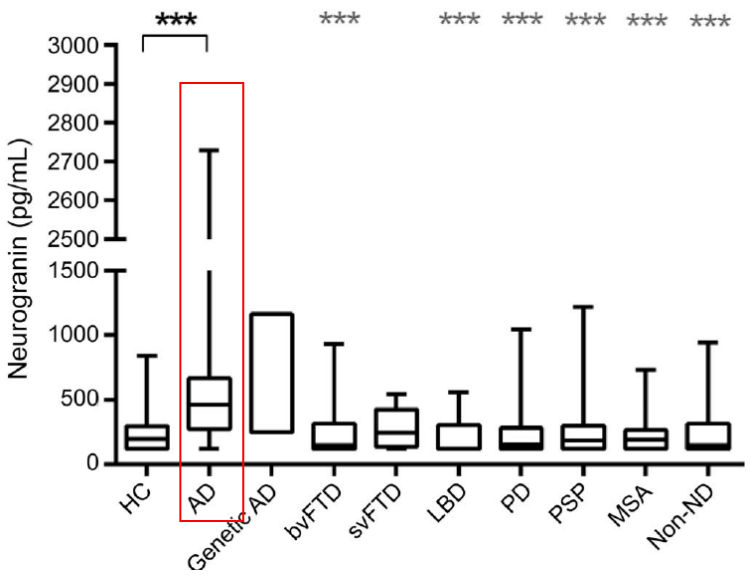

Figure 16. **Increased CFS Ng concentrations in patients with AD (Wellington et al., 2016).** Boxplots showing CSF Ng concentrations across different diagnostic groups. Ng concentrations were significantly higher in the AD group compared to control participants. The whiskers extend to the minimum and maximum Ng data points. AD - Alzheimer disease; FTD - behavioral variant frontotemporal dementia; genetic AD - those with confirmed PSEN1 mutations; HC - healthy controls; LBD - Lewy body dementia; MSA - multiple system atrophy; Ng - neurogranin; non-ND - non-neurodegenerative patients with mood disorder; PD - Parkinson disease; PSP - progressive supranuclear palsy; FTD - speech variant frontotemporal dementia.

In addition to the three core biomarkers ($A\beta_{42}$, T-tau, and P-tau), a meta-analysis conducted by Olsson et al. found neurofilament light protein (NFL), a protein likely released from neurons during acute axonal damage, to have high association with AD (Figure 8) (Mattsson et al., 2016; Olsson et al., 2016). This meta-analysis also revealed moderate performance of neuron-specific enolase (NSE), visinin-like protein 1 (VLP-1), heart fatty acid binding protein (HFABP), and human cartilage glycoprotein 39 (YKL-40). Though neither of these markers specifically reflect the pathology of AD, they could be useful in clinical trials for the prevention of AD (Olsson et al., 2016). The biomarkers performance is shown in Figure 17.

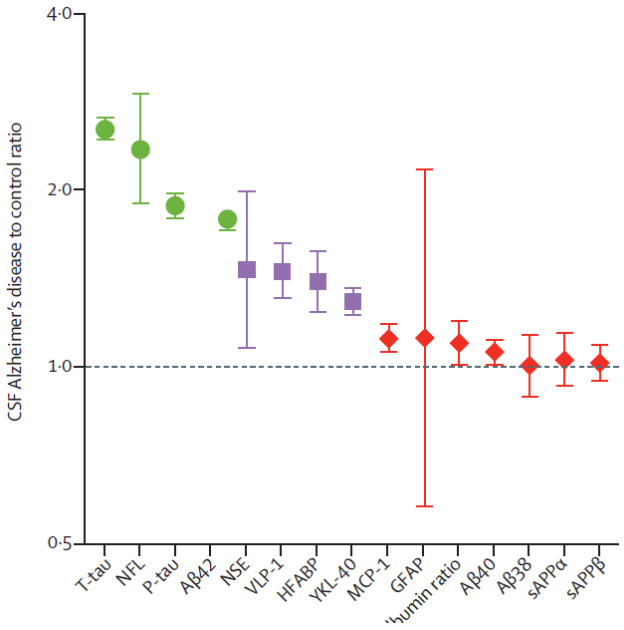

***Figure 17*. CSF biomarker performance rating in patients with Alzheimer's disease versus controls (Olsson et al., 2016).** Head-to-head biomarker performance in CSF based on average Alzheimer's disease to control ratios. Biomarkers shown in green are significant with good effect sizes, in purple significant with moderate effect sizes, and in red non-significant or signifi cant with minor effect sizes. The Alzheimer's disease to control ratios of CSF $A\beta_{42}$, $A\beta_{40}$, and $A\beta_{38}$ were inverted to allow for a clear comparison with the other biomarkers.

Mattsson et al. tested the hypothesis that combinations of T-tau, Ng, and NFL may increase the diagnostic accuracy for AD. Although levels of NFL were also increased in MCI and AD patients, there was no correlation of NFL with amyloid positivity (Figure 18). In fact, NFL seems to correlate with cognitive decline regardless of Aβ pathology, such as inflammatory disease and frontotemporal dementia. Because increased NFL concentration seems to be a non-specific marker of injury, further work is needed to use NFL as a discriminating factor in the study of AD (Mattsson et al., 2016).

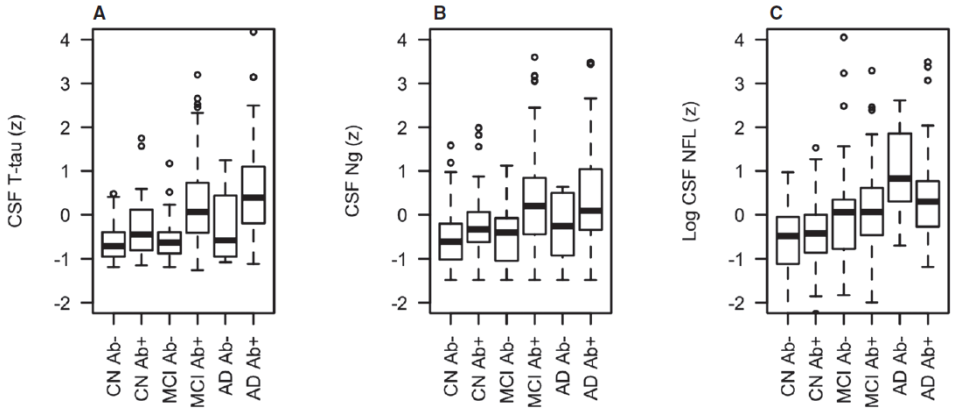

***Figure 18*. Biomarkers by diagnosis and amyloid pathology (Mattsson et al., 2016).** A–C CSF T-tau (panel A), Ng (panel B) and NFL (panel C) in different combinations of clinical diagnosis and Ab pathology (CN Ab-, n = 69; CN

Ab+, n = 40; MCI Ab-, n = 50; MCI Ab+, n = 137; AD Ab-, n = 8; AD Ab+, n = 85). Biomarker levels are standardized to z-scores and shown in box plots.

In addition to CSF biomarkers, blood-based biomarkers may add value to diagnosing and predicting AD. Because pathology precedes onset of AD symptoms by 10 years, identifying biomarkers that can identify the pathology before onset of symptoms may aid in prevention of debilitating AD symptoms. CSF biomarkers have made the most progress in AD diagnostics due to the direct reflection it has of brain metabolism. However, CSF collection requires an invasive lumbar puncture with potential side effects and poses difficulty with follow-up analysis (Humpel, 2011). The potential advantages of blood biomarkers would include sample collection convenience, lower processing cost, and the ability to separate blood compartments (plasma, serum, and cellular compartment). Therefore, the search for blood biomarkers associated with AD is ongoing.

Of the plasma and serum biomarkers reviewed, only plasma T-tau has been shown to be significantly elevated in AD patients compared to controls (Olsson et al., 2012). Aβ levels are detectable in plasma using ELISA. Nevertheless, attempts to measure Aβ subtypes in blood have produced inconsistent results (Anoop et al., 2010). Despite the potential advantages of using blood biomarkers in AD diagnosis, peripheral blood does not have a direct connection with the brain and may not reflect AD accurately. In addition, levels of components in the blood may change based on the environment, resulting in changes that are too dynamic (Thambisetty and Lovestone 2010). Still, the search for blood biomarkers may aid in a more non-invasive way of detecting AD.

In total, biomarkers are currently used as a tool to diagnose AD and MCI, and to predict conversion of MCI patients to AD. T-tau, P-tau, and $A\beta_{1-42}$ are CSF biomarkers that have yielded significant results among population studies and meta-analysis, and should be considered for clinical practice and clinical research (Olsson, 2016). These biomarkers may be used in combination for a more accurate reflection of the diagnosis. Presymptomatic detection of AD would facilitate the development of effective treatments. Therefore, there is a crucial need for research and development of other CSF and blood-based biomarkers. It is also necessary to further develop biomarkers more

specific to the pathology of AD to rule out other causes of dementia which also demonstrate a degree of Aβ presence.

Treatment

Management of AD is usually carried out through provision of safe environment with assistance for daily activities and personal care, nonpharmaceutical strategies such as social activities or exercise, symptomatic drugs, and psychoactive drugs. Neuropsychiatric drugs, such as antipsychotic, antidepressant, and anticonvulsive drugs, are commonly used to treat agitation, aggression, and psychosis in patients with dementia. Unfortunately, the benefits of the current drugs are moderate, and sometimes with serious adverse events including sedation, Parkinsonism, chest infections, edemas, and an increased risk of stroke and death (Ballard et al., 2011).

Established Symptomatic Treatments

To date, the treatments established for AD are all symptomatic in nature, aiming to balance the neurotransmitter disturbance occurring in the disease (Yiannopoulou and Papageorgiou, 2013). Symptomatic treatments for AD have been widely available since the mid-1990s and have been widely used (Ballard et al., 2011). Current approved treatments for AD include cholinesterase-inhibitors and memantine.

Cholinesterase-inhibitors (Cis) are target the cholinergic system, in which the loss of acetylcholine neurons result in memory loss. All CIs work by binding to and inactivating cholinesterase, thereby reducing acetylcholine degradation. The three approved CIs include donepezil, rivastigmine, and galantamine. Systematic reviews have shown that these three CIs delay decline in cognitive function as measured by the AD Assessment Scale. Compared to a placebo treatment, patients on CIs demonstrated an average cognitive improvement of 2.7 points on the ADAS-Cog after 6 months of treatment (Yiannopoulou and Papageorgiou, 2013). Initiation of CI treatment at earlier stages of the disease is more favorable. However, only half of the subjects treated with CIs show evidence of any improvement (Casey et al., 2010).

The other symptomatic treatment, memantine, is an N-methyl-D-aspartate (NMDA) antagonist that opposed the neurotransmitter glutamate. Excessive glutamate may

interfere with neurotransmission. Although memantine's mechanism is very different from CIs, the clinical effects appear to be similar (Casey et al., 2010). Multiple systematic reviews comparing use of memantine to placebo have shown memantine to improve cognitive function in patients with moderate to severe AD after 6 months of treatment. There is also some evidence of additive benefits to combining CI and memantine treatments (Ballard et al., 2011).

Despite its demonstrated efficacy, there remains a heavy controversy over the effectiveness of currently established symptomatic treatments. The amount of money spent on CIs and memantine exceeds $1 billion annually in the U.S. These drugs are not curative and do not alter the final outcome of the disease, and the benefits of treatment range widely from modest to drastic. Another dilemma includes the possibility of biased data within trials sponsored by drug companies (Casey et al., 2010).

Candidate Disease-modifying Treatments

The two targets for potential disease-modifying treatments of AD include Aβ and tau. These candidate disease-modifying treatments include Aβ inhibitors and proteinases, APP modulators, tau hyperphosphorylation and aggregation inhibitors, and microtubule stabilizers. These treatments are still in clinical stages, yet seem to produce promising results in the reversal of AD.

Aβ-targeting strategies include Aβ inhibitors, Aβ proteinases, and APP enzyme modulators. Compounds that prevent the aggregation of Aβ plaques have shown some promise. The only Aβ inhibitor to have reached phase III in clinical trials, called 3APS, has shown disappointing results and even increased tau aggregation. Other Aβ treatments, including colostrinin and scyllo-inositol have been shown to reduce Aβ aggregates and improve cognitive performance in animal models, but without significant results in human trials (Yiannopoulou and Papageorgiou, 2013). In addition to Aβ inhibitors, amyloid may be targeted via proteinases. Since amyloid plaque degrading enzymes gradually decrease in patients with AD, the proteinases serve as a replacement. These proteinases include neprilysin, insulin-degrading enzyme, plasmin, endothelin converting enzyme, angiotensin converting enzyme, and metalloproteinases. However, due to lack of specificity, these compounds have not reached the stage of clinical development. To date,

the only proteinase-based treatment of amyloid to reach clinical stages target the receptor for advanced glycation products RAGE, but have yet to be published (Folch et al., 2016).

As mentioned before, the overexpression and decreased clearance of Aβ is a result of APP cleavage. Modulators and inhibitors of APP processing enzymes, including secretase, have not yet been proven to be effective, due to lack of substrate specificity and adverse side effects. Selective γ-secretase modulators (SGSM) are being developed to avoid the adverse effects associated with γ- and β-secretase modulators. One effective SGSM is the microglial modulator, CHF5074, a compound understood to reduce inflammation, amyloid burden, and microglial activity. In a phase II clinical trial with MCI patients, administration of CHF5074 resulted in improved cognitive measures and decreased inflammatory markers (Folch et al., 2016). Another instance of a possible SGSM is the naturally occurring cyclic sugar alcohol, pinitol, purportedly modulates γ-secretase activity and reduces Aβ production, while preserving Notch activity. Although further replication is needed, pinitol and other SGSMs may be seen as a future topic for AD therapy and prevention.

Medications targeting tau include inhibitors of tau hyperphosphorylation, tau aggregation, and microtubule stabilizers. Tau hyperphosphorylation is caused by specific kinases that lead to the overexpression of tau. For example, human AD brains demonstrate an upregulation of a specific tau kinase, JNK3, in CSF levels and is associated with memory loss. Mouse models treated with SP600125, a pan-JNK inhibitor revealed reduced neurodegeneration and increased cognition. Inhibitors of GSK3β, another tau phosphorylator, are in the most advanced stages of advancement and have just reached phase II trials. Inhibitors of tau phosphorylation can also be attained using phosphatase activation. Sodium selenate, a protein phosphatase 2 activator currently in phase II trials in Australia, show reduction of tau phosphorylation in cell cultures and mouse models. Another method to reduce tau buildup is methylene blue, a compound that directly inhibits tau formation. A next generation version of methylene blue, TRx 0237, is currently being developed to not only inhibit aggregation but also to also dissolve already present tau aggregates (Folch et al, 2016).

The last technique to protect against tau toxicity is the use of microtubule stabilizers. In patients with AD, NFTs produce microtubule instability and neural dysfunction. As a result, the use of microtubule stabilizers protect against NFT's adverse effect on microtubules by blocking the cell cycle in its G1 or M phase, protecting against depolymerization. Paclitaxel is an example of a microtubule-stabilizing drug currently in use to help cancer patients and considered for use towards Alzheimer's disease (Folch et al., 2016). Another example is the drug Epothilone D, which has been seen to reduce hippocampal neural loss and axonal dystrophy in clinical trials.

Gene Therapy and Immunotherapy

Although recent in clinical applications, gene therapy and immunotherapy are currently making their way as alternatives to conventional treatments (Nobre and Pereira de Almeida, 2011). Currently, gene therapy directed for the neuroprotection of cholegernic neurons utilizing nerve growth factor (NGF) is being developed. Phase 1 clinical trials resulted in AD patients demonstrating improvement in the rate of cognitive decline, suggesting that *in vivo* NGF gene transfer may have a future role in the treatment of AD (Felgin and Eidelberg, 2007). Similar to NGF gene transfer, choligernic neurons may be targeted by the use of antisense RNA. Antisense RNA for acetylcholinersterase mRNA has shown some promise in mouse models. Other approaches of AD gene therapy involve targeting the genes and proteins responsible for the formation of amyloid plaques and NFTs using RNA interference.

Lastly of interest in the advancing of AD gene therapy is the BCL-2 protein family. Studies have found that expression levels of BCL-2 family proteins, such as Bax, Bak, Bad, Bcl-2, Bim, Bcl-w and Bcl-x are altered in affected neurons in individuals with AD. A recent study by Kudo et al. reported the importance of Bax in formation of Aβ plaques and neuronal death. Inhibition of Bax activity through Bax-inhibiting peptide and bax gene knockout significantly suppresses Aβ neurotoxicity ex vivo and in vivo, indicating that Bax is a critical mediator of Aβ neuronal cell death. Thus, Bax may serve as a new therapeutic approach for the treatment of AD (Kudo et al., 2012).

Another field of interest in the management of AD is targeted immunotherapy, which can be either active or passive. Whereas active immunotherapy utilizes vaccinations with either $A\beta_{42}$ or synthetic fragments to generate a response of antibodies against the antigen, passive immunotherapy utilizes antibodies to create short-term immunizations. Attempts for active immunity against Aβ are still in phase II of human trials. First-generation vaccinations utilizing a synthetic full-length $A\beta_{42}$ peptide resulted in 6% of patients developing cerebral inflammation and aseptic meningoencephalitis. Fortunately, a second-generation vaccine using a shorter $A\beta_{42}$ peptide fragment was designed by Novartis. This vaccination, CAD 106, was the first to reach clinical phase II and resulted in an antibody response of 75% without any adverse effects.

Passive immunizations utilize administration of monoclonal or polyclonal antibodies targeted against Aβ, rather than vaccinations. Animal models show that passive anti-Aβ immunotherapies neutralize soluble amyloid oligomers in the brain and improve cognitive function. Intracerbroventricular (icv) injection of anti-Aβ antibodies in mouse models have shown increases in synaptic plasticity in the hippocampus, reversing memory deficits (Thakker et al., 2009). Bapineuzumab and solanezumab are two monoclonal antibodies that reached late clinical trials in humans. Bapineuzumab has shown ability to reduce CSF P-Tau in AD patients, but is unable to improve cognitive function. Solanezumab suggested improved cognitive function but without statistical significance. Thus, for their lack of efficacy, bapineuzumab and solanezumab failed to phase III trials. Other monoclonal antibodies with affinity to Aβ fibers are currently being investigated in patients at risk for developing AD, with patient safety as a priority.

Potential Anti-inflammatory Treatments

According to the Neuroinflammatory Hypothesis, AD may be a result of an inflammatory response initiated by Aβ or tau aggregates. Several studies have aimed to evaluate the role of nonsteroidal anti-inflammatory drugs (NSAIDs), anti-oxidants, and cytokine modulators on the symptoms of AD.

NSAIDs have a potential role in neuroprotection by reducing the inflammatory response caused by microglial and astroglial cells. Epidemiological studies have established a link between (NSAIDs) and reduced risk of AD (Morales et al., 2014). For example,

Ibuprofen has shown to reduce amyloid plaque deposits in transgenic mice. Conversely, some clinical trials of the effect of NSAID treatment on cognitive decline in AD do not show clear results. For example, trials with Naproxen and Celecoxib indicate attenuation of cognitive decline.

Several molecules addressing oxidative damage have been evaluated as potential treatments for the symptoms of AD. Some antioxidants that have been examined to play a role in neuroprotection include *Andean Compound*, turmeric, and Resveratol. *Andean Compound* is a complex mix of natural antioxidants in which the major active ingredient, fulvic acid, is thought to have protective properties against neurodegenerative disorders. One study has found that fulvic acid is able to block tau self-aggregation *in vitro* (Morales et al., 2014). Additionally, the herb turmeric which contains the compound curcumin, has demonstrated prevention of neuronal death in animal models. Curcumin has also been found to stimulate hippocampal neurogenesis in adult mice. Another antioxidant, Resveratrol, has been investigated for its anti-inflammatory effects and has been recently found to inhibit Aβ formation *in vitro.* This compound has other potential properties including cardioprotection, anticancer, and antiaging effects. Numerous antioxidants, have received of attention, but after investigation did not show any significant effects on cognition. For example, Ginko biloba, folate, vitamin B6, and vitamin B12 fail to show any cognitive advantages compared to the placebo groups in large randomized trials (Ballard et al., 2011; Yiannopoulou and Papageorgiou, 2013).

Recent studies have shed light on the role of cytokine inhibitors to improve cognition. For instance, inhibition of tumor necrosis factor (TNF), which plays a role in the inflammatory response, has been shown to increase cognitive impairment. Multiple approaches are underway to find successful treatments for AD. Table 10 below summarizes evidence gathered from current and emerging treatment approaches.

***Table 10*. Current and Proposed Treatment for Alzheimer's Disease (Ballard et al., 2011).**

	Drugs	Status	Evidence
Symptomatic treatments			
Cholinesterase inhibitors	Donepezil, rivastigmine, galantamine	Licensed for mild-to-moderate Alzheimer's disease	More than 30 placebo-controlled randomised controlled trials, mainly of 6 months duration in patients with mild-to-moderate Alzheimer's disease (MMSE 10–26). Significant benefits in cognition, function, and global outcome, with MMSE gain of 1·5–2 points over 6–12 months. Several studies suggest similar benefit in severe Alzheimer's disease[74,75]
NMDA receptor antagonist	Memantine	Licensed for moderate-to-severe Alzheimer's disease	Significant benefit in cognition, function, global outcome, and neuropsychiatric symptoms over 6 months in three trials of moderate-to-severe Alzheimer's disease[76]
Treatments for neuropsychiatric symptoms			
Atypical antipsychotics	Risperidone, quetiapine, olanzapine, aripiprazole	Risperidone licensed for short-term treatment of severe aggression in Alzheimer's disease; other treatments are used off licence	Significant but modest efficacy for the treatment of aggression (SES 0·2–0·25) and psychosis (SES 0·15–0·2) over 6–12 weeks. Limited evidence of longer term benefits. Atypical antipsychotics associated with significant increase in stroke (RR 2·5–4·0) and death (RR 1·5–1·8)[77]
Antidepressants	Citalopram, sertraline	All antidepressants used off licence in Alzheimer's disease	Evidence not clear-cut. The largest trial with sertraline suggested no benefit for the treatment of depression in patients with Alzheimer's disease.[78] Severe depression should be treated, probably with a selective serotonin reuptake inhibitor
Anticonvulsants	Carbamazepine	Used off licence	There is preliminary evidence from small randomised controlled trials that carbamazepine might be an effective treatment for agitation or aggression in Alzheimer's disease[79]
Proposed disease-modifying treatments			
Immunotherapy	Bapineuzumab	In phase 3 clinical trials	Passive immunotherapy treatments show some benefit in animal models of Alzheimer's disease.[80] Bapineuzumab is in phase 3 clinical trials
Sectretase inhibitors	Tarenflurbil, semagacestat	In phase 3 trials	Tarenflurbil failed in phase 3 trials.[81] Semagacestat is in a phase 3 clinical trial programme at present
Amyloid aggregators	Tramiprosate	Discontinued	Failed in phase 3 trials[82]
Copper or zinc modulators	PBT2	Phase 2 clinical trials	PBT2 resulted in a decrease in cerebrospinal fluid amyloid and provided significant clinical benefit in a phase 2 clinical trial.[83,84] Phase 3 trials are awaited
Tau aggregation inhibitors	Methylthioninium chloride	Phase 2 clinical trial	A promising phase 2 trial suggested significant cognitive benefit over 52–78 months of follow-up, but there were major methodological limitations[85]
GSK3 inhibitors	Lithium	Early-phase clinical trials	The mood stabilising drug lithium inhibits the enzyme GSK3 and reduces the phosphorylation of tau in animal models.[86] Early-stage clinical trials are in progress
Natural products and vitamins	Vitamin E, ginkgo biloba, omega 3 fatty acids, and docosahex aenoic acid	Phase 2 and phase 3 clinical trials	Despite initial promise, a more recent randomised controlled trial in mild cognitive impairment did not report any benefit with vitamin E[87,88] A meta-analysis suggested ginkgo biloba might provide moderate benefit, but a large randomised trial did not show any advantage of ginkgo biloba compared with placebo[89,90] A large randomised controlled trial of omega 3 fatty acids did not report any benefit on function or cognition, but did suggest some possible benefit on neuropsychiatric symptoms in a post-hoc subgroup analysis.[91] A National Institute on Aging phase 3 trial of docosahexaenoic acid is in progress

MMSE=mini mental state examination. SES=sodoeconomic status. RR=relative risk. GSK3=glycogen synthase kinase 3.

Results of various treatments for AD: symptomatic treatments, treatment for neuropsychiatric symptoms, and proposed disease-modifying agents.

III. Hypotheses

Every year, millions of new cases of new AD cases are diagnosed, resulting in dementia and death of elderly individuals and increasing expenses of medical treatment. There remains a strong need for the early detection and screening of the disease as well as statistical evidence of biomarker specificity. I have chosen to focus on examining the value of neuroimaging, selected cognitive tests, genotype and biomarkers in diagnosing, detecting, and predicting AD.

My aims are in line with those of the Alzheimer's Disease Neuroimaging Initiative (ADNI), a collaborative longitudinal study that serves to aid researchers and clinicians in the development of new AD treatments and to increase the effectiveness of clinical trials. One of the primary goals of ADNI is the development of standardized neuroimaging and biomarker methods for AD clinical trials, as well as using these to measure changes longitudinally in control, MCI, and AD subjects.

Based on the research gathered, I have conducted statistical analyses to test the following hypotheses:

1. Temporal lobe atrophy (specifically hippocampal volumes) significantly correlates with progression of AD and serves as a marker for measuring progression of AD within controls, MCI, and AD subjects. This will be assessed by evaluating the statistically significant difference of hippocampal volume between cognitive groups over time (Schuff et al., 2009).
2. There is a statistically significant correlation between hippocampal atrophy, cognitive testing scores (MMSE and ADAS-Cog13) between groups over time. Because the ADAS-Cog 13 includes domains for testing various types of cognition, it is expected that this test will demonstrate more sensitivity to hippocampal volume changes over time.
3. The APOE gene is associated with the reduced ability to clear Aβ from the brain. It has been found that individuals with a single copy of the ε4 allele have 5-fold risk of developing LOAD. Subjects who are APOE-ε4 positive subjects will reflect greater disease progression than APOE-ε4 negative subjects (Shaw, 2009).

This will be assessed by evaluating longitudinal hippocampal loss by genotype, as well as the correlation of having a positive genotype to receiving differential diagnoses of AD.

4. The three core CSF biomarkers ($A\beta_{42}$, T-Tau, and P-tau) as well as combinations ($A\beta_{42}$/T-tau and $A\beta_{42}$ /P-Tau) will yield high sensitivity, specificity, and predictive value in the diagnosis of AD.

IV. Methods

Data Source

The Alzheimer's Disease Neuroimaging Initiative (ADNI) is a collaborative longitudinal study that serves to aid researchers and clinicians in the development of new AD treatments and to increase the effectiveness of clinical trials. ADNI researchers seek to measure the progression of MCI and early AD by identifying relationships between neuroimaging, biomarker, and cognitive assessment data over time. The identification of analyses with high statistical power of detection would act as tools for earlier and more accurate diagnosis of AD, allowing for a slowing of its progression. Clinical, neuroimaging, genetic, and biomarker data are collected from subjects from over 50 sites in North America. ADNI is organized into eight cores; each with separate responsibilities. The cores are comprised of the Clinical Core, the MRI and PET Cores, the Biomarker Core, the Genetics Core, the Neuropathology Core, the Biostatistics Core, and the Informatics Core. The ADNI study has been conducted in 4 phases: ADNI1 (5 years from October 2004), ADNIGO (2 years from September 2009), ADNI2 (5 years from September 2011), and the ADNI3 (6 years from September 2016). New participants are recruited in each phase of the study, while existing participants are tracked over time to monitor progression. An ADNI Data Use application and agreement were sent and approved of.

Data discussed in this report were obtained from the freely available ADNI database (www.adni.loni.usc.edu). Datasets downloaded include the ADNIMERGE package (on September 23rd, 2017), which includes all categories of results from each phase, minus genetic (SNP and biomarker) data. Biomarker data was downloaded also from the ADNI database. This dataset, titled "UPENNBIOMK9," includes the UPENN CSF Biomarkers

Elecsys data, which shows $A\beta_{42}$, P-tau, and T-tau results for ADNI1, ADNIGO, and ADNI2 subjects. Roche Elecsys immunoassays were performed in a series of 36 runs for each biomarker test. A total of 2401 samples were included in this analysis (Shaw et al., 2017). Technical limits, statistical adjustments and other details may be found at (www.adni.loni.usc.edu). The data needed for inclusion within the biomarker test were the three CSF biomarker concentrations (AB42, P-tau, and T-tau), baseline diagnoses, and differential diagnoses. Diagnoses data were matched from within the ADNIMERGE dataset.

Subjects

From the ADNIMERGE package, subjects from only the ADNI1, ADNIGO, and ADNI2 phases were selected. The ADNI3 cohort was excluded because this study is ongoing until 2022 and results will be inconclusive. Each ADNI phase consists of subjects given baseline diagnoses of the following: cognitively normal (CN), significant memory impairment (SMC), early MCI (eMCI), late MCI (lMCI), or AD. Subjects were excluded if they had any history of coexisting neurological disease, brain trauma, or psychoactive drug use (http://www.adni-info.org/Scientists/doc/ADNI_review_update2013-manuscript.pdf). Subjects were given a baseline diagnosis of CN if they show no signs of depression, MCI, or dementia. Diagnosis of SMC indicates that the subject scored within normal range on cognitive tests, but exhibited "slight forgetfulness." MCI subjects were given a diagnosis of eMCI or lMCI as determined by the Wechsler Memory Scale Logical Memory II. Lastly, subjects diagnosed as AD met the NINCDS/ADRDA criteria for probable AD (http://adni.loni.usc.edu/study-design/background-rationale/). Group specific inclusion criteria for each cohort can be found below in Table 11. Further screening information can be found within procedure manuals specific to the cohort at http://adni.loni.usc.edu/methods/documents/.

***Table 11*. Group Specific Inclusion Criteria.**

Baseline Diagnosis	Cohort	Criteria
CN (Cognitively Normal)	ADNI 1/GO/2	• Absence of impairment of cognition or daily function. • Normal memory function determined by Wechsler Memory Scale (delayed paragraph recall) II (maximum score is 25). • Mini-Mental State Exam Score between 24-30. • CDR = 0, Memory Box = 0
SMC (Significant Memory Concern)	ANDI 2	• Absence of impairment of cognition or daily function but with concern of slight forgetfulness. • Normal memory function determined by Wechsler Memory Scale (delayed paragraph recall) II (maximum score is 25). • Mini-Mental State Exam Score between 24-30. • CDR = 0, Memory Box = 0
eMCI (Early Mild Cognitive Impairment)	ADNI GO/2	• Memory complaint by subject or study partner. • Abnormal memory function determined by Wechsler Memory Scale (delayed paragraph recall) II (maximum score is 25). • Mini-Mental State Exam Score between 24-30. • CDR = 0.5, Memory Box at least 0.5 • General cognition and functional performance does not meet criteria for probable AD.
MCI (Mild Cognitive Impairment)	ADNI 1	
lMCI (Impairment)	ADNI GO/2	
AD (Alzheimer's Disease)	ADNI 1/GO/2	• Memory complaint by subject or study partner. • Abnormal memory function determined by Wechsler Memory Scale (delayed paragraph recall) II (maximum score is 25). • Mini-Mental State Exam Score between 20-26. • CDR = 0.5, Memory Box = 1.0 • NINCDS/ADRDA criteria for probable AD.

Diagnoses per cohort including the following criteria: memory complaint, cognitive impairment, daily function impairment, memory function determined by Weschler Memory Scale II, MMSE score, CDR score, and probable AD criteria (http://adni.loni.usc.edu/study-design/background-rationale/).

All ADNI subjects underwent a series of neuropsychological tests. Tests were given at baseline, as well as every few months to monitor the progression of their cognitive states. These cognitive tests include 1) the MMSE, as a global measure of mental status 2) the ADAS-Cog (11 and 13 item), the most frequently used battery of cognitive tests in clinical trials 3) the Clinical Dementia Rating Scale (CDR), which rates the severity of the dementia, 4) the Functional Activities Questionnaire, which serves as a measurement of independence and 5) the Geriatric Depression Scale (GDS), a self-report tool used to identify depression and 6) the Wechsler Memory Scale, designed the measure different memory functions (http://www.adni-info.org/Scientists/doc/ADNI_review_update2013-manuscript.pdf).

All subjects included were also tested for *APOE* genotyping and were given a series of cognitive tests each time they received 1.5T or 3T MRI scans, for measuring progression. ADNI1 cohorts received imaging including MRI and FDG-PET. ADNIGO/2 participants received MRI and FDG-PET, as well as fMRI and imaging for microhemorrhage detection.

The ADNI1 cohort consisted of 200 elderly controls, 200 MCI and 400 AD subjects. ADNI1 subjects moved to the ADNIGO phase at month 36, 48, or 60.The ADNIGO phases consisted of 208 subjects from ADNI1 as well as 200 new MCI recruits. Lastly, the ADNI2 cohort included of 258 ADNI1 and 115 ADNIGO subjects, plus new subjects (150 elderly controls, 250 MCI, and 150 AD). Subjects willing to receive lumbar punctures were tested for CSF biomarker analysis, including $A\beta_{1\text{-}42}$, T-tau, and P-tau. Biomarker analysis was conducted by the University of Pennsylvania School of Medicine, which collects DNA, blood, urine, and CSF samples from all ADNI sites.

Of the subjects within the ADNI cohorts, subjects were excluded if they did not meet all of the following criteria: 1) APOE genotyping, 2) MMSE at each 6 month period for 24 months, 3) ADAS-Cog at each 6-month period for 24 months, and 4) hippocampal volume scores at each 6 month period for 24 months. Month 18 for each subject was also excluded due to lack of hippocampal volume scores globally. The subjects used for biomarker analysis were different that those selected for the other tests. The data needed

for inclusion within the biomarker test were the three CSF biomarker concentrations (AB42, P-tau, and T-tau), baseline diagnoses, and differential diagnoses.

Data Analysis

Datasets downloaded include the ADNIMERGE package, which includes all categories of results from each phase, minus genetic (SNP and biomarker) data. Subjects were excluded if they had missing data (i.e. blank APOE, MMSE, ADAS, or MRI fields). The demographics and clinical data at baseline were calculated in Excel 2016, using the descriptive statistics feature of the Analysis ToolPak add-in. The data from Excel was imported and analyzed in SPSS Statistics GradPack (Version 23.0).

The diagnostic value of hippocampal atrophy over time was explored within SPSS via analysis of variance (ANOVA). First, baseline hippocampal volume was regressed against baseline diagnoses via univariate general linear model at a 95% confidence interval with age as a covariate. The equality of variances were tested between groups using the Levene's Test of Equal Variances, where $p > 0.05$ would result in the conclusion that variances are equal. Significant differences were then explored between cognitive levels via pairwise post-hocs with Bonferroni adjustment. Hippocampal volume was analyzed longitudinally via repeated measures ANOVA (RMANOVA) per cognitive group, where cognitive group was determined by post-diagnosis. The times were selected at 6-month intervals, from baseline to 24 months of monitoring (excluding the 18 month interval due to lack of descriptive information within the database). Mauchly's Test of Sphericity was employed to detect violations of sphericity between all possible groups over time. Sphericity values > 0.75 were then corrected using the Greenhouse-Geisser correction made available within SPSS. Pair-wise comparisons using estimated measures with Bonferroni adjustment were evaluated between each possible group (time, cognitive group, and time* cognitive group). Lastly, a line plot was generated for visualization of hippocampal differences between groups.

The two neuropsychological tests that were chosen to compare with hippocampal data included the ADAS-Cog 13 and the MMSE, due to their ability to diagnose and monitor progression of the disease over time. The ADAS-Cog 13 is scored out of 85, where higher scores indicate increased cognitive impairment. The MMSE is scored out of 30,

where lower scores indicate increased cognitive impairment. To evaluate the correlation of cognitive test scores to hippocampal volumes, Pearson's correlation and 2-tailed T-tests were calculated via canonical correlation tests. The test scores for each interval were then regressed over time via repeated measures ANOVA (RMANOVA). Each RMANOVA was performed, where independent variable (time) was regressed against the dependent variables (MMSE scores or ADAS scores) between each cognitive group (CN, MCI, and AD). Each test employed the Mauchly's Test of Sphericity with corrections. Pair-wise comparisons using estimated measures with Bonferroni adjustment were evaluated between each possible group (time, cognitive group, and time*dependent variable). Linear plots were generated for both RMANOVAs for visualization.

Descriptive statistics of genotype frequencies within cognitive levels were attained. This was followed by obtaining descriptive statistics of genotype frequencies grouped by disease progression (CN-stable, MCI-stable, AD-stable, CN-MCI, or MCI-AD). A histogram was created to visualize relationship between number of APOE- ε4 alleles and progression. To test the effect of AD progression due to APOE-ε4 genotyping, a multinominal regression was employed where APOE-ε4 genotype was the independent variable and progression was the dependent variable. Lastly mixed-model RMANOVAs were employed, where the independent variables (time and genotype) were regressed against hippocampal scores between each cognitive group. The same measures of sphericity were taken and were adjusted for using the Greenhouse-Geisser correction. Pairwise and multiple comparisons between each possible group were evaluated after Bonferroni adjustment. Line plot were then generated for each cognitive group for visualization of slopes.

Lastly, CSF biomarker positivity diagnostic value was assessed in normal, MCI, and AD subjects. Receiving Operator Characteristic (ROC) curves were employed utilizing concentrations of CSF biomarkers ($A\beta_{42,}$ T-tau, and P-tau) and combinations ($A\beta_{42}$/T-tau and $A\beta_{42}$/P-Tau$_{)}$ at a 95% confidence interval. Optimal cutoff values were assessed utilizing the coordinates of the curve with minimal distance to the top left corner of the box. This is the point where the true positivity rate (sensitivity) is 1 and the false

negativity rate (1-specificity) is 0. This distance was calculated in Excel using the formula below where SE = sensitivity and SP = specificity:

$$\text{Distance} = \sqrt{(1\text{-SE})^2+(1\text{-SP})^2}$$

After the optimal cutoff value was attained for each test, sensitivity (SE), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV) were calculated utilizing the following formulas:

- SE = TP/(TP+FN)
- SP = TN/((TN+FP)
- PPV = TP/(TP+FN)
- NPV = TN/(TN+FN)

Where TP, FP, TN, and FN represent true positive, false positive, true negative, and false negative scores, respectively. Scores with the highest values were selected as accurate predictors of AD.

V. Results

Hippocampal Volume Regressions

The main demographic and clinical data at baseline for all 3 cohorts are summarized in Table 12 below. Though each group demonstrated comparable age and sex distributions, and cognitive scores were markedly different. Subjects with AD have more than two-fold higher rates of APOE-ε4 carriers than the normal subjects. Chi-square values indicated that gender does not play a significant role.

Table 12. **ADNI Participant Demographics and Clinical Data at Baseline.**

Measure	Cognitively Normal	Late Mild Cognitive Impairment	Alzheimer's Disease
N	125	122	54
Women (%)	49.6	38.5	48.2
Age (years)	75.2±8.5	73.8±9.7	73.6±12.3
MMSE [a]	29.1±2.8	27.1±3.0	23.3±3.8
ADAS-Cog 13 [b]	8.8±3.9	18.3±6.7	28.0±8.9
APOE-ε4 Carriers (%) [c]	27.2	51.6	66.7

a) MMSE; Mini-Mental State Examination; range 0–30 points. b) ADAS-Cog 13; Alzheimer's disease Assessment Scale—Cognitive Subscale (13-item); range 0–85 points. c) APOE-ε4 Carriers (%); the percentage of subjects with 1 or 2 APOE-ε4 alleles (http://www.adni-info.org/Scientists/doc/ADNI_review_update2013-manuscript.pdf)**.**

Baseline hippocampus volumes were regressed against baseline diagnoses. Variances between groups were equal, per the Levene's test of equal variances. Differences between hippocampal volumes between baseline cognitive levels were significant ($p < 0.005$). Mean differences of volumes per group after Bonferroni adjustments via pairwise comparison can be seen below in Table 13 below.

Table 13. **Pairwise Comparisons of Baseline Hippocampal Volumes between Baseline Cognitive Levels.**

(I) DX_bl	(J) DX_bl	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
AD	CN	-1613.356*	161.804	.000	-2002.917	-1223.794
	LMCI	-657.227*	161.826	.000	-1046.841	-267.613
CN	AD	1613.356*	161.804	.000	1223.794	2002.917
	LMCI	956.129*	126.570	.000	651.396	1260.861
LMCI	AD	657.227*	161.826	.000	267.613	1046.841
	CN	-956.129*	126.570	.000	-1260.861	-651.396

Significant differences based on estimated marginal means at the 95% confidence interval. Adjustment for multiple comparisons: Bonferroni. AD = Alzheimer's Disease; CN = cognitively normal; LMCI = late mild cognitive impairment.

To evaluate the progression of hippocampal volumes between cognitive groups longitudinally, RMANOVA was performed. Table 14 below shows mean hippocampal volumes per cognitive group at each interval. Mean baseline hippocampal volumes (cm^3)

for CN, MCI, and AD were 7244.90±928.96, 6649.08±1030.60, and 5814.50±1169.255, respectively.

Table 14. **Descriptive Statistics of Hippocampal Volume (cm^3) per Group by MRI Scan Interval**.				
Baseline Diagnosis		Mean Hippocampal Volume (cm^3)	Standard Deviation	N
Baseline	AD	7244.90	928.957	120
	CN	6649.08	1030.596	78
	MCI	5814.50	1169.255	103
	Total	6601.03	1207.717	301
Month 6	AD	7223.27	1004.617	120
	CN	6592.32	1051.938	78
	MCI	5690.21	1175.697	103
	Total	6535.17	1260.829	301
Month 12	AD	7189.83	975.562	120
	CN	6490.79	1063.629	78
	MCI	5584.60	1132.723	103
	Total	6459.39	1257.193	301
Month 24	AD	7078.12	995.571	120
	CN	6324.37	1167.706	78
	MCI	5392.22	1145.001	103
	Total	6305.89	1309.243	301

Volumetric data was taken at baseline, 6-month, 12-month, and 24-month intervals for cognitively normal (CN), mild cognitive impairment (MCI), and AD subjects.

Hippocampal volume values were significant between time intervals and cognitive groups ($p < 0.005$). The differences in hippocampal volume atrophy between each cognitive group can be seen in Figure 19.

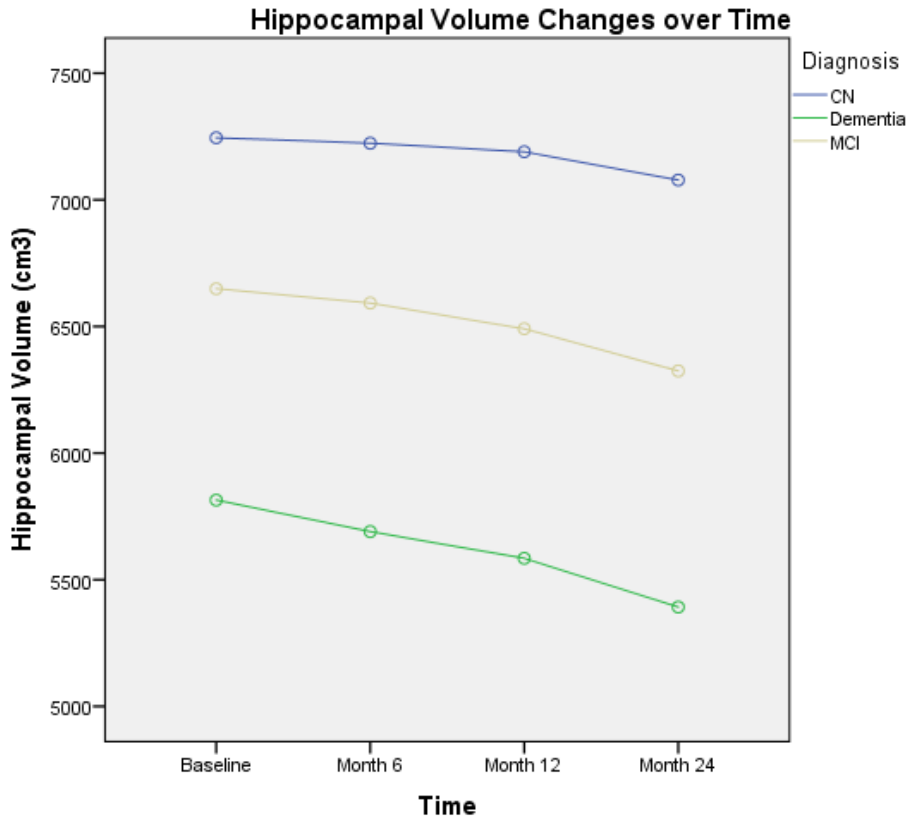

Figure 19. **Hippocampal Volume Changes over Time.** Mean hippocampal volume change (cm^3) per cognitive group (CN, MCI, and AD) over four intervals (BL – baseline, m06 – month 6, m12 – month 12, and m24 – month 24).

Cognitive Tests Score Regressions

Cognitive test scores were regressed against hippocampal volumes per group for each interval via RMANOVA. The same subjects were used for this analyses as for the hippocampal volume regressions. Descriptive statistics for ADAS-Cog 13 scores and MMSE scores are shown in Table 15 below. The ADAS-Cog 13 is scored out of 85, where higher scores indicate increased cognitive impairment. The MMSE is scored out of 30, where lower scores indicate increased cognitive impairment.

Table 15. **Descriptive statistics of cognitive test scores (ADAS-Cog 13 and MMSE) per group by intervals.**

Time	DX	Mean	SD	N
Baseline	CN	8.43	3.635	116
	Dementia	24.05	7.275	95
	MCI	16.32	5.689	75
	Total	15.69	8.722	286
Month 6	CN	8.82	3.952	116
	Dementia	26.53	8.093	95
	MCI	16.56	6.023	75
	Total	16.73	9.738	286
Month 12	CN	8.01	3.966	116
	Dementia	27.82	9.076	95
	MCI	16.28	7.047	75
	Total	16.76	10.882	286
Month 24	CN	8.58	4.316	116
	Dementia	33.27	11.315	95
	MCI	18.59	7.123	75
	Total	19.41	13.226	286

Time	DX	Mean	SD	N
Baseline	CN	29.11	1.027	120
	Dementia	24.95	2.534	102
	MCI	27.51	1.756	78
	Total	27.28	2.567	300
Month 6	CN	29.12	1.006	120
	Dementia	23.95	3.078	102
	MCI	27.65	1.960	78
	Total	26.98	3.110	300
Month 12	CN	29.22	1.139	120
	Dementia	22.93	4.101	102
	MCI	27.74	2.003	78
	Total	26.70	3.860	300
Month 24	CN	29.11	1.052	120
	Dementia	20.59	5.211	102
	MCI	27.12	2.604	78
	Total	25.69	5.046	300

Left: Descriptive statistics of ADAS-Cog scores by group. Right: Descriptive statistics of MMSE scores by group. Cognitive test scores taken at baseline, 6-month, 12-month, and 24-month intervals for cognitively normal (CN), MCI, and AD subjects. Groups determined by differential diagnosis.

The Pearson's correlation of ADAS-Cog 13 scores and hippocampal volumes over time can be viewed in Table 16. Scores on the ADAS-Cog 13 test negatively correlated with hippocampal volumes at each interval. MMSE scores and hippocampal scores exhibited a positive correlation with significance.

Table 16. **Canonical Correlations of ADAS-Cog 13 and MMSE Scores to Hippocampal Volume over Time.**

Interval	Test	ADAS-Cog 13	MMSE
Baseline	Pearson Correlation Sig. (2-tailed)	-.479 .000	.490 .000
Month 6	Pearson Correlation Sig. (2-tailed)	-.495 .000	.468 .000
Month 12	Pearson Correlation Sig. (2-tailed)	-.552 .000	.502 .000
Month 24	Pearson Correlation Sig. (2-tailed)	-.546 .000	.492 .000

Pearson's correlation and 2-tailed T-test significance between test scores and hippocampal volume at each interval (baseline, month 6, month 12, and month 24).

ADAS-Cog 13 and MMSE scores were significant different ($p < 0.005$) between each cognitive group (CN, MCI, and AD). However, ADAS scores did not demonstrate significant values between the month 6 and month 12 intervals ($p = 1.000$). ADAS scores for each cognitive level (CN, MCI, and AD) ranged from 7.37-9.55, 15.58-18.29, and 26.72-29.12, respectively.

As stated earlier, MMSE scores were statistically significant between all cognitive groups. Conversely, MMSE scores were not statistically different from each other between baseline and month 6 ($p = 0.055$) as well as month 6 and month 12 ($p = 0.163$). MMSE were statistically different from each other between all other intervals (baseline-month 12, baseline – month 24, month 12 – month 24, and month 6 - month 24). Mean MMSE scores for each cognitive group (CN, MCI, and AD) ranged between 28.76-29.31, 27.04-27.97, and 22.70-23.513, respectively. ADAS-Cog 13 and MMSE score changes are displayed in Figure 20 and 21 below, respectively.

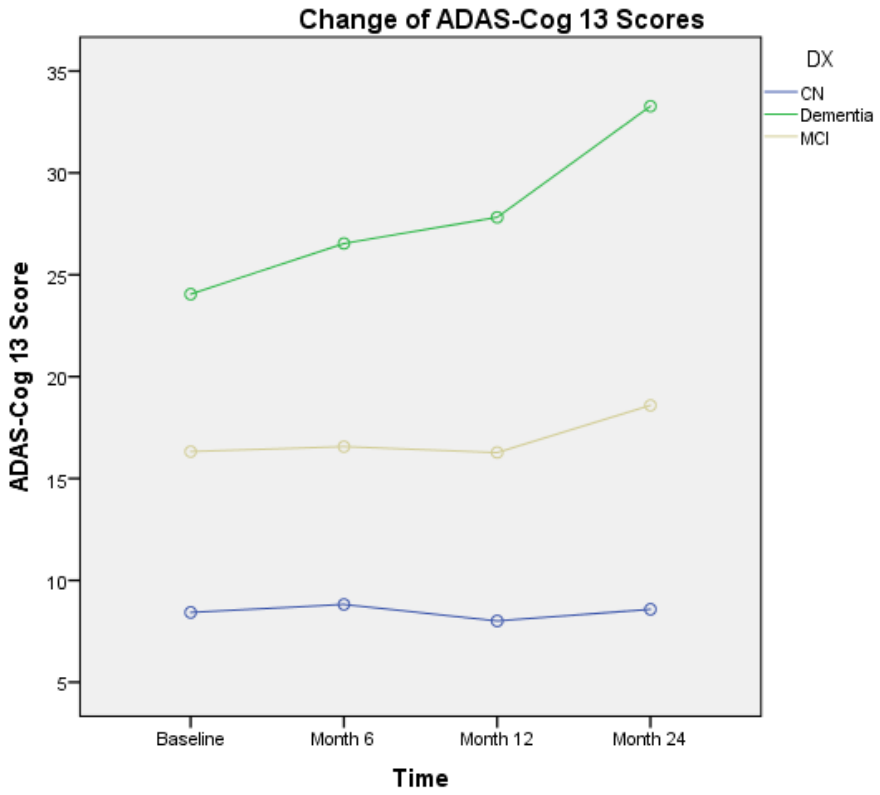

Figure 20. **Change of ADAS-Cog 13.** Mean ADAS-Cog 13 scores within cognitive groups (CN, MCI, and AD) over four intervals (baseline, month 6, month 12, and month 24).

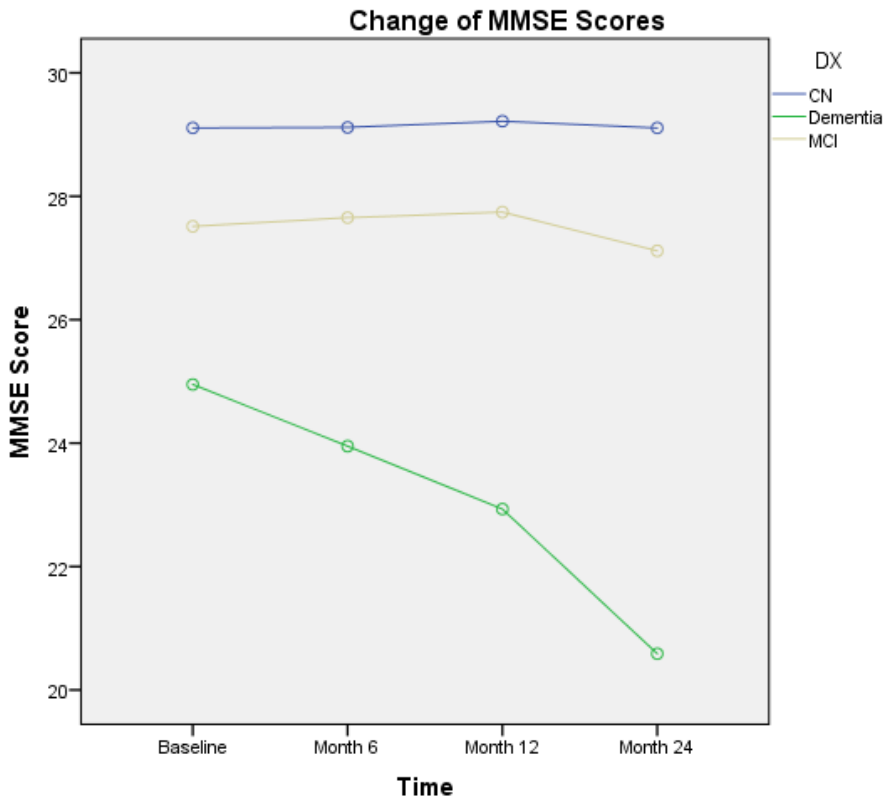

Figure 21. **Change of MMSE Scores.** Mean MMSE scores within cognitive groups (CN, MCI, and AD) over four intervals (baseline, month 6, month 12, and month 24).

Regression between APOE-ε4 Genotype and Time on Hippocampal Volume

Descriptive statistics for each genotype (0 APOE-ε4 alleles, 1 APOE-ε4 allele, and APOE-ε4 alleles) can be found in Table 17. Frequencies of subject disease progression (stable CN, stable MCI, stable AD, CN-MCI, or MCI-AD) grouped by genotype are found in Figure 22.

Table 17. **APOE-ε4 Descriptive Statistics by Group.**

		DX			
		CN	Dementia	MCI	Total
Number of APOE-ε4 Alleles	0	90	36	42	168
	1	28	47	28	103
	2	2	20	8	30
Total		120	103	78	301

Frequencies and percentages of subjects with each genotype (0 APOE-ε4 alleles, 1 APOE-ε4 allele, and APOE-ε4 alleles) by cognitive level (CN, MCI, and AD).

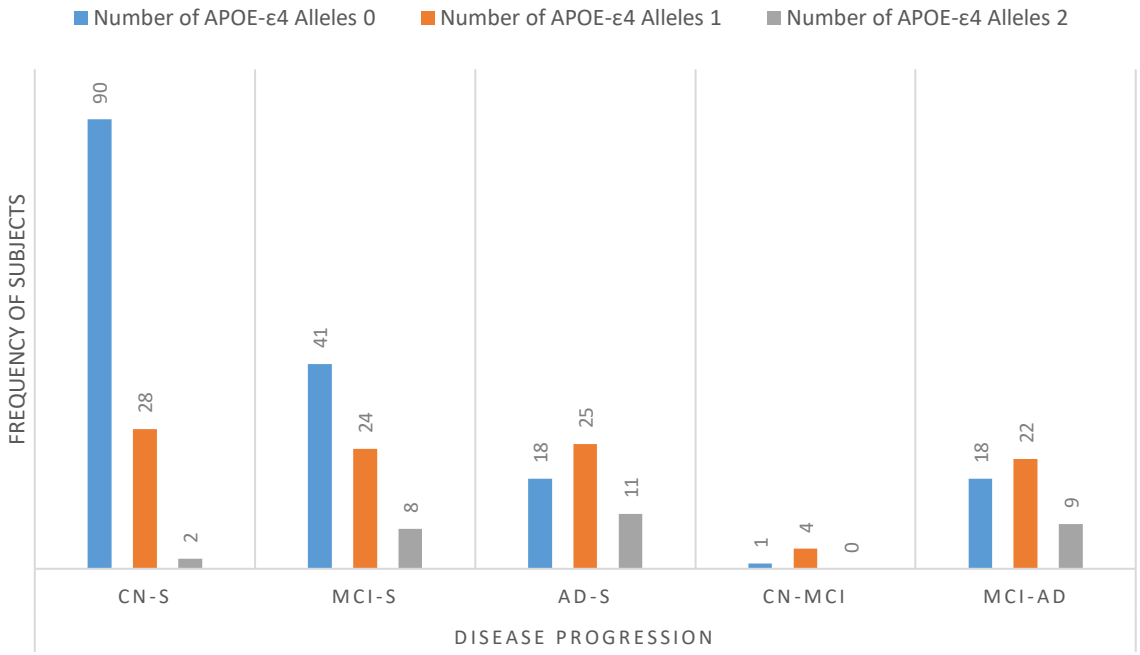

***Figure 22*. Frequencies of Subject disease progression Grouped by APOE-ε4 Genotype**. CN-S = stable CN; MCI-S = stable MCI; AD-S = stable AD; CN-MCI = progression from CN to MCI; and MCI-AD = progression from MCI to AD. Stable is used to describe lack of diagnosis change. Genotypes are represented by number of APOE-ε4 alleles (0 APOE-ε4 alleles, 1 APOE-ε4 allele, and APOE-ε4 alleles).

Multinominal logistic regression revealed there was no significance between number of APOE-ε4 allele and disease progression. Parameter estimates depicted significance only for the APOE-ε4 negative genotype. However, RMANOVA Multivariate Tests revealed statistically significant hippocampal volumes over time as a result of genotype within MCI subjects (p = 0.004). Hippocampal volume differences were not significant between genotypes within the CN or AD groups. Individual line plots of longitudinal hippocampal volume grouped by genotypes are shown below in Figures 23-25, separated by diagnosis.

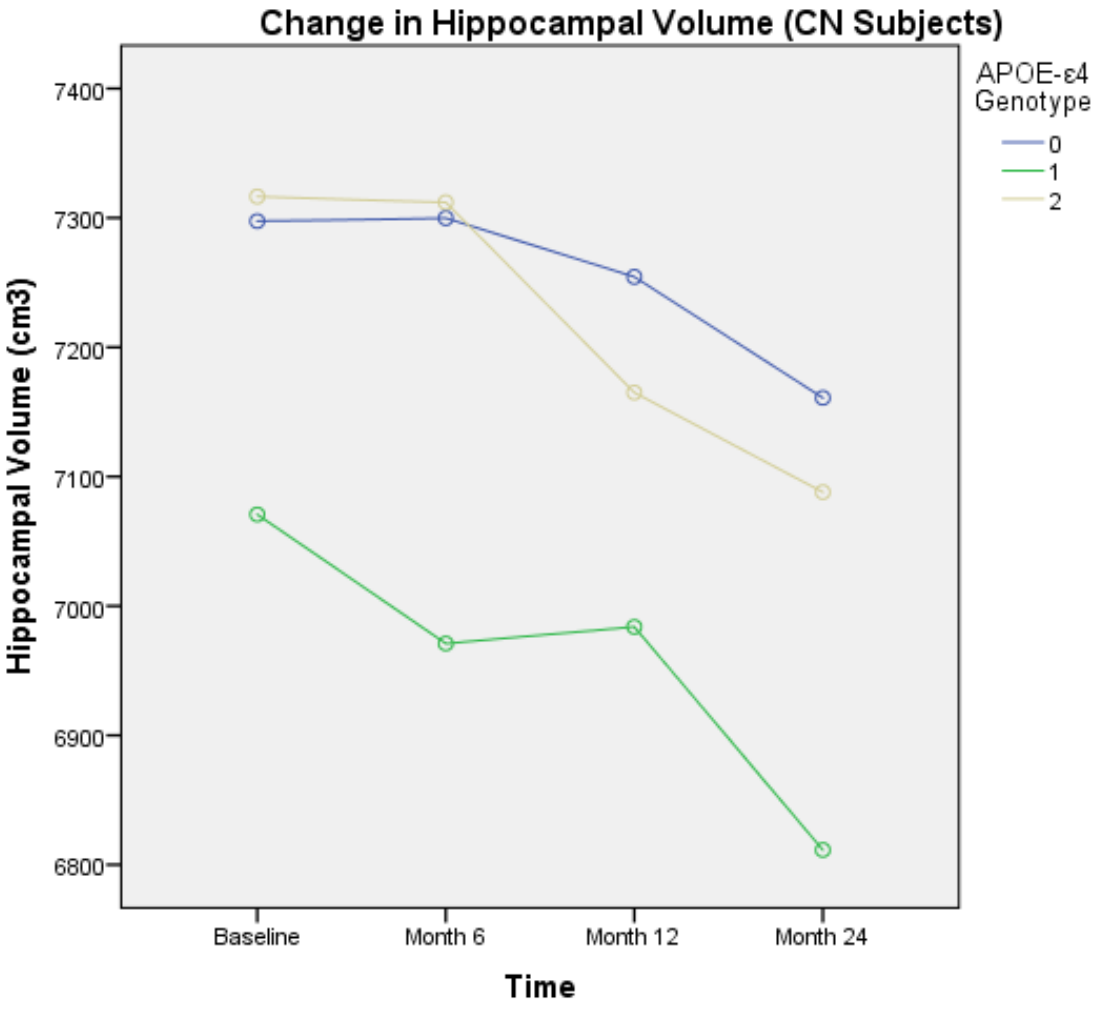

Figure 23. **The Change in Hippocampal Volume between CN subjects by APOE-ε4 Genotype.** 0; no APOE-ε4 alleles, 1; one APOE-ε4 allele, and 2; two APOE-ε4 alleles.

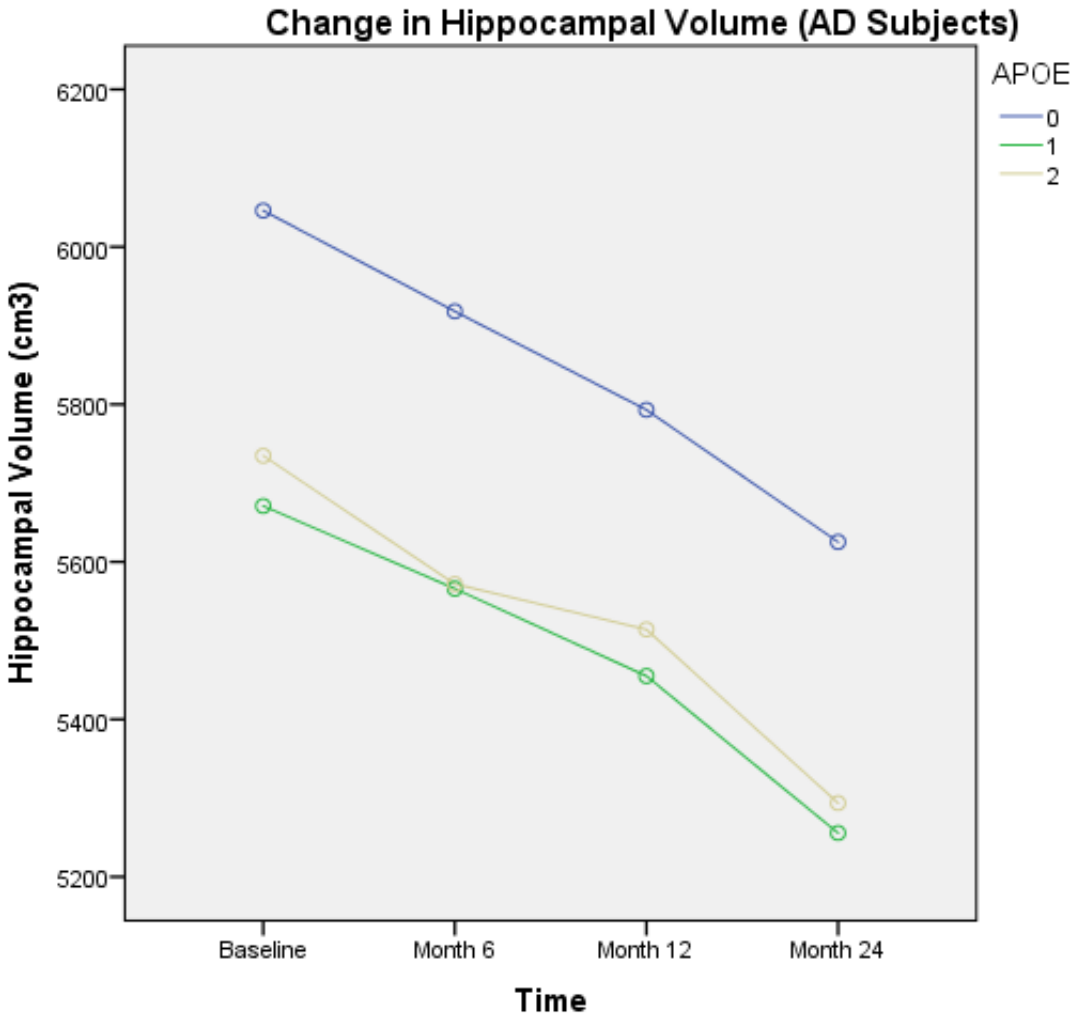

Figure 24. **The Change in Hippocampal Volume between MCI subjects by APOE-ε4 Genotype.** 0; no APOE-ε4 alleles, 1; one APOE-ε4 allele, and 2; two APOE-ε4 alleles.

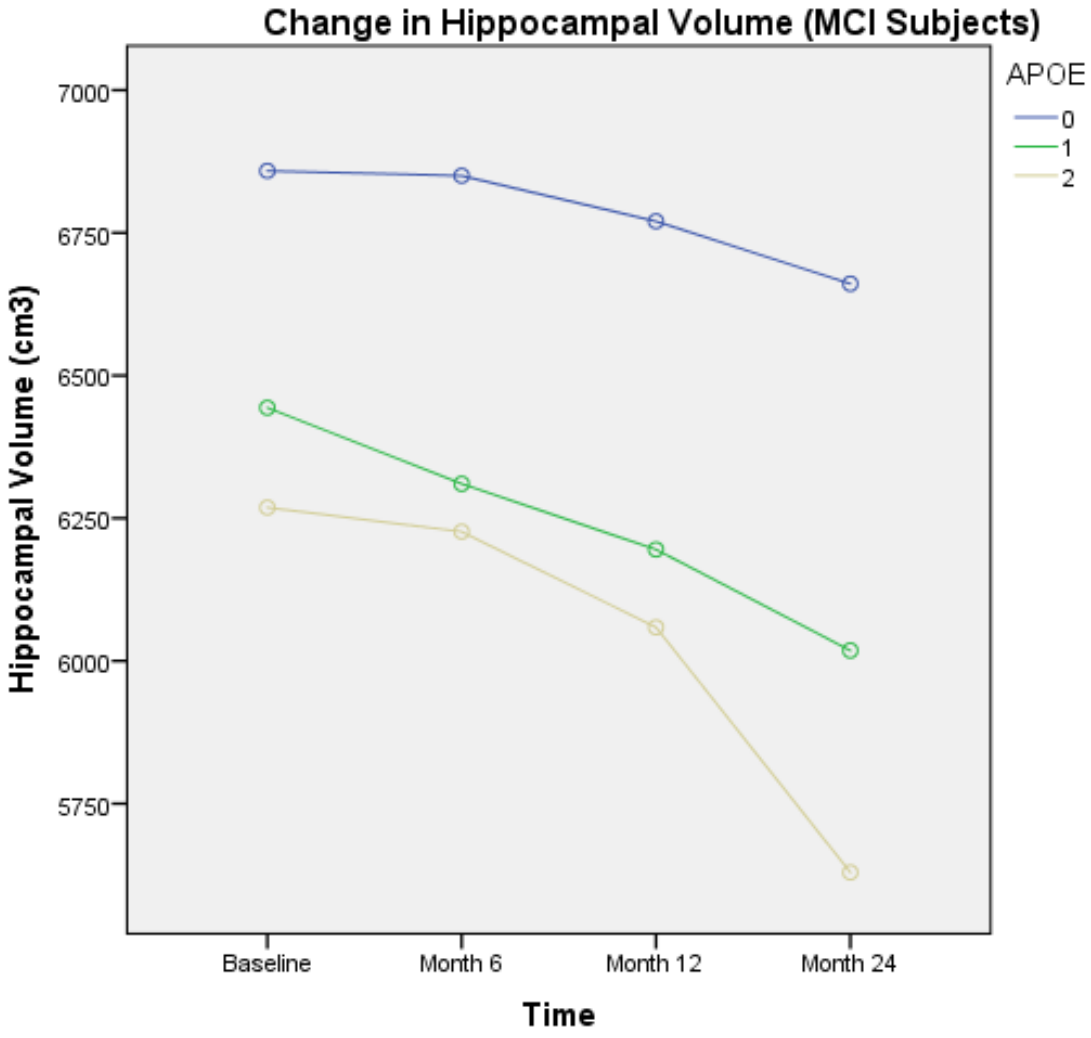

Figure 25. **The Change in Hippocampal Volume between AD subjects by APOE-ε4 Genotype.** 0; no APOE-ε4 alleles, 1; one APOE-ε4 allele, and 2; two APOE-ε4 alleles.

Biomarker Sensitivity, Specificity, and Predictive Values

The ROC Curve analyses of biomarkers included 211 subjects of various cognitive levels. ROC curves were employed to assess optimal cutoff values for each biomarker ($A\beta_{42}$, T-Tau, and P-tau) and ratios ($A\beta_{42}$/T-tau and $A\beta_{42}$/P-tau). Cutoff values discriminating AD subjects from controls are as follows: $A\beta_{42}$ < 741.75 pg/mL (SE: 84.09%) (SP: 33.33%) (PPV: 72.55%) (NPV: 88.14%); T-Tau > 256.85 pg/mL (SE: 77.27%) (SP: 78.79%) (PPV: 70.83%) (NPV: 83.87%); P-tau > 26.38 pg/mL (SE: 50%) (SP: 83.33%) (PPV: 50%) (NPV: 83.33%); $A\beta_{42}$/T-tau < 1.9905; (SE: 79.55%) (SP: 92.42%) (PPV: 87.5%) (NPV: 87.14%); and $A\beta_{42}$/P-tau < 17.26; (SE: 70.45%) (SP:

95.45%) (PPV: 91.18%) (NPV: 82.89%). Cutoff values for biomarker and biomarker ratios can be found in Table 18 below.

Table 18. **Cutoff values for each biomarker signature diagnostic accuracy and prediction.**

Biomarker Signature	Optimal Cutoff Value	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
$A\beta_{42}$	< 741.75 pg/mL	84.09%	33.33%	72.55%	88.14%
T-tau	256.85 pg/mL	77.27%	78.79%	70.83%	83.87%
P-tau	> 26.38 pg/mL	50%	83.33%	50%	83.33%
$A\beta_{42}$/T-tau	< 1.9905	79.55%	92.42%	87.5%	87.14%
$A\beta_{42}$/P-tau	< 17.26	70.45%	95.45%	91.18%	82.14%

Cutoff values for each biomarker **(**$A\beta_{42}$, T-tau, P-tau) and combination ($A\beta_{42}$/T-tau and $A\beta_{42}$/P-tau) with sensitivity, specificity, positive predictive value, and negative predictive value. $A\beta_{42}$ = amyloid-beta 42 peptide; T-tau = total tau protein; and P-tau = hyperphosphorylated tau.

VI. Discussion

The goal of this present study was to assess the diagnostic value of hippocampus volume, the Mini-Mental State Evaluation test, the Alzheimer's Disease Assessment Scale, APOE-ε4 genotype screening, and CSF biomarkers, to detect changes in cognitive impairment of CN, MCI, and AD subjects within the ADNI database. Significant linear correlations of hippocampal atrophy between intervals and groups suggest that structural MRI is a powerful tool in the detection of hippocampal changes over time and therefore should be used for monitoring disease progression. Since the mean volumes were significantly different between cognitive groups, utilizing structural MRI as a biomarker for detecting early AD in MCI subjects may be promising.

It was expected that both the MMSE and ADAS-Cog would accurately reflect hippocampal volumes as a measure of cognitive impairment. As anticipated, there was a negative correlation of ADAS-Cog 13 scores to hippocampal volume, where higher scores indicated increased cognitive impairment and lower hippocampal volumes. Conversely, a positive correlation of MMSE scores to hippocampal volume was revealed, where lower MMSE scores indicated increased cognitive impairment and lower hippocampal volumes. This association suggests that the two cognitive test scores do

reflect AD pathology. However, since the ADAS-Cog 13 was more sensitive to change over time, it may be beneficial to use both tests in diagnostic screening, to complement the each other.

It was expected that the number of APOE-ε4 alleles would significantly correspond to cognitive impairment, due to its role in binding to Aβ. Yet the frequencies of subjects with each APOE-ε4 genotype (0 alleles, 1 allele, and 2 alleles) did not linearly correlate to their level of cognitive impairment, as shown in Table 13 and Figure 13. However, when regressed to hippocampal volume by group, the number of APOE-ε4 alleles yielded significant effects within MCI subjects (Figure 14). This suggests that the APOE-ε4 allele may have diagnostic value in the evaluation of MCI to AD conversion, when paired with structural MRI scans.

The sensitivity, specificity, and predictive values of the CSF biomarkers ($A\beta_{42}$, T-Tau, and P-tau) and combinations ($A\beta_{42}$/T-tau and $A\beta_{42}$ /P-Tau) were assessed. Of all 5 signatures, AB_{42} yielded the highest sensitivity (84.09%) and negative predictive value (88.14%) but low specificity (33.33%). AB_{42}/P-tau demonstrated the highest specificity (95.45%) and positive predictive value (91.18%). T-tau and AB_{42}/T-tau yielded intermediate values throughout. Thus, the combination of the AB_{42} and P-tau may serve as accurate predictors of disease progression.

VII. Conclusion

Alzheimer's Disease a progressive neurodegenerative disease characterized by the formation of neurofibrillary tangles and senile plaque deposits. The prevalence of Alzheimer's Disease is expected to triple by the year 2050. However, it has been estimated that the delay of onset by 5 years may reduce AD prevalence by 50% over the next 50 years (Shaw, 2008). The identification of analyses with high statistical power of detection acts as tools for earlier and more accurate diagnosis of AD, allowing for a slowing of its progression. Such detection may be most beneficial when cognitive impairment at its earlier stages (prodromal and MCI). Currently, the best classifiers for early detection include MRI, FDG-PET, CSF biomarkers, and clinical tests (Weiner et al., 2015). The goal of this present study was to evaluate the diagnostic value of early

detection strategies. These included hippocampal MRI scans, two cognitive tests (the MMSE and the ADAS-Cog 13), *APOE-ε4* genotyping, and three CSF biomarkers (AB-42, P-tau, and T-tau).

The medial temporal lobe (including the hippocampus) is typically the first location to demonstrate atrophy in the presence of AD. Structural MRIs have been utilized as a non-invasive tool to track the progression of the disease. The present statistical analyses have shown that structural MRI does provide valuable information of cognitive changes between 6-month intervals by groups (based on diagnoses). Thus, hippocampal volume may serve as a powerful tool for detecting atrophy in short periods of time. Moreover, MRIs are safe from carcinogenic effects because they do not involve ionizing radiation exposure, allowing subjects to receive multiple scans.

Consistent with previous literature, the MMSE and the ADAS-Cog are used as complementary screening tests. As expected, the ADAS-Cog 13 is more sensitive to cognitive changes over time. For cross-validation and avoidance of extraneous variable effects, this regression should be repeated, adjusting for age as co-variates.

The *APOE* gene is associated with the reduced ability to clear Aβ from the brain by binding to Aβ. In comparison to individuals with APOE-ε4 negative subjects, those with a single copy of the ε4 allele have increased risk of developing the disease (Simic et al., 2016). However, even with the knowledge of APOE-ε4 being a significant risk factor for the heritability of late-onset AD, roughly only 27% of individuals with LOAD have this genotype (Cauwenberghe et al., 2016). This information is consistent with the current genotype regressions, where the number of APOE-*ε*4 alleles did not correlate with the frequency of having the differential diagnosis of AD. However, when combined with hippocampal volume data, the *APOE-ε4* genotype did seem to have an effect within MCI patients. Thus, *APOE-ε4* genotype must be used in conjunction with other biomarkers of AD to produce any significant answers.

Lastly, the three core CSF biomarkers ($A\beta_{42}$, T-Tau, and P-tau) as well as combinations ($A\beta_{42}$/T-tau and $A\beta_{42}$ /P-Tau) did yield a high combined sensitivity, specificity, and predictive value of AD diagnosis. Despite its success, lumbar puncture may not be

recommended for routine clinical use unless in the case of suspected early onset AD, due to potential side effects. For this reason, the ongoing research of blood-based biomarkers in the detection of AD is necessary. The potential advantages of blood biomarkers would include sample collection convenience, lower processing cost, and the ability to separate blood compartments (plasma, serum, and cellular compartment).

Though each test did produce results consistent with the hypotheses, limitations of this study included the inconsistency of data availability between time intervals. For example, the lack of hippocampal data at the month-18 interval prevented its use within regression studies. Although tracking hippocampal volume data over a longer period of time would have increased accuracy, data availability was inconsistent. Nevertheless, hippocampal activity still followed a linear pattern and produced significant effects.

To summarize, the use of pathological markers, including structural neuroimaging, cognitive screening tests, genotyping, and biochemical markers should be used in combination for increased accuracy of early detection and disease monitoring. Future avenues of research include the effect of anti-inflammatory treatments, gene therapy and immunotherapy on the progression of AD, as well as an updated overview of biomarker research. The increased ability to accurately diagnose and quickly detect Alzheimer's will aid in the development of more effective treatments and clinical trials, thereby reducing the prevalence of the debilitating disease.

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