Chapter 1: Introduction to Alzheimer's Disease

Neurodegenerative and psychiatric disorders taken together account for more chronic suffering than all other disorders combined (Kale 2001). Neurodegeneration is an umbrella term developed to describe the progressive loss of structure and function of neurons eventually leading to neural apoptosis or cell death. Many neurodegenerative diseases including Parkinson's disease, Huntington's Disease, and Alzheimer's disease occur as the result of the degeneration of neurons in the brain. Current research indicates that there are many parallels between these neurodegenerative disorders specifically in the neuropathology of each leading to atypical protein assemblies as well as induced neuronal death (Cowan and Kandel 2001). Discovering these similarities could lead to therapeutic advancements that could ameliorate many diseases simultaneously. This thesis will focus on Alzheimer's disease by discussing both the predispositions that lead to onset and possible preventative measures currently being researched in the field of neurodegeneration. Since neurodegenerative disorders involve the most pertinent organ in the body, the brain, any information involving how to avoid its degeneration is of the utmost importance.

Epidemiology and Significance

The study of the neuropathology of Alzheimer's disease is of great interest in the field of neurodegenerative disorders. Ever since its discovery by Alois Alzheimer in 1901, hundreds of thousands of research articles have been published identifying different mechanisms that when disrupted could cause Alzheimer's disease (Dahm 2006). Even with all this research, much is still unknown about the intricacies of this disease especially in terms of environmental stimuli. A couple of correlations between

depression, diet and the development of Alzheimer's symptoms have been identified but none of these were strong enough to be conclusive (Wuwongse et al. 2009, Shobab et al. 2005). The goal of my literature review is to survey the current scientific literature in the field of Alzheimer's research and identify connections between genetic predispositions and environmental factors. Presently, Alzheimer's disease is an incurable and irreversible disorder, and connections discussed in my thesis could lead to a better understanding of how to avoid different environmental factors in order to limit the chances of developing symptoms of Alzheimer's disease

Alzheimer's dementia is a disorder in which individuals, normally over 50, develop a progressive deterioration of cognitive functions clinically indistinguishable from senile dementia (Coyle et al 1983). The course of the disease is fluctuant with symptoms ranging from a loss of memory and learning ability to personality changes, psychotic attributes, and motor disturbances such as seizures and myoclonus (Fodale et al 2006). Most cases result in the death of the patient when cognitive impairment becomes too great and mental function is no longer possible. Post-mortem analysis of many patients clinically diagnosed with Alzheimer's disease have identified two major pathological hallmarks: the extracellular senile plaque and the intercellular neurofibrillary tangles, and each are composed of clusters of amyloid beta protein and hyperphosphorylated tau respectively (Dong et al 2012).

Neuropathology

Despite the astounding improvements in the comprehension of the pathogenesis of Alzheimer's disease (AD) have been made over the last decades, the exact mechanism leading to its onset still remains unclear. One proposed theory involves disruption of

homeostatic processes that regulate the proteolytic cleavage of the amyloid precursor protein (APP) and is commonly known as the amyloid cascade hypothesis (Dong et al 2012). It has been hypothesized that genetic, environmental, and age-related factors influence a metabolic shift favoring the beta-amyloidogenic processing of APP deviating from the regulative secretion pathway. This beta-amyloid fibril is generated by the successive cleavage of APP by beta-secretase and gamma-secretase located in neural membranes. Figure 1 on the next page provides an illustration of this APP cleavage. Among the several different beta-amyloid isoforms that obtain their differences via differing numbers of amino acids in their C-terminus, Aß-42 plays the most pivitol role in the pathogenesis of AD. The neurotoxic potential of this fiber comes from its biochemical "sticky" properties favoring aggregation into insoluable oligomers. Beta-secretase and gamma-secretase favor the production of Aß-42 leading to its over-production and eventual extracellular aggregation. Once Aß-42 oligomers accumulate in the extracellular space of the brain, a series of neurotoxic cascades are initiated leading to cytoskeletal changes, neuronal dysfunction, and cellular death (De-Paula et al 2012). Researchers indicate these changes could be due to the focus of Aß-42 accumulation in neural synapses and the induced oxidative stress favored by this detrimental aggregate, but much is still unknown (Dong et al 2012).

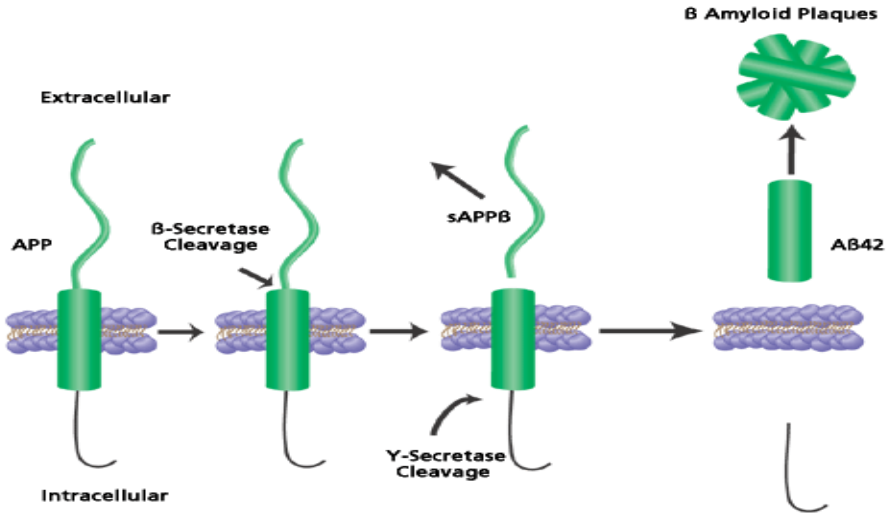

Figure 1. Cleavage of Amyloid Precursor Protein. Amyloid precursor protein (APP) is sequentially cleaved first by β-secretase (BACE1) and then by γ-secretase to form soluble amyloid precursor protein β (sAppβ) and the amyloid β42 peptide fragment (Aβ42). The Aβ42 fragments then aggregate and form the extracellular senile plaques common to Alzheimer's disease (Dong et al 2012).

In parallel with senile plaques, the hyperphosphorylation of tau protein and formation of intraneuronal neurofibrillary tangles (NFTs) represents another characteristic neuropathological feature in AD brain. Tau is a microtubule associated protein (MAP) that is highly influential in the association of tubulin allowing the formation of a microtubule. In a neurofibrillary tangle (NFT), tubulin can not associate together in a linear fashion, causing microtubules to agglomerate into helical bundles. Since the microtubule serves as the main "highway" to transport nutrients, neurons with NFT's can not function properly and eventually die. In brain biopsies of deceased AD patients, it has been found that abnormally phosphorylated Tau is the main constituent of the helical bunches forming a NFT allowing researchers to conclude that the causation of a NFT is indeed the hyperphosphorylation of Tau (Duan et al 2012).

Within a conventional neuron, the homeostasis of the phosphorylation and dephosphorylation of Tau is in strict regulation by different protein kinases. During beta-amyloidosis in brains inflicted with Alzheimer's disease (AD), Aß triggers the transcriptional up-regulation of a gene named dual-specificity tyrosine-regulated kinase 1A (DYRK1A), which disrupts the homeostasis and leads to the hyperphosphorylation of Tau (Kimura et al 2007). This research indicates that both hallmark pathologies of AD mediate each other allowing the production of symptoms. Without beta-amyloidosis, the homeostasis of Tau is a lot less likely to be effected making the genetic and environmental predispositions of beta-amyloid the most important for the onset of Alzheimer's disease.

How one actually succumbs to Alzheimer's disease (AD) is one of the most controversial topics in the study of neurodegenerative disorders. AD is a dichotomous disorder meaning there are two separate types of Alzheimer's based on age of onset (early-onset and late-onset). Early-onset Alzheimer's Disease (EOAD) is also referred to as familial AD because the primary course of developing symptoms is from the inheritance of mutated genes. The mechanism to which EOAD develops is very well researched and understood in the scientific community (Tanzi 2012, Schellenberg and Montine 2012). The second type of AD or late-onset Alzheimer's disease (LOAD) develops via combination of genetic and environmental influences. Much less is understood about LOAD, but there are a variety of theories on how it develops from genome wide association studies (GWAS) and animal models. The GWAS has pointed to several different genetic suspects: angiotensin converting enzyme 1 (*ACE*), sortilin-related receptor (*SORL1*), and with the most significance, the e4 allele of apolipoprotein

E (*APOE*) (Tanzi 2012). In addition, animal models (mainly mice) have pointed to depression, diet, and presence of diabetes mellitus as the predominant environmental influences on the development of LOAD (van den Berg et al 2006, Takayoshi et al 2012, Wuwongse et al 2009). More recent studies have even pointed to head trauma as a possible environmental predisposition to LOAD as well (Daneshvar et al 2011). The genetic influences on EOAD as well as the connections between genetic predispositions and environmental influences will be thoroughly examined in the following pages. In the perusal of this thesis, one will be able to fully comprehend the development of AD and will learn certain environmental factors to circumspect in order to better their chances of avoiding this personality altering disorder.

2. Genetic Predispositions of Alzheimer's Disease.

Aggregation of misfolded proteins appear to play a substantial role in the onset of Alzheimer's disease (AD). The production of proteins arises from gene expression in that functional DNA gets transcribed into mRNA, which then gets translated into a polypeptide or protein via ribosomes. When the nucleotide sequence of a gene is altered via nonsynonymous mutations, the resulting polypeptide is altered as well. These alterations can cause improper adhesion of alpha helixes and beta sheets in a three-dimensional structure leading to protein misfolding. In AD, misfolded Tau leads to neurofibulary tangles and misfolded APP leads to the increased production of Amyloid-Beta. Since both hallmarks of AD have genetic attributes, the study of genetic predispositions exceptionally important.

For AD, early work between 1988 and 1995 identified three genes that harbor mutations that cause the disease. The term "cause" is used because individuals with these mutations inevitably develop symptoms of AD, often at a remarkably young age (Tanzi 2012). While these mutations only in rare cases lead to onset (5% of AD cases), they provide a substantial amount of information on the mechanisms underlying the onset of AD and also provide potential therapeutic approaches currently being studied today. Starting in the early 1990's, technology was being developed to examine the causative genes that when non-synonymously mutated could increase an individual's risk for developing AD. Knowledge obtained in the last five years from genome wide association studies and the human genome project have drastically changed the outlook by identifying genes that not only mechanistically contribute to AD, but also the mutations in these genes which increase risk. (Schellenberg and Montine 2012). The following section is going to review early genetic findings, and then move on to the more recent findings on genetic risk factors for both early onset and late onset Alzheimer's disease.

A. Genetic Aspects of Early-Onset (familial) Alzheimer's Disease

Alzheimer's disease is a dichotomous disorder divided into 2 separate clinical categories: Familial and Non-familial. Familial Alzheimer's disease is normally diagnosed in individuals under 65 years of age, and since it occurs so early in life, it is also called early-onset Alzheimer's disease (EO-AD). This classification of AD is predisposed by purely genetic factors according to Mendelian inheritance, meaning that children of an affected parent are at 50% risk for developing EOAD (Tanzi 2012). These

factors are very rare, yet fully penetrant and consist of mutations in three different genes: *APP*, *PSEN1*, and *PSEN2*.

Identification of *APP* was made possible by the seminal work of Glenner and Wong who isolated a partial amino acid sequence from AD brain containing cerebrovascular amyloid (Glenner and Wong 2012). This partial sequence was later designated *APP* at a gene locus on chromosome 21 (Tanzi 2012). *APP* is the protein that gives rise to the toxic derivative amyloid-beta and consists of a very specific amino acid sequence that guides the cleavage by the transmembrane proteolytic enzymes β-and γ-secretase (Tanzi 2012). β-and γ-secretase function by making two endoproteolytic cleavages on either side of amyloid-beta causing this toxic derivative to be formed. Figure 2 provides an illustration of this cleavage. On the other hand, α-secretase cleaves in the middle of the amyloid-beta polypeptide segment prohibiting a complete amyloid-beta protein from forming (Schellenberg and Montine 2012)

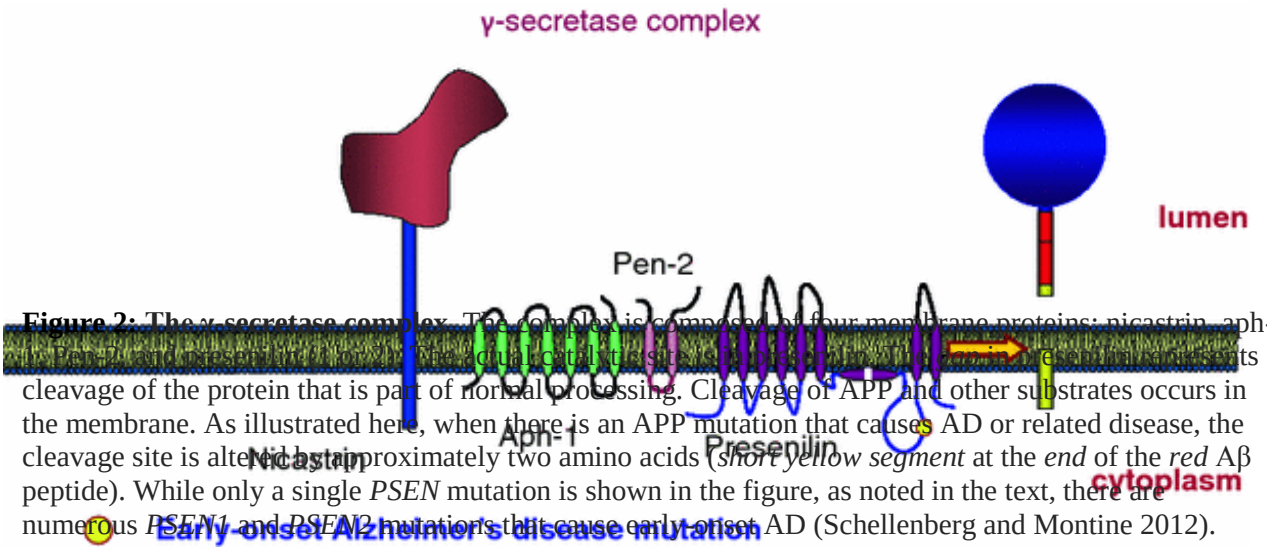

Figure 2: The γ-secretase complex. The complex is composed of four membrane proteins: nicastrin, aph-1, pen-2, and presenilin. The dual catalytic site is provided by two transmembrane aspartates in presenilin and mediates cleavage of the protein that is part of normal processing. Cleavage of APP and other substrates occurs in the membrane. As illustrated here, when there is an APP mutation that causes AD or related disease, the cleavage site is altered by approximately two amino acids (*short yellow segment* at the *end* of the *red* Aβ peptide). While only a single *PSEN* mutation is shown in the figure, as noted in the text, there are numerous *PSEN1* and *PSEN2* mutations that cause early-onset AD (Schellenberg and Montine 2012).

When mutations occur in the gene producing APP, mutated mRNA is created thus generating an improper sequence of amino acids in APP. For example, the Swedish mutation (achieved its name from first being discovered in the Swedish population) is a double substitution that changes the two amino acids immediately before the amyloid-beta domain in the promoter region on APP (lysine-methionine is replaced with asparagine-leucine). The functional consequence of this mutation results in the amount of amyloid beta being produced are double to triple that of normal, non-mutated APP (Schellenberg and Montine 2012). This statement leads to the fundamental biological question whether excess amyloid-beta production leads to the onset of AD. This conclusion is supported when looking at existing research indicating that people with down's syndrome (trisomy 21) also develop AD-like neuropathology that occurs very early in life. By age 40, virtually all individuals with DS also show evidence of senile plagues in their cerebrum (Leverenz and Raskind 1998). This evidence indicates not only a comorbidity, but also one extra copy of the amyloid-beta producing *APP* is sufficient enough to result in the development of EO-AD.

Even though mutations in *APP* are significant in the development of AD symptoms like decreased retention of events and altered personality, the genes with the strongest correlation to familial Alzheimer's disease (EO-AD) are Presinilin 1 (*PSEN 1*) and Presinilin 2 (*PSEN 2*). Over 180 mutations in the *PSEN1* gene are known to cause autosomal dominant inheritance of AD with onset as early as 30 years of age. Penetrance of *PSEN1* is complete by the age 60, meaning all infected individuals containing mutations in this gene will develop EO-AD. Less is known about *PSEN2* with only 15 mutations being confirmed to cause autosomal dominant EO-AD with penetrance being

more variable (Schellenberg and Montine 2012). Mutations in the *PSEN1* and *PSEN2* genes result in defective (or altered) proteins (mutated proteins) that influence the production of AD in a very similar manner because both proteins interact with the gamma secretase complex in APP cleavage as shown in Figure 2. Normally, the non-mutated presinilin proteins combines with the gamma-secretase to catalyze endoproteolysis at the C- and N-terminal end of beta-amyloid peptide sequence forming a normal Aß40 peptide. However, when presinilin is mutated, a different cleavage product of beta-amyloid is formed producing Aß42 instead of Aß40. This toxic derivative of beta-amyloid is more amyloidogenic, thus making it more prone to aggregate in neural synapses. Individuals who die of EO-AD from mutations in *PSEN1* and *PSEN2* are noticed to have hippocampuses littered with senile plaques (Schellenberg and Montine 2012).

B. Genetic Aspects of Late-Onset (Non-familial) Alzheimer's Disease

Unlike familial (early-onset) Alzheimer's disease, for which the genes have been identified, the development of non-familial (late-onset) Alzheimer's disease has been a source of great debate in the modern field of neuroscience. The age of onset is later than that of familial (over 65) and is highly variable meaning symptoms could arise as soon as age 65 or as late as age 85 (Elias-Sonnenschein et. al. 2012). This varying age spectrum of onset has kept research in the field very theoretical allowing controversy to remain high. What genetically predisposes one to acquiring late-onset Alzheimer's disease (LOAD)? A study by Bertram and others in 2012 says that the answer lies within genome wide association studies.

The use of genome wide association studies (GWAS) has become much more prevalent in the research of neurodegenerative disorders. The advent of this technology has revolutionized genetics research because you can sequence the entire genome for SNP's highly associated with the phenotype (in this case Alzheimer's disease). Genes containing SNPs, if they are nonsynonymous, can affect the amino acid sequence of the protein they produce. This could in turn affect amyloid beta aggregation or neurofibulary tangle generation (Elias-Sonnenschein et. al. 2012). To determine the significance of SNP's, a rigorous criterion has been developed in the form of p-values. If a SNP is influential in the production of LOAD, then it is given a smaller p-value based on statistical significance in the GWAS. Figure 3 provides an illustration of multiple GWAS completed on the main LOAD association genes.

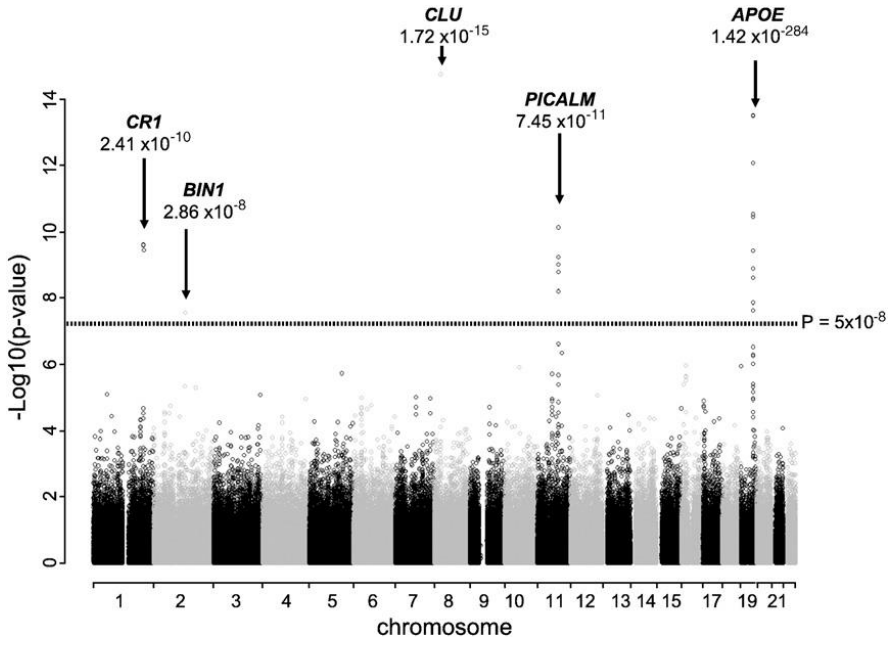

Figure 3: A Sample Genome Wide Association Study (GWAS) Identifying the Significance of some LOAD Associated Genes. The joint p-values for each of the genome-wide significant SNPs and the name of the gene to which they lie closest are given. The top six most significant APOE locus SNPs have been removed, but the best p-value is given (rs2075650). The other SNPs are CR1, rs6701713; BIN1, rs744373;CLU, rs11136000; PICALM, rs3851179 and APOE, rs2075650 (Jones et al 2010).

The genes granted the smallest p-values, thus the most significant in the onset of LOAD are as follows: *CR1* (complement component (3b/4b) receptor 1), *CLU* (clusterin or apolipoprotein J), *PICALM* (phosphatidylinositol binding clathrin assembly protein), and with the most significance, *APOE* (apolipoprotein E gene) (Tanzi 2012, Schellenberg and Montine 2012, Elias-Sonnenschein et. al. 2012). The table below illustrates all of the genes found in association to LOAD including the genes with the most significance (smallest p-values) already listed.

Gene	Chromosome	Top SNPs	*P*-Values
APOE	19	rs4420638 rs2075650b	1.1×10^{-266} 1.04×10^{-295}
CLU	8	rs1532278 rs11136000	8.3×10^{-8} 1.62×10^{-16}
CR1	1	rs6701713 rs6656401	4.6×10^{-10} 3.5×10^{-9}
PICALM	11	rs561655 rs3851179	7.0×10^{-11} 3.16×10^{-12}
BIN1	2	rs7561528 rs744373	4.2×10^{-14} 3.16×10^{-12}
CD33	19	rs3865444	1.6×10^{-9}
TRIM15	19	rs3752246 rs3764650	5.8×10^{-7} 5.0×10^{-21}
EPHA1	7	rs11767557	6.0×10^{-10}

CD2AP	6	rs9349407	8.6 × 10−9
MS4A4A *MS4A6A* *MS4A4E*	11	rs4938933 rs610932 rs670139	8.2 × 10−12 1.2 × 10−16 1.1 × 10−10
SORL1	11	rs668387 rs3781835	0.001 2.9 × 10−4

Table 1: Summery of Genetic Findings for LOAD. Comprehensive GWAS were completed and these are all the genes that were reported with slight to significant association to LOAD. The second column states the chromosome on which the gene is found, the third column states all the LOAD associated SNPs found with each gene according to GWAS, and finally the fourth column illustrates the p-values associated with these SNP's (Schellenberg and Montine 2012).

In 2009, two large GWA studies from the UK (Harold et. al. 2009) and France (Lambert et. al. 2009) were published highlighting three novel LOAD genes: *CLU, CR1,* and *PICALM*. All three genes have received overwhelming support from independent follow up studies (Carrasquillo et. al. 2010, Jun et. al. 2010) and currently rank at the very top of the AlzGene meta-analyses (a scale rating genes associated with Alzheimer's disease based on their statistical significance) meaning they have SNPs with very low p-values: *CLU* (p-value=1.62 x 10^{-16}), *CR1* (p-value=4.6 x 10^{-10}), and *PICALM* (p-value= 3.16 x 10^{-12}) (Table 1). Now that it is established that these genes have statistical significance in the development of LOAD, the question of how mechanistically do SNPs in these genes generate Alzheimer's symptoms is posed. Research has indicated that *CR1* and *CLU* influence LOAD via the immune response and *PICALM* has more to do with the endocytosis pathway of neurons (Schellenberg and Montine 2012).

Components of the immune response have access to all tissues in the body, including the brain. The brain acquires its defenders from microglial cells that release a variety of pro-inflammatory molecules in response to pathogens or cellular irregularities

(Rivest 2009). *CLU* is a transcriptional gene found within microglial cells that is used to generate clusterin (a chaperone molecule, which is generally associated with the clearance of cellular debris and apoptosis). The LOAD associated SNP (rs11136000) of *CLU* has been shown possibly to have an involvement in the clearance and aggregation of amyloid-beta. In addition, research has shown that the mutated clusterin produced from this SNP has involvement in amyloid-beta fibrillization, regulation of brain cholesterol and lipid metabolism, and initiation or neural apoptosis (Nuutinen et. al. 2009). Furthermore, microglial cells have the ability to initate a mechanism of innate immunity. The immune system can be beneficial when the brain contains an extracellular pathogen, but detrimental to a brain under the influence of LOAD. *CR1* is a gene that codes for the protein CR1, a cell surface receptor that is part of the innate immune response in the brain (Schellenberg and Montine 2012). CR1 has binding sites for the complement factors C3b and C4b and participates in clearing immune complexes containing these two proteins. Since amyloid-beta oligomers can bind C3b, CR1 may participate in the clearance of amyloid-beta from the extra-neuronal space (Schellenberg and Montine 2012). The LOAD associated SNP for *CR1* (rs6701713) has been shown to have inhibitory effects on the clearance of amyloid-beta in mouse models. In addition, this SNP has been shown to have a role in neuroinflammation, a prominent feature of LOAD (Jun et. al. 2010).

The third gene highlighted in the two GWAS from Europe is *PICALM* and instead of affecting the progression of LOAD extracellularly like *CLU* and *CR1*, research has shown that this gene actually attacks from inside a neuron (Bertram et. al. 2010). *PICALM* plays a role in clatherin-mediated endocytosis of substrate into a neuron

(Schellenberg and Montine 2012). Interestingly it has been discovered that the C-terminal fragment of APP generated by beta-secretase cleavage undergoes clatherin-mediated endocytosis before being cleaved by gamma-secretase. This process means in the case of LOAD, dysfunctional PICALM protein could interfere with this process (Elais-Sonnenschein et. al. 2010). The SNP found with the highest association to LOAD for *PICALM* (rs3851179) is still under investigation with a concrete mechanism for neurodegeneration yet to be discovered.

Finally, the gene with the most significance, which has shown the highest correlation with late-onset Alzheimer's Disease is *APOE* (Mirra 1999, Tanzi 2012, Ghebranious et. al. 2011, Schellenberg and Montine 2012). Multiple GWAS have confirmed *APOE* has a heavy association to LOAD with the SNP (rs2075650b) reaching a p-value as low as 1.04×10^{-295} (Schellenberg and Montine 2012). APOE is a lipid-transporting protein that synthesized in the liver and brain. A polymorphism occurs in the gene resulting in three different isoforms: e2, e3, and e4 (Mirra 1999). Each of these isoforms differ by only 1 amino acid which makes them prone to isoform switching when point mutations occur in APOE (Tanzi 2012). Because each individual inherits one of these APOE alleles from each parent, 6 genotypes could arise with e3/e3 being the most common. Research has shown that with every inherited e4 allele, the chances of developing Alzheimer's symptoms increase exponentially with the most dangerous being the e4/e4 genotype (Mirra 1999). How these different alleles and combinations of alleles in APOE exactly lead to the generation of the disorder is still under speculation, but the most conclusive evidence points to the resulting protein being involved in the transportation of senile plaques to neural synapses (Ghebranious et al 2011). All three of

the genes described above seem to have neurospecificity to cholinergic neurons of the hippocampus and prosencephalon (forebrain). These regions have a high specificity to memory and learning, both of which are heavily affected in Alzheimer's patients (Fodale et al 2006)

3. Environmental influences on Late-Onset Alzheimer's Disease

Alzheimer's Disease (AD) is a detrimental neurodegenerative disorder, which is the fourth most common cause of death in the developed world. It is characterized by plague formation, neuronal loss, and cognitive decline. As already discussed above, the pathological hallmarks of AD are the senile plaque that result from the misfolding of the Amyloid Precursor Protein (*APP*) and the neurofibrillary tangle caused by altering the functional properties of *Tau*. Research has shown that since amyloidogenesis occurs outside of the neuron, the senile plague or, more specifically, *APP* is more susceptible to environmental influences (Tsuang et. al. 2004). The main question that still perplexes scientists to this day is how the environment influences the generation of neurodegenerative disorders like AD? It seems that AD, like many normal physiological conditions (blood pressure) and cognitive abilities (intelligence), probably results from the combined action of multiple genes of small effect together with a variety of environment factors (Tsuang et. al. 2004). Thus, late-onset Alzheimer's Disease (LOAD) most likely results from a combination of epigenetic and genetic factors. This thesis has already discussed the variety of genetic factors that can predispose one to the generation of AD and is now going to transition into environmental influences leading to onset. The influences with the most research backing them are depression, diet, and external stimuli (head trauma).

A. Depression: Influences onset and is a co-factor increasing severity

The neurodegenerative features caused by both depression and Late Onset Alzheimer's Disease (LOAD) are very similar when examining animal models and post-mortem brains of infected patients. Researchers have found correlations between neuronal death, neurotrophic factors, and neuroinflammation leading to decreased cognitive ability and eventually death (Figure 4). The majority of research points to comorbidity between depression and LOAD, but when the proper genetic predispositions are in place (SNP's previously described and the e4 allele of *APOE*) depression can lead to onset (Wuwongse et al 2010). The following section describes the correlations between depression and LOAD indicating the prospect of possibly using anti-depressants to co-treat LOAD along with depression.

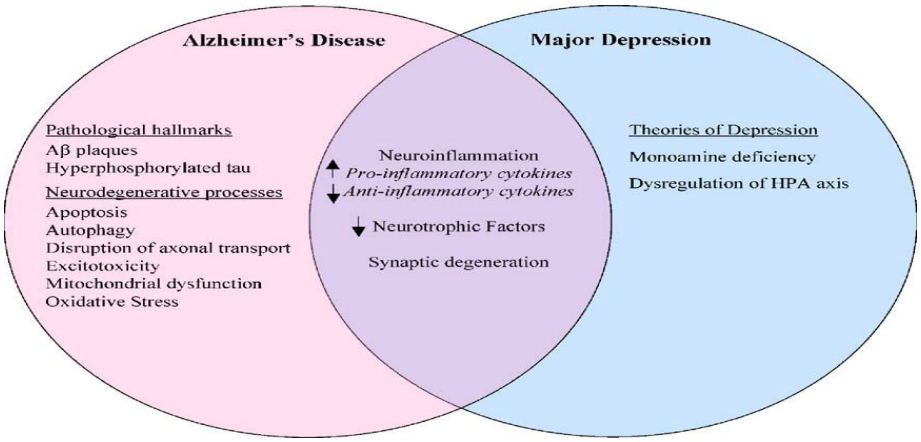

Figure 3. Current understanding on the common factors shared between depression and AD. AD is pathologically characterized by Ab plaques and NFT which results in neurodegeneration via several mechanisms including excitotoxicity and mitochondrial dysfunction. Depression on the other hand is characterized by monoamine deficiency and HPA-axis dysregulation. These two conditions appear to share common factors including inflammation and reduction in neurotrophic support. (Wuwongse et al 2010).

Neuronal death, which plays an important role in the pathogenesis of LOAD, was believed to be caused exclusively by both amyloid-beta plagues and neurofibrillary tangles. Current research indicates that this outcome could also be triggered by the pro-

apoptotic markers produced during depression (Li and Luo 2004). Several studies using animal models have shown that mice kept in depressing environments (dark, secluded enclosures) have an increase in pro-apoptotic markers and a decrease in anti-apoptotic markers specifically in the hippocampus and prefrontal cortex (regions for memory and personality respectively) (Bachis et al 2009). In addition, postmortem brains of depressed subjects also show reduced levels of extracellular signal-related kinase (an enzyme involved in a signal transduction cascade responsible for neuroplasticity and cell survival) (Dwivedi et al 2001). This research provides substantial evidence that there is a co-morbidity between depression and LOAD producing accelerated neuronal death.

The second correlation found between depression and LOAD is an increase of pro-inflammatory cytokines in brain regions associated with both diseases (Wuwongse et al 2010). Pro-inflammatory cytokines influence neuronal functioning in brain regions associated with both depression and LOAD, namely the prefrontal cortex and the hippocampus (Leonard and Myint 2006). Significant increases in the production of cytokines (IL-1B, IL-6, IL-12, and TNF-alpha) by cerebral monocytes have been found in patients in the preliminary stages of LOAD when they are showing minor cognitive impairment. These pro-inflammatory cytokines have been reported to modulate central neurotransmitters and growth factors, which is highly implicated in depression and is associated with the severity of the disease (Wuwongse et al 2010). Pro-inflammatory cytokines also induce oxidative species increasing the prevalence of neurodegeneration in the entire brain. Since monocytes can be stimulated to produce these cytokines by either amyloidosis in LOAD or neuronal stress in depression, inflammation has to be explained as a co-morbidity of both LOAD and depression.

The third and final correlation found was between the disruption of neurotrophic factors in LOAD producing symptoms of depression. This disruption was the only correlation researched in which depression initiates the Alzhiemer's process. Neurotrophic factors are described as those which promote neuronal growth and survival. A reduction in these factors results in decreased neurogenesis and impairment of neuroplasticity. In LOAD, disruption in brain-derived neurotrophic factor (BDNF) signaling has been shown to promote the amyloidogenic pathway in hippocampal neurons, subsequently leading to the activation of apoptosis (Matrone et al 2008). The activation of amyloidgenesis due to decreased BNDF in depressed brains can be observed in Figure 4. The synthetic introduction of more BNDF to the hippocampus has also shown to exhibit neuroprotective properties in several animal models of LOAD. In amyloid transgenic mice, BNDF gene delievery can reverse synaptic loss, improve cell signaling, and in some cases restore cognitive functioning (Beglopoulos et al 2004). Depression produces psychological stress that is manifested in the brain by oxidative species. These reactive molecules can alter many cognitive functions, but mainly decrease BNDF signaling, thus making these molecules one of the targets of antidepressant drugs like fluoxetine (main chemical in Prozac). Animal models for depression indicate that in administration of fluoxetine to mice, up regulation of BNDF occur in neurons throughout the hippocampus (Baj et al 2012). This evidence provides support that depression could be another factor pushing someone toward surpassing the threshold into LOAD. Depression cannot initiate the process alone, but could work with genetic factors (like the e4 allele in APOE) to produce onset.

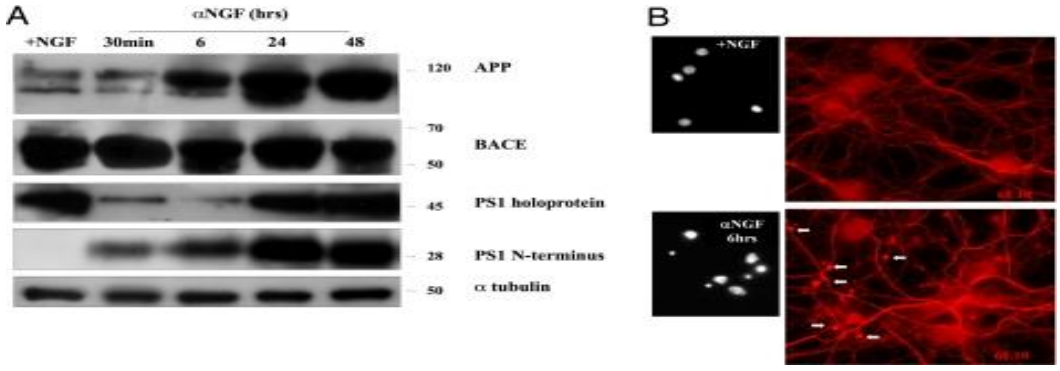

Figure 4: The interruption of NGF (BNDF) signal activates amyloidogenesis. *A*) Western blot analysis performed with antibodies against APP, BACE, and PS1 (see *Methods*) of lysates from hippocampal neurons of controls (+NGF) or of neurons exposed to anti-NGF antibodies (αNGF) in a time ranging from 30 min to 48 h. (*B*) (*Right*) Immunofluorescence analysis performed with anti-Aβ antibody against amino acid residues 1–17 (MAb 6E10). Arrows mark varicosities forming along neurites after 6 h of NGF removal (Matrone et al 2008).

As already discussed, depression causes the brain to undergo psychological stress that produces oxidative species. These reactive molecules like superoxide are normally broken down into benign molecules by superoxide dismutase. Under cases like depression where the brain is stressed, superoxide does not get broken down and is able to combine with and alter membrane enzymes like gamma-secretase on neurons. This reaction induces gamma-secretase to dysfunctionally cleave APP into AB42. This process then cascades when an individual has dysfunctional Apolipoprotein E (APOE) produced when the e4 allele of APOE is on chromosome 19. This altered APOE cannot effectively clear all the access AB42 due to the high superoxide concentrations in the cerebrum thus initiating amyloidosis producing Alzheimer's disease.

From the evidence presented in this section, it is easy to see that depression and Alzheimer's disease are mostly viewed as co-morbidities unless when discussing neurotrophic factors. If depression is present in high enough severity, it can psychologically stress the brain into producing oxidative species. These species in turn

can work with genetic predispositions (e4 allele in *APOE*) to produce Alzheimer's symptoms. It is important to note that depression cannot initiate onset single-handedly, but can help to escalate the process.

B. External Stimuli: Severe Head Trauma and its correlations to the onset of Late onset Alzheimer's Disease (LOAD).

In the United States, approximately 1.7 million people sustain a traumatic brain injury (TBI) annually; these injuries account for 1.365 million emergency room visits and 275,000 hospitalizations each year. These numbers reflect only the serious occurrences requiring hospitalization. This means the statistics reported above could vastly underestimate the total incidence of brain trauma since many individuals suffering from mild or moderate TBI do not seek medical advice (Daneshvar et al 2011). Since TBI is a common injury, the idea that it can lead to the inception of Late-Onset Alzheimer's Disease (LOAD) is traumatic in its own way. This following section is going to examine the correlations between brain trauma and LOAD in order to see if simply being more careful could prevent the acquisition of this neurodegenerative disorder.

One of the supported theories regarding the pathogenesis of LOAD is an "aging hypothesis" that regards dementia of the Alzheimer's type to just be manifestation of accumulated aging changes, an inevitable part of old age. These aging changes are just the natural deterioration of neural processes that happens over time as neurons "wear out" and die. One way to accelerate normal neuronal loss is by TBI, which reduces the safety factor provided by the brain (Nandoe et al 2002). As a result, LOAD will clinically start to express itself by producing symptoms in TBI victims as illustrated by Figure 5.

Another contributing factor is genetic predispositions an individual's genome contains toward the onset of LOAD. The apolipoprotein E (APOE) protein, which is engaged in growth and repair of the nervous system, may influence the deterioration after TBI (Nandoe et al 2002). Specifically the presence of an e4 allele could take advantage of the damaged state the brain is in post-TBI and produce Alzheimer-like symptoms (plaques and tangles). The following paragraph illustrates a temporal relationship observed between TBI and the occurrence of LOAD in a patient who developed LOAD after minor head trauma.

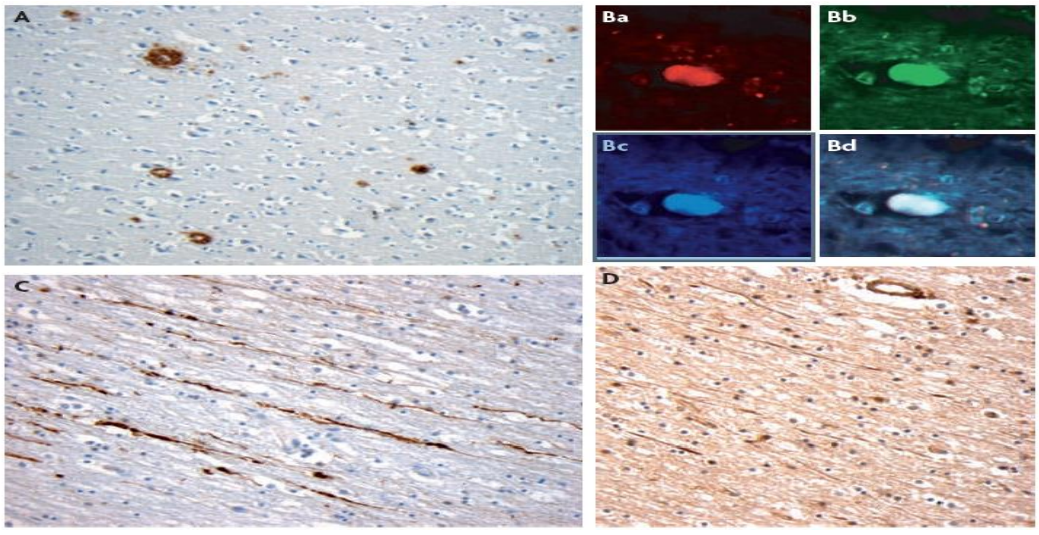

Figure 5: Immunohistochemical Findings Exploring Mechanisms of amyloid‑β plaque ormation following Traumatic Brain Injury. A) Representative amyloid-β (Aβ) plaques (brown) found acutely following a single incidence of traumatic brain injury (TBI) caused by a fall in an 18 year old male. The survival time from injury was just 10 hours. Plaques were identified using an antibody specific for Aβ. B) Representative immunohistochemistry showing amyloid precursor protein (APP) (Ba), β-site-APP cleaving enzyme (BACE) (Bb) and presenilin-1(PS-1) (Bc) co-accumulating (Bd) in the disconnected terminal of an axon, known as an axon bulb. C) Demonstration of axonal pathology using APP immunohistochemistry. APP (brown) accumulates within the tortuous varicosities along the length of damaged axons. D) Increased neprilysin immunoreactivity (brown) is also observed in damaged axons following TBI (Johnson et al 2010).

A 55-year old woman was admitted to the hospital after a traffic accident in which she bumped her head against the front window, when driving in her car without safety belts. She experienced a brief loss of consciousness and was brought to the emergency department, which diagnosed her with minor TBI. She was admitted for observation. Her skull X-ray revealed no fractures, thus she was discharged the next day after she rested and recovered. Almost 1.5 years after the accident she checked into a neurological clinic because she complained of "progressive forgetfulness". Upon further questioning she also disclosed problems concerning writing and mathematics, as well as word finding difficulties. Her spouse and two children were convinced that she had displayed no signs of cognitive impairment before the head trauma, which she blamed it on the accident. Physical examination showed no abnormalities with her pulse and blood pressure both normal. Neuropsychological examination showed global impairment in all cognitive domains, including executive dysfunction. In addition, she exhibited difficulties in simple arithmetic, reading aloud, and word associations. Since these are all signs of clinical dementia, a brain MRI was completed in order for confirmation. This test was completed along with an APOE genotyping and the results were mild cortical and moderate hippocampal atrophy in addition to an e4 homozygositiy in the *APOE* gene. These results provided enough evidence to clinically diagnose the now 56-year old woman with Alzheimer's disease. She was admitted to a home and died 5 years after the diagnosis (Nandoe et al 2002).

Epidemiological studies clearly show that head trauma is a risk factor for the development of dementia, but a temporal case study providing evidence of a single

individual developing LOAD after an episode of traumatic brain injury (TBI) had not been provided until the study completed by Nandoe et al. Since a formal neurophychological examination was not completed until 1.5 years after the accident, it remains unclear in what state the subject was in exactly at the time of the accident. Friends, family, and employer disclosed that there were no obvious signs of cognitive impairment at all before the head trauma. This evidence indicates that Alzheimer's disease was indeed triggered by the head trauma. Now, the main questions of research are how does TBI trigger the onset of Alzheimer's disease? It is suggested by Allsop et. al. 1986 that the pathogenesis of LOAD is initiated by an inflammation process where genetic factors play an important role. Until recently, the brain was thought to be an immunologically privileged site with limited mechanisms to induce an immune response. This thought was changed when studies were released indicating that head trauma may cause an inflammation process through damage to the blood brain barrier (BBB) allowing the extravasation of serum proteins into the brain parenchyma (Schofield et. al. 1997). These proteins could then sensitize the immune system of the brain, permitting a secondary response to the same antigens many years later. There is considerable evidence that the amyloidosis, which occurs during the formation of amyloid-beta plaques, will provide the signals for this secondary response (Nandoe et. al. 2002). Such an "acute phased response" occurs within days of TBI normally by the formation of interleukin-1 (Il-1). There is significant evidence displaying that Il-1 leads to increased synthesis of amyloid precursor protein (APP) thus producing more amyloid-beta (Abraham 1992, Nandoe et. al. 2002).

Now as was already discussed in the genetic section of this thesis, the synthesis of amyloid-beta from APP is normally kept in homeostasis by several key genetic factors (presenilin 1/2 and Apolipoprotein E). *PSEN 1* and *PSEN 2* produce proteins that combine to make the gamma-secretase complex that cleaves APP into amyloid-beta. When these genes are mutated, they produce hyper-active proteins that cleave APP in to the toxic derivative AB42. This is where Apolipoprotein E (APOE) comes in, as it works to remove the access AB42 from cerebral circulation. When an e4 allele of *APOE* is within the genome, the protein product APOE is not effective in removing AB42 allowing it to aggregate. The 55-year old woman in the case study previously described was homozygous for e4 in *APOE,* but normal in *PSEN 1* and *PSEN 2*. This evidence basically means that the severe Alzheimer's symptoms observed in her could not have arisen organically and had to be from some external stimulus. She had the incapability to remove AB42 in the e4/e4 genotype of *APOE,* but did not have the mutations in *PSEN* to produce enough AB42 to see anything substantial. This provides proof that the inflammation produced from the TBI in the case study had to produce the cerebral environment needed for *APOE* to fabricate the onset of LOAD (Nandoe et. al. 2002).

The case study provided by Nandoe et al, along with epidemiological research (Johnson et. al. 2010) is substantial enough to conclude that head trauma is associated with the incidence of Late-Onset Alzheimer's disease (LOAD). A reduction in TBI risky activities later in life like football, rugby, or driving without a seatbelt is recommended especially if an individual has Alzheimer's disease in their family history.

C. Diet: Can Cholesterol Intake, Omega-3 fatty acids, and The Insulin Pathway Modulate Late-Onset Alzheimer's Disease?

Eating and what not to eat is a source of controversy in the United States. Existing in an environment surrounded by processed foods at bargain costs makes it difficult to choose the healthy option over the unhealthy one especially when one has no conception of what "unhealthy" means. According to the National Institute of Health, sixty-five percent of American adults are currently overweight or obese. The incidence of type-2 diabetes mellitus is also increasing in this country with around 21 million people being clinically diagnosed in 2010. Even though diabetes and obesity are problems, what do they have to do with acquiring late-onset Alzheimer's disease (LOAD)? Current research indicates that risk factors associated with obesity and the acquisition of diabetes do have correlations to the development of LOAD (Puglielli et. al. 2003). Factors like high cholesterol intake, a diet low in Omega-3's, and insulin pathway dysfunctions all seem to contribute to not only obesity, but also LOAD. This fact has lead researchers to investigate these cofactors producing a variety of different theories on how diet could contribute to Alzheimer's disease. This following section is going to discuss how diet and diabetes mellitus can be environmental predispositions to Late-onset Alzheimer's disease.

1) Cholesterol Intake:

Cholesterol is commonly discussed as an individual increases in age. Over the age of 50, visiting the doctor becomes a common theme since high LDL (low-density lipoprotein) levels can contribute to heart disease. Modern research has denoted that heart disease does not exclusively result from high cholesterol levels in that dementia

(specifically of the Alzheimer's type) can also be an outcome (Puglielli et. al. 2003). As already discussed in this thesis, the primary genetic risk factor for the acquisition of LOAD is the e4 allele of the Apolipoprotein E gene (*APOE*). This glycoprotein is also the major protein component of a cholesterol transport unit called a very-low density lipoprotein (VLDL), in addition to being the major apolipoprotein of the brain. This evidence indicates that cholesterol may play a direct role in the pathogenesis of the disease leading researchers to investigate (Puglielli et. al. 2003).

In order to investigate whether cholesterol has a role in the pathogenesis of LOAD, Puglielli et. al. first had to identify what role cholesterol plays at homeostatic circumstances within the brain. Approximately 25% of the total amount of cholesterol is localized to the brain, most of it present in myelin sheaths surrounding neurons. Almost all brain cholesterol is a product of local synthesis, with the blood brain barrier (BBB) efficiently protecting it from exchange with lipoprotein cholesterol in the body circulation (Leoni et. al. 2003). Because of this reason, ingestion of excessive cholesterol (high cholesterol foods) normally cannot contribute to the onset of LOAD exclusively making cholesterol levels mainly a co-morbidity. This fact aside, a high cholesterol diet can contribute to the development of LOAD when the proper predispositions are in place. Recent research has shown that hypercholesterolemia can work with the e4 allele of APOE to generate symptoms of LOAD (Puglielli et. al. 2003).

As already discussed, APOE is involved in the clearance and transport of amyloid-beta. There are three common alleles of the *APOE* gene: e2,e3, and e4. The protein isoforms produced by these different alleles differ in amino acids at positions 112 and 158 in the 299 amino acid sequence. E3 is the most common allele, and e4, the

LOAD associated allele, exists in about 25% of the population (Tanzi 2012). What makes these isoforms different from each other mechanistically is in the different ways they transport and dispose of amyloid-beta. The e3 of APOE (APOE3) captures the extracellular amyloid-beta produced from the gamma-secretase cleavage of amyloid precursor protein (APP) and internalizes the aggregate within a neuron for digestion in the lysosomal compartment. On the other hand, the e4 of APOE (APOE4) collects the amyloid-beta (specifically Aß42) and brings them together for aggregation into plaques. In addition, APOE4 is less inclined to clear free amyloid-beta from the extra-cellular space adding to the aggregation potential (Puglielli et. al. 2003). Now the question posed by researchers is how does this system work with cholesterol? Pugielli et. al. found that APOE4 also increases the production of cholesterol inside of neurons by activating oxysterol 24S-hydroxycholesterol (oxy-24S-hc). After receptor-mediated internalization of APOE4, it interacts with oxy-24S-hc, which initiates a signal cascade producing cholesterol. This free cholesterol is then released to cellular membranes, which amalgamates with the gamma-secretase complex up regulating the production of

amyloid-beta (Puglielli et. al. 2003). Figure 6 illistrates how cholesterol contributes to amyloid-beta aggregation.

Now that the genetic correlations have been reviewed, can an individual eat enough cholesterol to drive themselves toward onset of Alzheimer's? The answer is complicated because of the blood brain barrier (BBB). In normal circumstances, the BBB would be sufficient to block intravenous cholesterol from entering cerebral circulation. The onset of LOAD can damage the BBB allowing the admittance of very-low-density lipoproteins (VLDL's) which can interact with APOE releasing the cholesterol into cerebral circulation (Puglielli et. al. 2003). When this happens, the free cholesterol can interact with neural membranes and up-regulate the production of amyloid-beta accelerating the production of plaques. Thus from the research observed, the only conclusion that can be made is to avoid foods high in cholesterol when one has a family history of Alzheimer's.

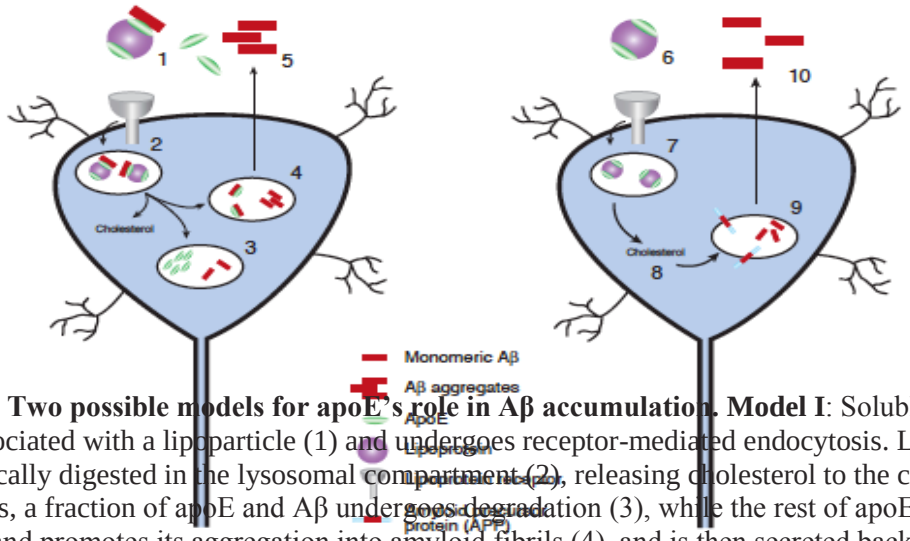

Figure 6. Two possible models for apoE's role in Aβ accumulation. Model I: Soluble Aβ interacts with apoE associated with a lipoparticle (1) and undergoes receptor-mediated endocytosis. Lipoproteins are then enzymatically digested in the lysosomal compartment (2), releasing cholesterol to the cell. In the lysosomes, a fraction of apoE and Aβ undergoes degradation (3), while the rest of apoE remains associated with Aβ and promotes its aggregation into amyloid fibrils (4), and is then secreted back into the extracellular milieu (5). Given that apoE4 has more affinity for Aβ than apoE2 and apoE3, it would be expected to accelerate this process. **Model II:** In addition to directly facilitating Aβ internalization and aggregation, apoE may also up-regulate the rate of Aβ generation by increasing cellular cholesterol. After receptor-mediated internalization (6) and enzymatic digestion of the lipoproteins (7), cholesterol is released to cellular membranes (8). ApoE4 lipoproteins tend to contain more cholesterol. The increased sterol content of intracellular membranes promotes the rate of Aβ generation (from its precursor APP) (9), resulting in increased secretion into the extracellular milieu (10) (Puglielli et. al. 2003).

Cholesterol intake is purely a co-factor which can work with genetic predispositions to produce the onset of LOAD.

2) Omega-3 Fatty Acids: Docosahexaenoic Acid (DHA)

In modern society a stigma has been associated with the ingestion of fats because ingestion of foods with a high concentration of fat leads to the excessive production of adipose tissue. In most cases fatty foods lead to fat production, but there is such a thing as "good fats". Specifically, Omega-3 fatty acids like docosahexaenoic acid (DHA) are known as "essential fatty acids" because they cannot be made synthetically inside of the body. Omega-3s are needed for the body to function properly and have even been found to play an important role in reducing inflammation throughout the body – in the blood vessels, the joints, and even the brain (Cole et. al. 2005). Omega-3s are normally found in fatty fish like salmon, in certain seeds like flaxseed, and in plant sources like algae. Since the ingestion of these fatty acids seem to be beneficial for the body, researchers started to examine whether they have any effect on reducing the severity or even preventing neurodegenerative disorders like Alzheimer's disease. This following section is going to review the research completed studying the effects of Omega-3s on Late-Onset Alzheimer's Disease (LOAD).

Omega-3s, more specifically DHA, play an essential role in brain growth and development. They are critical modulators of neuronal function and the regulation of oxidative stress mechanisms in brain health and disease (Jicha and Markesbery 2010). A plethora of *in vitro* studies have been completed in the last decade which highlight the important role DHA may play in the development of Late-Onset Alzheimer's disease

(Serini et al 2012, Hashimoto and Hossain 2011, Grimm et al 2011). An example of one of these *in vitro* studies showing the beneficial effects of DHA on decreasing γ-secretase activity is shown in Figure 7. Cross sectional and prospective cohort data have demonstrated that reduced dietary intake or low brain levels of DHA are associated with accelerated cognitive decline in the progression of LOAD (Cole et al 2005). For example, the Three-City cohort study in 2007 examined a total of 8,085 subjects from Bordeaux, Dijon, and Montpellier, France. This study consisted of two groups: one which was instructed to eat a hearty serving of fish once a week (experimental) and another with a history of eating a high omega-6 diet and received no instructions on what to eat (control). This study found that the group with the weekly fish consumption was associated with reduced risk for developing LOAD (in APOE e4 non-carriers) when compared to the control group (Barberger-Gateau et al 2007).

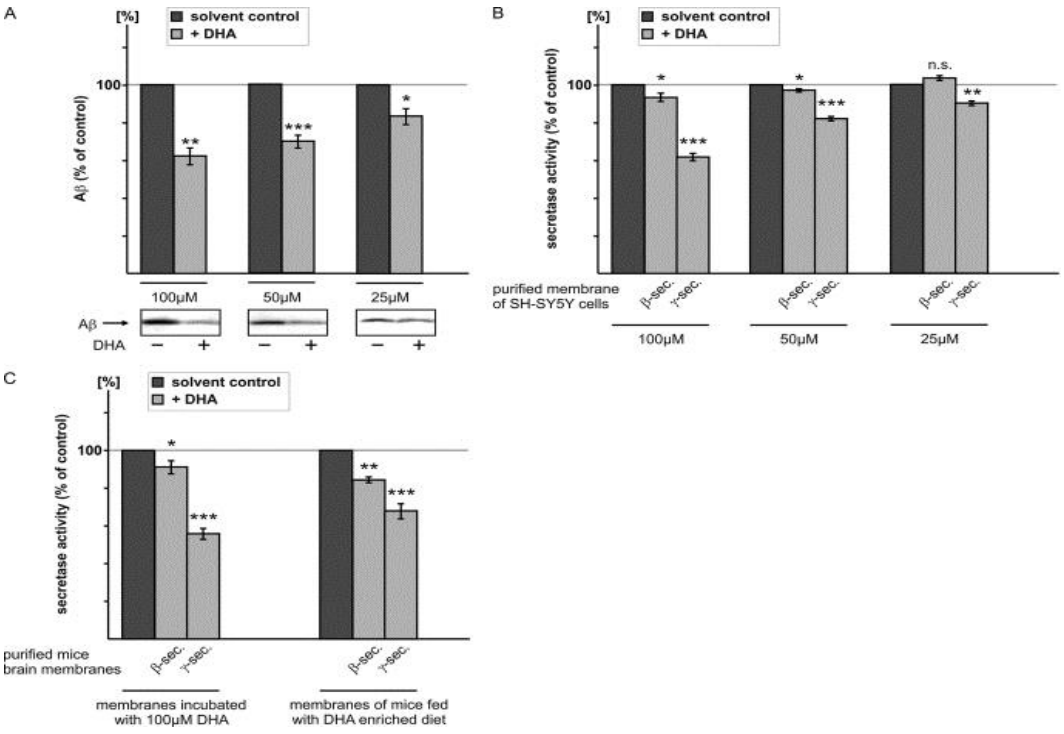

Figure 7: Influence of DHA on β- and γ-secretase activity. A) Human neuroblastoma cells stably expressing APP were incubated with 25, 50, and 100 µm DHA or solvent control. Equal volumes of conditioned media were immunoprecipitated with antibody W02, recognizing an epitope between amino acids 1 and 10 of Aβ. Immunoprecipitated Aβ peptides were detected by WB analysis with W02. DHA decreases dose-dependent Aβ generation. B) Purified membranes of wild type neuroblastoma cells were incubated *in vitro* with 25, 50, and 100 µm DHA or solvent control, and β- and γ-secretase activities were determined by a fluorometric assay. DHA directly decreases β- and γ-secretase activity. C) Left: purified membranes of mouse brain were incubated with 100 µm DHA or solvent control, and β- and γ-secretase activities were determined. DHA also decreases β- and γ-secretase activities in membranes of mouse brain. Right: membranes of mice fed a DHA-enriched diet were prepared, and β- and γ-secretase activities were measured. Membranes of mouse brain fed the DHA-enriched diet show reduced β- and γ-secretase activities compared with membranes of mouse brain fed a calorie-matched control diet. (Grimm et al 2011).

In addition *in vitro* assays, cell culture systems, and transgenic animal models of AD (Tg2576) have revealed that Omega-3 fatty acids (specifically DHA) can mechanistically prevent the aggregation of amyloid-beta produced by the e4 allele of *APOE*. Since DHA is an integral membrane component of neurons, it may act to alter amyloidogenic processing in several distinct and possibly interrelated ways as illustrated by Figure 8 (Jicha and Markesbery 2010).

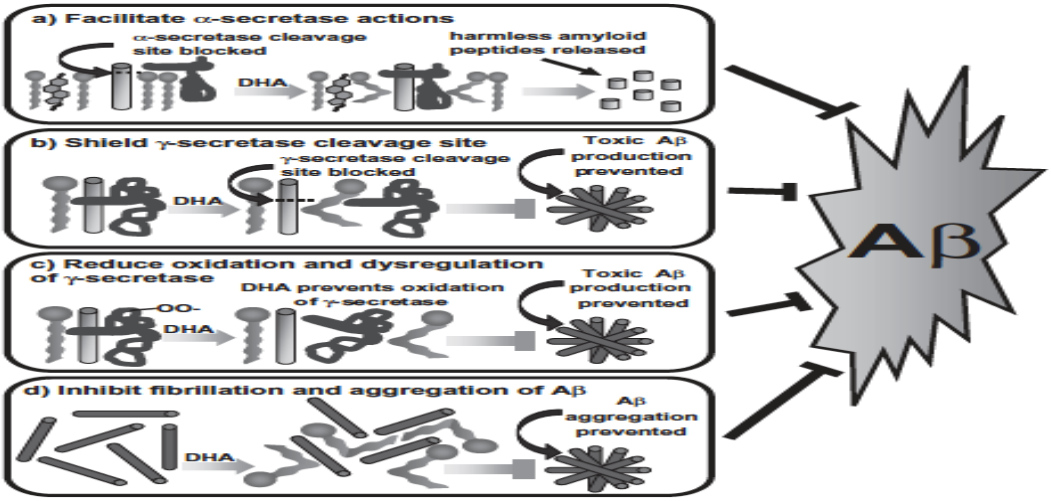

Figure 8. Omega-3 PUFAs influence amyloidogenic processing through several distinct and interrelated mechanisms: a) facilitation of the interaction of α-secretase with APP to produce non-toxic fragments and prevent the formation of Aβ, b) shielding the essential recognition sequence and intramembrane cleavage site for γ-secretase, c) serving as a local sink for free radicals that reduce the enzymatic augmentation of γ-secretase activity that can be induced by free radical damage to the protein complex important for the regulation of normal γ-secretase function, and d) directly inhibiting fibrillation and formation of toxic oligomeric species of Aβ (Jicha and Markesbery 2010).

Even with all this positive evidence, no solid correlation has been drawn for the prevention of LOAD by eating DHA. The ingestion of this Omega-3 has only shown to decrease the severity of symptoms (memory loss and personality alterations) and reduce risk for those without genetic predispositions. This fact aside, DHA is still a very beneficial nutrient in brain functionality and neural connectivity. Eating at least a serving

of fish a week can definitely improve cognitive functions and reduce the risk for neurodegeneration.

3) Is Alzheimer's Disease Type 3 Diabetes? The Relation between Late-Onset Alzheimer's and the Insulin Pathway

Digestion occurs after the ingestion of food mainly to separate nutrients (like glucose) for absorption into the body. Glucose is the most important nutrient when it comes to energy metabolism (how cells get energy to perform cellular functions) especially in the brain. The internalization of glucose into cells and neurons is regulated by the hormone insulin. This hormone is released by pancreatic beta cells in response to elevated blood glucose levels after a meal. Failure to uptake and store glucose results in Diabetes Mellitus. Type 1 diabetes is characterized by the inability to synthesize insulin from beta cells and is normally genetic. However, Type-2 diabetes is environmental in results in the body becoming resistant to the effects of insulin presumably from becoming obese and failure to exercise. Current research indicates that dysfunction in the insulin and insulin-like growth factor (IGF-1/2) pathways in the brain can produce Alzheimer's like symptoms (de la Monte and Wands 2008). The following section is going to describe how insulin can affect the progression of Late-Onset Alzheimer's Disease (LOAD).

Diabetes mellitus is associated with changes in cognition. In type-1 diabetes, this association is shown by mild to moderate slowing of mental speed and diminished metal flexibility. In type-2 diabetes, changes mainly affect learning and memory, mental

flexibility, and mental speed (Biessels et al 2006). Although the association between diabetes and cognition is well established, the relation between diabetes and LOAD is shrouded in controversy. A systemic review of populations studies done by Biessels et al indicates that the acquisition of type-2 diabetes does increase the risk of acquiring LOAD as well as increase the severity of cognitive decline when someone is already under the influence of LOAD (Biessels et al 2006). This conclusion can also be mechanistically backed up when looking at what hyperinsulinaemia, produced by type-2 diabetes, does to the brain. Insulin is transported actively across the blood brain barrier and travels to the insulin receptors (receptor tyrosine kinases) throughout the brain (Biessels et al 2006). An abundance of these receptors have been reported in the hippocampus and prefrontal cortex indicating that they could be involved in learning and memory (Banks 2004). Brain autopsies samples were taken from patients who died of Alzheimer's disease and researchers found that the activation of insulin receptor tyrosine kinases was impaired. These results lead researches to make the conclusion that LOAD was an "insulin resistant brain state", thus indicating that type-2 diabetes has a role (Biessels et al 2006).

Alternatively, another study which has received a multitude of recognition from the scientific community proposed that the insulin pathway dysfunction within the brain occurred independently from diabetes mellitus. Suzanne de la Monte and Jack Wands examined postmortem cases of advanced Alzheimer's disease and concluded that endogenous brain deficiencies in insulin, insulin-like growth factor-1 (IGF-1), insulin-like growth factor-2 (IGF-2), and their corresponding receptors in the absence of type-2 diabetes, is linked to the most common form of dementia-associated neurodegeneration in the Western Hemisphere or LOAD (de la Monte and Wands 2008). The mechanistic

proof behind this conclusion comes from several *in vitro* and *in vivo* studies completed testing insulin/IGF signaling in neurons. These studies reported that neurons placed in a medium consisting of access insulin and deficient insulin from homeostatic conditions resulted in deficits in energy metabolism, increased oxidative stress, mitochondrial dysfunction, proinflammatory cytokine activation, and APP expression (de la Monte and Wands 2008, Rivera et al 2005, and de la Monte et al 2006). As already reported in this thesis, pro-inflammatory cytokines can induce the production of oxidative species like superoxide leading to oxidative damage of neurons. This damage has been reported to increase amyloid-beta cleavage and *APP* gene expression leading to amyloid-beta aggregation. In addition, oxidative damage increases the methylation of Tau which causes it to malfunction leading to the production of neurofibrillary tangles. Since impaired insulin/IGF signaling leads to increased oxidative stress and the release of pro-inflammatory cytokines, it also increases the production of senile plaques and neurofibrillary tangles (de la Monte and Wands 2008).

Even though there is controversy on whether diabetes specifically contributes to Alzheimer's disease, the evidence is concrete when it comes to the involvement of the insulin pathway. It is a fact that insulin can freely cross the blood brain barrier, which means intravenous insulin can have an effect. In addition, there are insulin receptor tyrosine kinases located on neurons with a high concentration of these receptors located on neurons within the hippocampus (Biessels et al 2006; de la Monte and Wands 2008). Since the research indicates that insulin modulates neuronal well-being by promoting growth and strengthening synaptic connections (aiding in long term potentiation), it is a necessity for proper learning and memory. On the other hand, too much insulin can cause

competitive inhibition of the insulin-degrading enzyme (IDE) which is also used to degrade free amyloid-beta (Biessels et al 2006). This makes regulation of the diet very important because intake of foods exclusively with high sugar contents can cause a spike in insulin sufficient to reach the brain. Conversely, if a diet consisted of no glucose, the brain wouldn't get the insulin needed to maintain proper neuronal function. For these reasons, diet consisting of moderate glucose levels would be optimum since your brain is overloaded with glucose yet it has enough insulin to function properly.

4. Possible Preventative Measures

Alzheimer's Disease is a disorder that evokes more apprehension in patients than cancer, cardiovascular disease, and metabolic disease. This apprehension is due to the neurodegeneration induced since it robs individuals of their greatest human qualities—reasoning, memory, abstraction, language, and emotion—and it appears that a treatment is out of reach. Drugs applying NMDA receptor antagonists and acetylcholinesterase inhibitors (like Huperzine A) have been examined since both mechanisms are employed in the creation of memories and synaptic plasticity (Selkoe 2012). Clinical trials have been completed using these drugs with only slight cognitive improvement shown as a benefit. When placed under a CT scan, the patients who received Huperzine A did not show a decrease in the classical Alzheimer's legions (senile plaques and neurofibrillary tangles) leading researchers to drop the drug as effective (Selkoe 2012).

This failure led researchers to try and combat the disease from another angle by using the body's natural immune system to break up amyloid-beta plaques. These monoclonal amyloid-beta clearing antibodies (bapineuzumab) were synthetically

constructed and introduced to Alzheimer affected rats. Although these trials applying the antibodies did enhance clearance of amyloid-beta and other amyloid-beta lowering agents, such as inhibitors of the α-secretase complex (promotes cleavage inside of the amyloid-beta fragment), the results did not significantly slow the cognitive decline over an 18-month period (Selkoe 2012). Because of the continued failures in treating the disease during onset, the AD field has moved toward a consensus that secondary prevention (diagnosing and treating the disease before overt symptoms) is more likely to slow the pathogenic process (Selkoe 2012).

5. Discussion

The purpose of this thesis was to identify environmental factors to aid in the secondary prevention of Alzheimer's disease. It not only provided a list of the environmental factors, but also discussed how they work with genetic predispositions to paint a full picture on the transgression of neurodegenertation. As individual genome sequencing becomes more affordable (about $1000), people can indentify their individual genetic risk factors and plan their lives accordingly. This thesis has proven that the avoidance of excessive head trauma and ingestion of cholesterol, a sunny disposition, and ingestion of Omega-3's can all increase one's chances of evading the onset of Alzheimer's disease. Since no treatments have been identified, the only way decrease the possibility of developing Alzheimer's disease is to live smart and to know what to avoid. This honors thesis has provided sufficient information to educate an individual on how to do exactly that.

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