Contributions of Apolipoprotein E and Environmental Factors in Alzheimer's Disease

Kelly C. McMullen

A Senior Thesis submitted in partial fulfillment
of the requirements for graduation
in the Honors Program
Liberty University
Spring 2013

Acceptance of Senior Honors Thesis

This Senior Honors Thesis is accepted in partial fulfillment of the requirements for graduation from the Honors Program of Liberty University.

_____

Gary Isaacs, Ph.D.
Thesis Chair

_____

Randall Hubbard, Ph.D.
Committee Member

_____

Chad Magnuson, Ph.D.
Committee Member

_____

Marilyn Gadomski, Ph.D.
Honors Director

_____

Date

Abstract

Alzheimer's disease (AD) is a debilitating neurodegenerative disorder, which currently affects nearly 5.5 million people in the United States alone. Clinical features often exhibited in AD include memory loss, unusual behavior, personality changes, and impaired cognitive function. The primary molecular hallmarks of AD include deposits of senile plaques and neurofibrillary tangles in brain tissue. A myriad of risk factors are associated with the disease, but this review will focus on Apolipoprotein E polymorphisms and certain environmental factors. Understanding the role of Apolipoprotein E in AD pathology may aid in the development of certain drug therapies and possible cures for AD. Moreover, epigenetic mechanisms such as deoxyribonucleic acid (DNA) methylation are equally important in understanding AD pathology. Environmental factors may have the potential to induce the epigenetic mechanisms associated with AD. As a result of these new findings, the focus of some AD research has recently shifted to a preventive approach in understanding AD pathology. The relationship between Apolipoprotein E polymorphisms and environmental factors in AD pathology will address the importance of preventive measures that can be taken in regard to AD.

Contributions of Apolipoprotein E and Environmental Factors in Alzheimer's Disease

History and Background of Alzheimer's Disease

Alzheimer's disease (AD), the most common form of dementia, is a progressive neurodegenerative disorder characterized by memory loss, unusual behavior, personality changes, and impaired cognitive function. The hallmarks of the disease include loss and damage of neurons, intracellular protein deposits known as neurofibrillary tangles, and extracellular protein deposits referred to as amyloid-beta plaques or senile plaques (Parihar & Hemnani, 2004). Despite advanced imaging techniques such as positron emission tomography (PET), functional magnetic resonance imaging (fMRI), computer-assisted tomography, hydrogen magnetic resonance spectroscopy (^{1}H MRS), and magnetic resonance spectroscopy (MRS), a definitive diagnosis of AD can only be made post-mortem by way of a brain autopsy, which can confirm the relationship between clinical features and the presence of senile plaques and neurofibrillary tangles in brain tissue (Brody, 2011; Parihar & Hemnani, 2004).

Alzheimer's disease was first described by Alois Alzheimer, a German neuropsychiatrist and neuropathologist. Alzheimer firmly believed that clinical work and laboratory research, although separate disciplines, were essential to the development of both. Taking this approach, Alzheimer showed keen interest in his patients and their behavior as well as the examination of their brains after their death. One of Alzheimer's patients was Auguste Deter, a 50-year-old woman exhibiting significant cognitive decline in the form of delirium, hallucinations, memory problems, apathy, and ultimately muteness and unresponsiveness. Although Alzheimer was not the first to report on amyloid plaques, he was the first to notice neurofibrillary tangles. Alzheimer first noticed

the characteristic neurofibrillary tangles in Deter's brain after her death. Subsequent findings similar to Deter's case soon became more common, and Alzheimer's coworker, Emil Kraepelin, considered these findings to be consistent with his own ideas on brain psychiatry. Kraepelin soon dubbed this new illness a disease and referenced it as Alzheimer's disease in his next edition of *Textbook of Psychiatry* (Verhey, 2009).

The dawn of a newly recognized disease always feeds a heightened interest and desire by both the medical and scientific communities to meticulously research and solve the new problem. In the past 100 years, great measures have been taken to understand and combat AD. The past 30 years have met significant success in AD research as many advancements have been made in the disciplines of genetics, epigenetics, molecular biology, and biochemistry (Alzheimer's Association, 2012). Despite the advancements made in AD research, many people still continue to suffer from the disease.

Since the case of Auguste Deter, millions of people have been diagnosed with AD. Worldwide, the disease has affected over 25 million people (Dalvi, 2012). In the United States alone, nearly 5.4 million people had AD in 2012, with 5.2 million of those people being 65 and older. These numbers are expected to rise significantly in the coming years as the baby boomer generation ages and as technological advances allow for longer life expectancy. It has been projected that by the year 2050, 11 million to 16 million people in the United States will have AD, ignoring the possible development of cures for the disease by that time. These predictions are illustrated in Figure 1. With cases of AD on the rise, the economic impact is daunting. The cost of care is expected to rise significantly.

In 2012, the total cost for health care, long-term care, and hospice for patients with AD amounted to $200 billion and is expected to reach $1.1 trillion by 2050 (Alzheimer's Association, 2012).

AD presents in two forms, early-onset and late-onset. Individuals with early-onset AD show symptoms before 65 years of age, while people with late-onset AD show symptoms at 65 years or older. Late-onset AD constitutes the majority of AD cases (Koedam et al., 2010). Symptoms progress within three domains. Cognitive symptoms present first followed by behavioral symptoms and finally ending in functional symptoms (Dalvi, 2012). Although symptoms vary from person to person, memory loss is typically the first symptom that people develop. Because neuron function is impaired in regions of the brain used for forming new memories, short-term memory function is severely affected. Memory loss then leads to other challenges such as having difficulty in completing familiar tasks in daily life, being confused with time and place, misplacing items and being unable to retrace one's steps to find those items, and having difficulty understanding visual images and spatial relationships. Symptoms progressively become worse as behavioral changes become more apparent. People may show drastic changes in mood and personality and may become socially withdrawn. In the final stages, people with AD lose functional abilities and do not recognize loved ones. Assistance must be provided in bathing, dressing, eating and using the bathroom. Ultimately, the individual becomes bed-ridden and becomes extremely susceptible to infection, notably pneumonia. At this point, AD is fatal (Alzheimer's Association, 2012).

A variety of risk factors are associated with AD, age being the leading risk factor. By the time a person reaches 65 years of age, the chances of developing AD doubles

every 5 years thereafter. By age 90, an individual has a 35%-40% chance of having the condition. Genes also serve as a risk factor for AD. The most well known genetic risk factors are mutations in genes such as amyloid precursor protein (*APP*), presenilin-1 (*PSEN-1*), and presenilin-2 (*PSEN-2*) and the ε4 allele of apolipoprotein E (*APOE*) (Welsh-Bohmer, Plassman, & Hayden, 2010). Familial and twin studies have confirmed the large role that genetic factors play in AD. In addition to age and certain genes, environmental factors working through epigenetic mechanisms are also thought to contribute to AD risk (Dalvi, 2012).

Until more can be revealed about the causes of AD and the genetic and molecular influences involved in its pathology, AD will remain insatiable in its course of robbing societies not only economically but also relationally. The future, however, is not entirely grim. New findings continue to be made regarding the role of genetics in AD pathology. Moreover, how gene expression can be altered through various environmental exposures is a promising avenue to understanding the disease more fully. Genes do not have to seal a person's fate. To see a glimpse of both the genetic and environmental factors involved in AD pathology, a protein directly involved in the process known as apolipoprotein E (ApoE) will be investigated in detail followed by an explanation of how environmental factors alongside ApoE may influence AD pathology.

Biology of Apolipoprotein E (ApoE)

Knowing that genetics significantly contribute to the pathology of AD, scientists have intensely studied and searched out genes thought to be associated with AD. To date, researchers have identified over 660 genes that are suspected to be associated with AD.

One gene in particular, accounting for up to 50% of the known genetic contribution to AD, is known as *APOE*, the first gene discovered to have a correlation with late-onset AD. Consequently, *APOE* has been extensively researched (Welsh-Bohmer et al., 2010). *APOE* is responsible for encoding apolipoprotein E (ApoE), a protein involved in the transport of lipids throughout the body.

Structure of Apolipoprotein E

To better understand the function of ApoE, its structure must first be understood. The *APOE* gene is found on the long arm of chromosome 19 at position 13.2 and consists of 1223 base pairs and is made of four exons. The protein product is a 34-kDa protein consisting of 299 amino acids, which are arranged into two structural domains, an N-terminal domain and a C-terminal domain. A hinge region separates the two domains. Four amphipathic α-helices make up the N-terminus, and of particular interest is the fourth α-helix, whose characteristic kinks are responsible for constituting the low-density lipoprotein (LDL) receptor-binding region of ApoE. Many basic amino acids constitute this region of the protein and account for the evident kinks in the N-terminus. The binding ability of ApoE in the N-terminus is heavily influenced by the presence of many basic amino acids (Hsieh & Chou, 2011). The C-terminal domain, made up of amino acids ~225-299, contains the lipid binding region which is comprised of amino acids ~244-272. The amino acid sequence is crucial to the binding ability of the protein. Figure 2 illustrates the ApoE characteristics described above (Mahley, Weisgraber, & Huang, 2009).

Function of Apolipoprotein E

Understanding the function of a protein is essential, but knowing where that protein is made and used in the body is equally important. Primarily, the liver and brain are responsible for synthesizing ApoE. Lesser amounts of ApoE are synthesized in the adrenal glands and the kidneys (Elshourbagy, Liao, Mahley, & Taylor, 1985). Originally, astrocytes, oligodendrocytes, and ependymal layer cells were the only known source of ApoE synthesis in the brain. However, according to recent studies, neurons may produce ApoE in small quantities when under pathophysiological stress (Xu et al., 2006).

Apolipoprotein E performs various functions, but its primary function is to catabolize and transport triglyceride-rich lipoprotein constituents thus allowing for proper transport and circulation of lipoproteins, fat-soluble vitamins, and cholesterol throughout the body but primarily in the brain (Singh, Singh, & Mastana, 2002). Cholesterol, for example, is essential for proper brain function, considering cholesterol is a component of cellular membranes and myelin sheaths within the brain. Ultimately, synaptic integrity and neuronal function depend heavily on cholesterol transport within the brain by way of the contributing activities of ApoE (Pfrieger, 2003). Astrocytes hold the primary responsibility of producing brain ApoE/lipoprotein particles. After astrocytes have produced ApoE/lipoprotein particles, cholesterol and other lipids are delivered to neurons to support neuronal synapses by way of interactions between ApoE receptors and ApoE (Bu, 2012). See Figure 3 for the role of ApoE in lipid transport within the brain.

To ensure that synapse formation is carried out successfully, the following steps must be met. First, astrocytes synthesize and secrete ApoE, which then combines with cholesterol and other lipids to form lipoprotein particles. The assembly of ApoE and

lipids to form lipoprotein particles is conducted by a plasma membrane transporter called ATP-binding cassette, sub-family A (ABCA1). After lipoprotein formation, one of two paths can be taken. The ApoE-lipoprotein particles will either bind to the neuronal ApoE receptors known as, low-density lipoprotein receptors (LDLRs) and low-density lipoprotein receptor-related protein 1 (LRP1), or will be transported to the cerebrospinal fluid (CSF). Moreover, instead of going directly to neuronal ApoE receptors or to CSF, ApoE-lipoprotein particles can be modified through a step involving recruitment of oligodendrocyte-specific lipids and additional ApoE molecules (Bu, 2009).

It has been suggested that AD pathology is heavily influenced by compromised cholesterol metabolism within the brain. For example, AD brains tend to have lower levels of cholesterol in comparison to healthy brains. With this in mind, it is proposed that the different isoforms of ApoE are responsible for compromised cholesterol metabolism within the brain (Steinberg, 2009).

The different alleles of *APOE* include ε2, ε3 and ε4, with ε3 being the most common. The ε3 allele is considered to be the normal form of *APOE* and is present within approximately 79% of all ethnic populations (Alzheimer Research Forum, 2010). Moreover, the ε3 allele is considered a protective agent against late-onset AD (Steinberg, 2009). The other alleles, ε2 and ε4 are less common and are found in 7% and 14% of the population respectively. The ε2 and ε4 alleles are known to be the culprit of various diseases. For example, individuals homozygous for ApoE ε2 are at risk for type III hyperlipoproteinemia, whereas those homozygous for ApoE ε4 are prone to develop atherosclerosis but more importantly late-onset AD (Alzheimer Research Forum, 2010).

The residues at positions 112 and 158 in ApoE account for the structural differences among ApoE ε2, ApoE ε3, and ApoE ε4. The most common isoform, ApoE ε3 has a cysteine at residue 112 and an arginine at residue 158. In contrast, ApoE ε2 has cysteines at both positions resulting in less binding ability of the protein. Similarly, ApoE ε4 has arginines at both positions thus affecting the proper function of ApoE (Ghebranious, Ivacic, Mallum, & Dokken, 2005). Essentially, because the isoforms exhibit different amino acid sequences, the functions of these isoforms will be altered. Again, isoforms ε2 and ε4 are considered abnormal and thus are incapable of carrying out the desired activity of the protein (Mahley et al., 2009). With pathology in mind, the main residues of concern include the residues associated with the N-terminus (1-191) and the C-terminus (225-299), residue 112 and residue 158. The identity of these residues ultimately affects the structure and function of the protein. Figure 2 depicts the basic structure of ApoE, noting key residues associated with the isoforms of the protein (Hsieh & Chou, 2011).

Role of Apolipoprotein E in APP processing and AD Etiology

As mentioned earlier, the ε2 and ε4 isoforms of ApoE account for a heightened predisposition to late-onset AD. Although many factors contribute to the pathology of the disease and various hypotheses regarding AD pathology have been proposed, the Amyloid Cascade Hypothesis is perhaps the most widely accepted view of AD pathology. The foundation of this pathological hypothesis highlights the two main pathological hallmarks associated with AD. These hallmarks include intracellular neurofibrillary tangles and extracellular amyloid plaques, both of which are found in the brain parenchyma (Potter & Wisniewski, 2012).

Vast amounts of research have been devoted to understanding the role of amyloid plaques in AD and how these plaques are processed from the amyloid precursor protein (APP). Essentially, the mechanism in which APP is processed undoubtedly serves a significant role in AD pathology. The production of amyloid plaques in the brain is often due to mutations within the *APP* and *PSEN* genes. However, new evidence suggests that the Amyloid Cascade Hypothesis would not be complete without considering the influence of amyloid-associated inflammatory proteins such as α1-antichymotrypsin (ACT) and ApoE. Both ACT and ApoE influence amyloid formation by assisting other proteins in forming the plaques. In a sense, ACT and ApoE serve as pathological chaperones (Potter & Wisniewski, 2012).

Before elaborating on how ApoE and other amyloid-associated inflammatory proteins play a role in amyloid formation, a basic overview of APP processing should be discussed. Two basic pathways exist in APP processing. One path results in the production of Aβ peptides while the other path does not produce Aβ peptides (O'Brien & Wong, 2011).

First, APP is sorted within the endoplasmic reticulum and golgi apparatus. Once sorting has been achieved in the golgi apparatus, APP is delivered to the axon, where it is transported by fast axonal transport to synaptic terminals. The next few steps in APP processing occur at the cell surface and in the trans golgi network (TGN). By way of clathrin-associated vesicles, APP is transported from the TGN to either the cell surface or directly to an endosomal compartment (O'Brien & Wong, 2011).

Mechanisms at the cell surface, although occurring at rapid paces, are extremely important in APP processing. Once at the cell surface, APP is proteolyzed by α-secretase

and subsequently by γ-secretase. This form of proteolysis does not generate Aβ. Proteolysis of APP by α-secretase and γ-secretase is not the only means of processing APP once it reaches the cell surface. Clathrin-coated pits can also reinternalize APP into an endosomal compartment containing β-secretase and γ-secretase, both of which act as proteases. If γ-secretase proteolyzes APP, Aβ is produced. The Aβ peptide is either subjected to vesicle recycling and ultimately dumped into the extracellular space, or will simply be degraded by the action of lysosomes. Whether APP will be proteolyzed by α-secretase or will be internalized by endosomes remains unclear. The final step to the APP processing cycle entails retromers, which influence communication between endosomal compartments and the TGN (O'Brien & Wong, 2011). See Figure 4 for APP processing and trafficking.

As stated before, ApoE and other amyloid-associated inflammatory proteins contribute significantly to AD pathology in the context of amyloid formation and inflammation via APP processing, but the main question to be answered is whether the plaques and inflammation actually contribute to AD or are merely pathological features of AD. In other words, do amyloid plaques and inflammation cause the disease, or does the disease promote inflammation and amyloid plaque formation? In an attempt to answer this question, research led by Potter and Wisniewski (2012) focused on the influence of ACT and ApoE in plaque formation. The main proposition made by Potter and Wisniewski was that ACT and/or ApoE stimulate the production of amyloid plaques. More specifically, the research done by Potter and Wisniewski revealed that the formation of amyloid plaques, stimulated by ACT and/or ApoE, is heavily dependent on dose size and isoform type.

The ApoE isoform most responsible for promoting plaque formation is ApoE ε4. In contrast, ApoE ε2 acts as an inhibitor in the process of plaque formation (Potter & Wisniewski, 2012).

One study in particular, led by Manelli and colleagues (2007), helped confirm that ApoE and other inflammatory proteins are heavily involved in the amyloid cascade. Manelli and colleagues (2007) demonstrated in their research that Aβ neurotoxicity significantly increased in the presence of ApoE ε4 as compared to ApoE ε2 or ε3. Based off of these findings, Manelli and colleagues confirmed that ApoE ε4 constitutes a negative gain of function but more importantly plays a significant role in the amyloid cascade mechanism. Considering ApoE is an integral component of the amyloid cascade, the absence of ApoE would halt the cascade at the harmless point of Aβ monomers. Essentially, AD would be nonexistent without the action of ApoE in the amyloid cascade (Potter & Wisniewski, 2012).

Despite coming across as a completely different function in relation to AD pathology, ApoE also acts as an agent in Aβ clearance. Rather than being viewed as a destructive agent in the case of contributing to plaque formation, ApoE in the context of Aβ clearance serves as a protective agent. When speaking of ApoE in relation to Aβ clearance, the ε2 and ε3 isoforms are more protective than the ε4 isoform, which yet again confirms that the ε4 isoform increases the risk of AD. The protective qualities of ApoE were confirmed by further experiments done by Potter and Wisniewski using APP transgenic mice carrying a second transgene expressing one or another human ApoE isoform. The human ApoE transgene did in fact inhibit the production of amyloid deposits confirming the protective qualities of ApoE. However, amyloid did happen to

develop in the mice with the ApoE ε4 isoform. This isoform ultimately caused plaque accumulation to occur earlier and more extensively. From these findings, Potter and Wisniewski assumed that human ApoE possibly serves as an inhibitor in the clearance of Aβ plaques, with the ε4 isoform exhibiting the strongest inhibition (Potter & Wisniewski, 2012).

With a basic understanding of two functions that ApoE serves in the amyloid cascade, a more detailed explanation of the actual amyloid cascade mechanism with an emphasis placed on the exact role of ApoE in the mechanism is worth mentioning. Amyloid precursor protein serves as the starting point in the production of Aβ plaques. However, the production of amyloid plaques is not always the final outcome of APP processing. The final outcome of APP depends on which proteins are interacting in the cascade mechanism. Essentially, as APP goes through the processing cascade as described in brief earlier, ApoE eventually participates in the process in its ability to bind Aβ (O'Brien & Wong, 2011).

ApoE is involved in the secretory pathway of APP processing and enters the pathway once the cell has internalized APP by the action of clathrin-mediated proteins. Once the cell has internalized APP, APP becomes a part of an early endosome with the assistance of ApoE and LRP1. Interestingly, amyloidogenic or non-amyloidogenic processing can occur at this point. The type of processing that will ensue depends primarily on which ApoE receptors are directly involved in the process. Specifically, ApoE receptor 2 allows for APP retention at the cell surface thus promoting non-amyloidogenic processing. In contrast, in the presence of ApoE ε4 and LRP1, amyloidogenic processing will ensue ultimately resulting in intraneuronal Aβ

accumulation. Moreover, research has shown that if APP is overexpressed in neuronal cells, ApoE ε4 will increase the production of Aβ. Keeping in mind the combined actions of ApoE ε4 and LRP1 in APP processing, one can further understand the reasoning behind the idea that ApoE ε4 stimulates plaque formation (Bu, 2009).

After reaching the early endosome stage, further APP processing is carried out by β-secretase and γ-secretase. Although either non-amyloidogenic or amyloidogenic processing can occur based on which ApoE receptors are involved, the type of proteases at work in the process also have a profound effect on the end result of APP processing. Both the non-amyloidogenic and amyloidogenic pathways and how α-, β-, and γ-secretases are involved in these pathways are clearly illustrated in Figure 5 (Wilquet & Strooper, 2004). In the non-amyloidogenic pathway, α-secretase first cleaves membranous APP producing APPsα. Next, γ-secretase cleaves the α-carboxy terminal fragment of APPsα generating a p3 peptide and APP intracellular domain (AICD). In the amyloidogenic pathway, β-secretase is used in place of α-secretase thus resulting in the production of APPsβ and membrane-anchored β-carboxy terminal fragment. Next, γ-secretase cleaves the membrane bound β-carboxy terminal fragment thus generating Aβ and AICD (Wilquet & Strooper, 2004). The generated Aβ then accumulates intraneuronally (Bu, 2009).

As mentioned earlier, ApoE acts not only in mediating APP processing but also in mediating Aβ clearance. Despite aggregation of Aβ in the brain, there is opportunity for Aβ to be cleared. Two pathways of Aβ clearance have been identified, both of which are depicted in Figure 6. The first pathway is receptor-mediated clearance and involves the combined effects of microglia, astrocytes, and neurons within the interstitial fluid

drainage pathway or the blood-brain barrier (BBB). ApoE also contributes significantly to this pathway. ApoE can elicit good or bad outcomes to Aβ clearance depending on which isoform serves as the mediator in the process. For example, isoforms ε2 and ε3 bind Aβ directly and have relatively high binding affinity for Aβ. The resulting ε2- and ε3-Aβ complexes are then transferred to the BBB where they will then be delivered to lysosomes to be degraded or to be transcytosed into the plasma for final clearance. In contrast, ApoE ε4 fails to successfully clear Aβ because it has very poor binding affinity for Aβ. Ultimately, clearance mediated by ApoE ε4 leads to highly toxic intraneuronal Aβ accumulation. The other Aβ clearance pathway is executed by proteolytic degradation. Various enzymes produced by neurons or glia act directly in proteolytic degradation, but ApoE ε4 reduces the expression of these enzymes thus affecting proper clearance of Aβ. Recognizing that ApoE has the potential to bind Aβ with high affinity, it is clearly demonstrated that Aβ essentially distracts ApoE from performing its duties in brain lipid metabolism (Bu, 2009).

Apolipoprotein E as a Therapeutic Target

To date, only five drugs are FDA approved to treat AD. These drugs include memantine and acetylcholinesterase inhibitors, tacrine, donepezil, rivastigmine and galantamine. These drugs improve overall cognition in people with AD but are not capable of slowing the progression of AD. Moreover, these drugs are of no value to those in advanced stages of AD (Fenili & McLaurin, 2005). In a sense, the worst damage has already been done, and the approved drugs on the market have minimal positive results. Developing therapies that target earlier pathological changes is greatly needed.

Considering ApoE is directly involved in earlier critical steps in the pathology of AD, ApoE stands as a promising therapeutic target.

Several different strategies have been proposed in developing effective therapeutic models for AD, most of which focus on regulating ApoE expression and function. One promising strategy is to change the structure of ApoE ε4 to better resemble ApoE ε3 since ApoE ε4 is more often responsible for AD pathology. Molecules such as GIND-25, a disulfonate, and GIND-105, a monosulfoalkyl have demonstrated the capability of altering the structure of ApoE ε4 to mimic the structure of ApoE ε3 thus limiting the production of Aβ plaques (Bu, 2009).

Regulating ApoE expression levels in the brain stands as a potential therapeutic strategy as well. It has been suggested that increasing the expression of all ApoE isoforms may slow down the progression of AD. In implementing this strategy, consideration should be taken in that increasing ApoE ε4 expression could result in harmful effects, primarily slowing down Aβ clearance. Ultimately, this approach in managing expression levels of ApoE should be approached carefully since ApoE ε4 can affect the brain in two different ways, either by loss of protection or gain of toxicity (Bu, 2009).

An additional therapeutic strategy is to take advantage of liver X receptors, which are oxysterol receptors acting as transcription factors. Being transcription factors, liver X receptors are responsible for upregulating ApoE in the brain, ultimately promoting cholesterol efflux in neurons and glia. Moreover, liver X receptors act as agonists, thus aiding in the clearance of Aβ (Bu, 2009).

Considering ApoE is directly involved in Aβ deposition, another promising therapeutic model could be disrupting ApoE-Aβ interaction. Disruption is possible by implementing a synthetic Aβ peptide that resembles the ApoE-binding site on a full-length Aβ molecule. This synthetic Aβ peptide has been tested in amyloid mouse models and has proved to be quite successful in that it was BBB permeable and non-toxic. Additionally, the synthetic Aβ peptide significantly reduced total brain Aβ levels and Aβ plaques and sharpened memory performance in two amyloid mouse models (Bu, 2009).

Other possible therapeutic targets are ApoE receptors as they are heavily involved in brain ApoE-lipoprotein metabolism and Aβ clearance. LRP1 and LDLR, specifically, serve as promising targets for therapeutic measures considering decreased levels of LRP1 are observed in AD brains. It is possible that increasing LRP1 expression may result in more effective Aβ clearance thus halting the progression of AD. Furthermore, finding a way to block the interaction between APP and ApoE receptors in APP processing has a promising outcome (Bu, 2009).

Epigenetics and Environmental Factors in Alzheimer's Pathology

Understanding the genetic risk factors of AD, such as the role of ApoE polymorphisms in AD pathology, is certainly a step forward in developing treatments and other clinical interventions for the disease. However, current knowledge of how epigenetics and environmental factors influence AD pathology heralds promising preventive measures in warding off the disease. More remains to be known about the link between genes and environmental factors, but recent evidence indicates that regardless of a person's genetic predisposition, his or her risk for AD can be substantially lowered by lifestyle changes.

General epigenetic mechanisms in relation to ApoE will be discussed in order to understand how lifestyle changes can potentially prevent AD.

Epigenetic Mechanisms

Epigenetics, an emerging field of study, seeks to explain how environmental factors have the potential to influence changes in phenotype through alterations in the transcriptional activity of various genes (Welsh-Bohmer et al., 2010). Essentially, gene expression is not entirely dependent on DNA sequence and can thus be modified by certain epigenetic mechanisms. Inhibiting transcriptional access to certain genes is the basis of epigenetic mechanisms, and some evidence shows that environmental factors such as diet, hazardous exposures, and certain life events are involved in these epigenetic mechanisms (Mastroeni et al., 2011). Interestingly, epigenetic modifications can occur in two different realms. In one case, specific gene loci in specific cells can be subject to modifications while in other cases, multiple genes in a variety of cells can be subject to modifications. The latter case is thought to be involved in aging, the greatest risk factor for AD (Mastroeni et al., 2011). To understand how environmental factors are translated to epigenetic modifications of certain genes, it is best to be aware of the different types of epigenetic mechanisms.

Epigenetic mechanisms can be subdivided into three primary categories, which include histone modifications, DNA methylation, and RNA-related mechanisms. For the sake of brevity, only DNA methylation will be covered in detail. First, however, a basic overview of histone modification will be discussed, considering DNA methylation is a form of histone modification (Mastroeni et al., 2011).

Histones are proteins responsible for packaging and ordering DNA into nucleosomes, the structural units of chromosomes. DNA winds around these histone proteins like thread on a spool. Although their primary function rests in DNA packaging and ordering, histones also play a significant role in gene regulation (Cramer & Wolberger, 2011). The principal means by which histones influence gene regulation is through conformational changes in protein structure of the histones. Moreover, how DNA wraps around the histones can influence gene regulation. The transcriptional machinery thus has altered access to the regions that have been modified by the two mechanisms previously mentioned. Various mechanisms are known to modify histones. Such mechanisms include acetylation, methylation, phosphorylation, ubiquitination, sumoylation, citrullination, and adenosine diphosphate ribosylation. Of these mechanisms histone acetylation and methylation are the most common forms of histone modification and the most well understood mechanisms. Histone acetylation causes conformational relaxation of the chromatin by neutralizing the histone proteins. Due to the neutralization, the histone protein tails interact more weakly with the negatively charged phosphate groups on the DNA. Now in a relaxed state, certain genes are more easily accessible to transcriptional machinery and DNA methylation is now possible. In reverse, if the histone proteins are deacetylated, the chromatin becomes more condensed, ultimately causing the genes in that region of DNA to be inaccessible (Mastroeni et al., 2011).

DNA methylation is perhaps the most well understood epigenetic mechanism. Interestingly, DNA methylation often acts in part with acetylation, as previously mentioned. Due to its heavy influence in histone modification, DNA methylation can be thought of as its own class of epigenetic mechanism despite being a form of histone

modification (Mastroeni et al., 2011). As the name suggests, DNA methylation modifies genome function and chromosomal stability by methylating DNA, specifically the cytosine in CpG dinucleotides (Bollati et al., 2011). Any CpG site, whether in coding or noncoding regions, has the potential to be methylated. Regions of DNA rich in CpG sites are often referred to as CpG islands, and interestingly, the human genome is known to have over 50,000 CpG islands. Furthermore, CpG shores, regions within 2 kb of CpG-enriched sequences have been shown to be methylated, but in a tissue specific manner (Mastroeni et al., 2011).

The enzymes responsible for carrying out DNA methylation include the DNA methyltransferases, DNMT1, DNMT2, DNMT3a/b, and DNMT4. Prior to methylation, histone acetylation must occur to allow the DNA to be accessible to methylation. With the DNA now accessible, the enzymes are able to incorporate methyl groups into the genome. The source of the methyl groups used in this process is methyltetrahydrofolate. The methyl group is then transferred from S-adenosylmethionine to the cytosine in the CpG site (Mastroeni et al., 2011). Methylation of the CpG regions is associated with gene silencing. However, active transcription ensues if the body of the gene is methylated (Balazs, Vernon & Hardy, 2011).

Although DNA methylation serves various functions, its primary purpose is to alter gene expression. DNA methylation can alter gene expression by several different mechanisms, but only three mechanisms will be mentioned here. The first mechanism in which methylation can alter gene expression is by inducing histone modifications that are involved in preventing transcriptional machinery from accessing a gene. Essentially, genes that are highly methylated become repressed, and genes that are scarcely

methylated undergo enhanced expression or overexpression. Some exceptions to this pattern do exist. The second mechanism in which DNA methylation alters gene expression is through methyl-CpG-binding proteins (MeCPs). For example, MeCP2 binds to methylated DNA triggering histone deacetylases to cause the chromatin to become more condensed ultimately preventing access to certain genes. In some cases, MeCP2 does not depend on DNA methylation or histone deacetylation to condense chromatin. The final epigenetic mechanism involved in DNA methylation is hydroxymethylation, which occurs when 5-methylcytosines are oxidized to 5-hydroxymethylcytosines. The transformation of 5-methylcytosine to 5-hydroxymethylcytosine ultimately causes certain genes to be highly restricted. Neurons have shown to exhibit hydroxymethlation, which is thought to be a result of oxidative damage and/or oxidative enzymes (Coppieters & Dragunow, 2011). See Figure 7 for a brief overview of DNA methylation

Epigenetic Mechanisms in Alzheimer's Pathology

As research continues to reveal the specifics of how histone modifications and DNA methylation function in altering gene function and transcription, more can begin to be explained in regard to the relationship between environmental factors and AD pathology. Although not officially confirmed in literature, environmental factors are very likely to be the cause of epigenetic modifications resulting in diseases ranging from cancer to AD. Regarding DNA methylation, several studies have shown that the genomes of people with AD tend to be hypomethylated. However, in some cases it has been observed that certain genes, primarily nucleolar rRNA genes, are hypermethylated. Hypermethylation of rRNA genes is thought to be a major contributor to AD pathology

due to ribosomal deficiencies that result from the hypermethylation. In short, people with AD tend to have altered DNA methylation patterns in comparison with healthy individuals (Leszek, Sochocka & Gąsiorowski, 2012). As more is understood regarding these DNA methylation patterns in normal aging brains versus AD brains, the relationship between DNA modifications and AD pathology can be better explained (Coppieters & Dragunow, 2011).

Equally important is the fact that DNA hypomethylation is known to be associated with aging, the greatest risk factor for developing AD. The correlation between aging and AD and how DNA methylation is involved in AD pathology opens up many new prospects in understanding both the aging process and AD pathology. To investigate this correlation, Ladd-Acosta et al., (2007) conducted a study using bisulfite conversion. The main conclusion drawn from this study was that DNA methylation levels at specific CpGi loci increased with increasing age (Ladd-Acosta et al., 2007).

Although DNA methylation is directly involved in tau and neurofibrillary tangle formations and in Aβ-related mechanisms, it does have possible association with ApoE as well. Not much research has focused on the methylation status of *APOE* thus far (Mastroeni et al., 2011). However, some evidence has suggested that the *APOE* promoter is poorly methylated. Interestingly, the degree of methylation does vary among the different *APOE* alleles. For example, the methylation of CpG sequences are evident in the ε4 allele but not in the ε2 or ε3 alleles (Wang, Oelze & Schumacher, 2008). In review, the ε4 allele stands as a significant risk for developing AD.

Interestingly, however, having the ε4 allele does not ensure the development of AD, which begs the question of whether or not methylation status at ε4 CpG sites is altered in ε4 carriers who end up developing AD (Mastroeni et al., 2011).

Inducing Epigenetic Changes via Environmental Factors

Much more can be said about epigenetic mechanisms, but of particular interest is identifying certain environmental factors that may induce epigenetic mechanisms. As mentioned before, aging is the greatest risk factor for AD and thus stands as a primary inducing factor in epigenetic mechanisms. Aging clearly cannot be evaded and therefore serves as a poor modifiable agent (Welsh-Bohmer et al., 2010). Other environmental factors, however, are promising modifiable agents. Many different environmental factors have been studied, including lead, arsenic, tobacco, education, diet, and engagement in physical activity to name a few.

Although all of the aforementioned factors are important in epigenetic mechanisms, the discussion here will focus on diet. A study conducted by Gu et al. (2010) demonstrated the effects of diet on AD development. The dietary patterns of 2000 subjects were analyzed. Only 253 people developed AD, but Gu et al. confirmed that diets rich in omega-3 and omega-6 polyunsaturated fatty acids, vitamin E, and folate, along with limited consumption of saturated fatty acids and vitamin B12, reduced the risk of AD. Moreover, those who ate more fruits and vegetables and less animal products lowered their chances of developing AD. The reason that eating less meat protects against AD is that meat contains high levels of vitamin B12, a risk factor for AD. Likewise, eating more fruits and vegetables protects against AD because of the high levels of vitamin C found in these foods.

Interestingly, vitamin C has been found to decrease DNA methylation, specifically in human embryonic stem cell lines, HES2 and HES3 (Coppieters & Dragunow, 2011).

Similar to the findings of Gu et al., Dr. Campbell (2006), author of *The China Study*, has found after a twenty-seven-year laboratory program funded by the National Institutes of Health, the American Cancer Society and the American Institute for Cancer Research, and after four decades of biomedical research that a healthy diet, primarily a plant-based diet, has the potential to prevent diseases such as AD. Such a diet has the potential to prevent not only AD but also other diseases such as heart disease and diabetes, both of which are risk factors for AD. Campbell provides several arguments that are consistent with one another and support the notion that diet has a profound effect on AD development. One argument rests on recent studies focusing on the prevalence of AD in Japanese men living in Hawaii versus Japanese men living in Japan. According to this study, The Japanese American men had much higher rates of AD than the Japanese men living in Japan. Similarly, an additional study found that African American men living in Indiana had significantly higher rates of dementia and AD than native Africans. A more broad study, focusing on dietary habits in eleven different countries, found that populations with high fat intake and low cereal and grain intake experienced higher rates of AD. As illustrated by the previously mentioned studies, dietary habits have a profound effect on AD pathology (Campbell & Campbell, 2006). In addition, one can gather from these findings that the typical American diet, which is commonly high in saturated fat and animal protein acts as a significant culprit in the development of AD.

Concluding Remarks

Since its initial discovery by Alois Alzheimer about 100 years ago, AD has left in its path a history of harrowing endings for many individuals and families. However, throughout the same historical timeline, much scientific advancement has been made both in the realm of therapeutic and preventive approaches, thus giving hope for a brighter future. AD is a complicated disease involving a myriad of risk factors, ranging from genetic factors to environmental factors. Of recent interest is the role ApoE plays in AD pathology and how lipid metabolism within the brain can be drastically impaired due to minor structural differences in ApoE. Further investigation of the direct impact that various ApoE isoforms have on lipid and cholesterol metabolism will help elucidate the questions yet to be answered regarding AD.

The relationship between epigenetic mechanisms and environmental factors is equally important in the study of AD pathology, and research in this realm has gained much more momentum in recent years. Despite the wealth of knowledge acquired in such a short amount of time in the discipline of epigenetics, much still remains to be known about the mechanisms involved. More is known how epigenetic mechanisms associate with APP, but very little is known about ApoE and epigenetic mechanisms. For example, future research could focus on the methylation status of ApoE isoforms and how this affects AD pathology. Likewise, delineating how environmental factors such as diet have a direct influence on the control of epigenetic mechanisms is greatly needed to advance our knowledge about AD. Much encouragement can be taken from the fact that environmental factors like diet play a significant role in AD pathology. Making simple lifestyle changes may possibly be the key to warding off AD.

Appendix

Table 1. Commonly used abbreviations.

ABCA-1	ATP-binding cassette, sub-family A
ACT	α1-antichymotrypsin
AD	Alzheimer's disease
APOE	Apolipoprotein E
APP	Amyloid precursor protein
BBB	Blood brain barrier
DNA	Deoxyribonucleic acid
LDL	Low-density lipoprotein
LDLR	Low-density lipoprotein receptor
LRP1	Low-density lipoprotein receptor-related protein 1
TGN	Trans golgi network

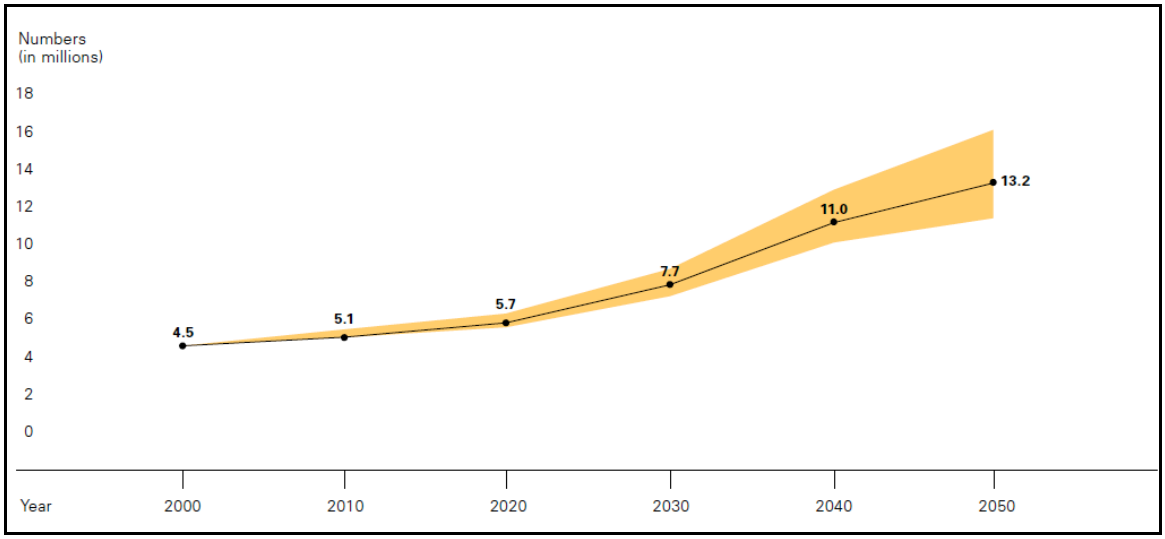

Figure 1. Projected Numbers of People Age 65 and Over in the U.S. Population with Alzheimer's Disease Using the U.S. Census Bureau Estimates of Population Growth*

*Numbers indicate middle estimates per decade. Colored area indicates low and high estimates per decade.

(Alzheimer's Association, 2012, p. 19).

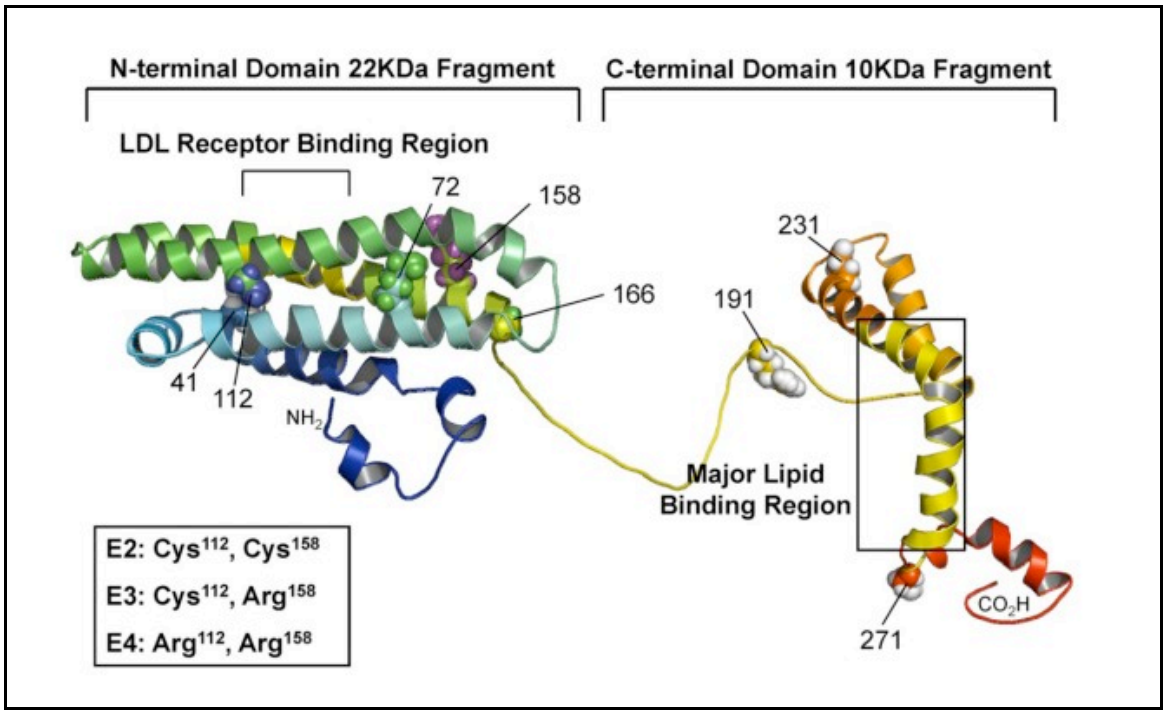

Figure 2. The N-and-C-terminal domains of human ApoE are important in both the structure and function of ApoE. The N-terminal domain of ApoE contains the LDL receptor binding region while the C-terminal domain contains the lipid binding region. Residues 112 and 158 of the E2, E3, and E4 isoforms are also important as amino acid differences among these isoforms account for altered structure and function of ApoE (Hsieh & Chou, 2011, p. 2).

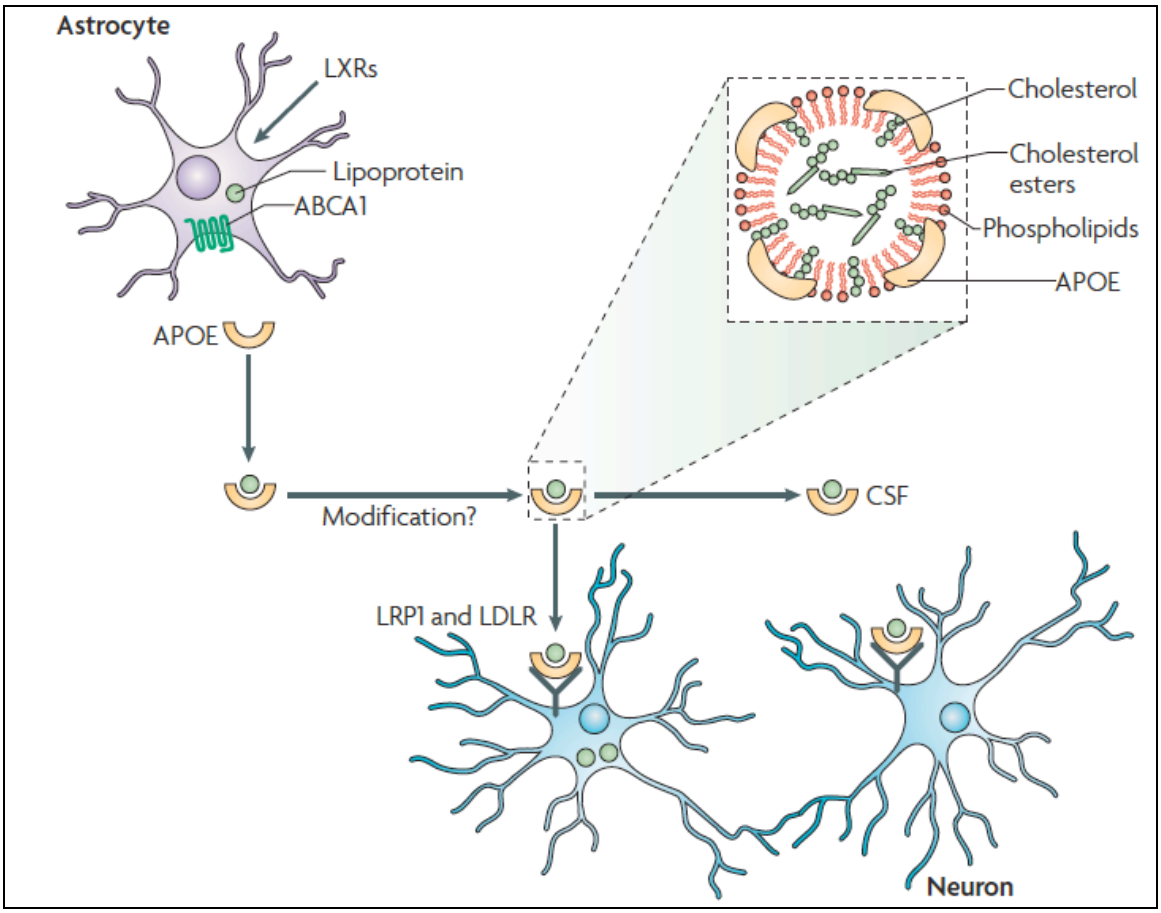

Figure 3. The action of ApoE is crucial to proper neuronal function. First, astrocytes secrete ApoE, which assembles lipids and cholesterol into lipoprotein particles. ABCA1 is a plasma membrane transporter responsible for loading the lipids onto ApoE. The newly assembled ApoE-lipoprotein particle (as depicted in the inset) can undergo modifications before binding to receptors on neurons. Once ApoE binds to receptors on neurons, synapse formation and repair can occur (Bu, 2009, p.8).

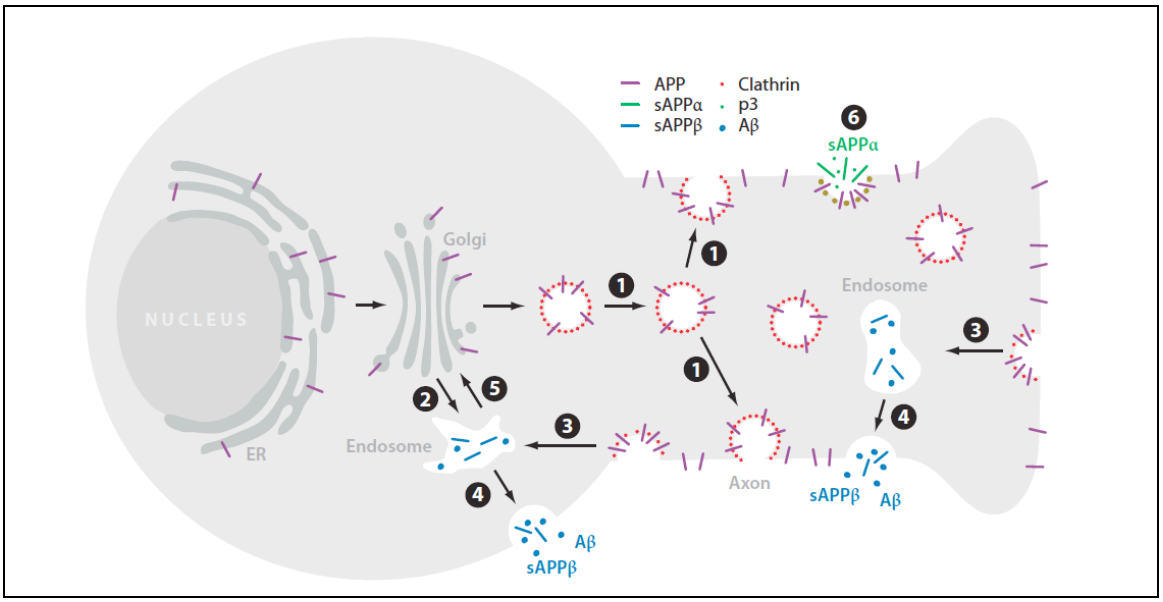

Figure 4. APP trafficking in neurons is a process necessitating various components. **"**Newly synthesized APP (*purple*) is transported from the Golgi down the axon (1) or into a cell body endosomal compartment (2). After insertion into the cell surface, some APP is cleaved by α-secretase (6) generating the sAPP α fragment, which diffuses away (*green*), and some is reinternalized into endosomes (3), where Aβ is generated (*blue*). Following proteolysis, the endosome recycles to the cell surface (4), releasing Aβ (*blue*) and sAPP β. Transport from the endosomes to the Golgi prior to APP cleavage can also occur, mediated by retromers (5)" (O'Brien & Wong, 2011, p. 191).

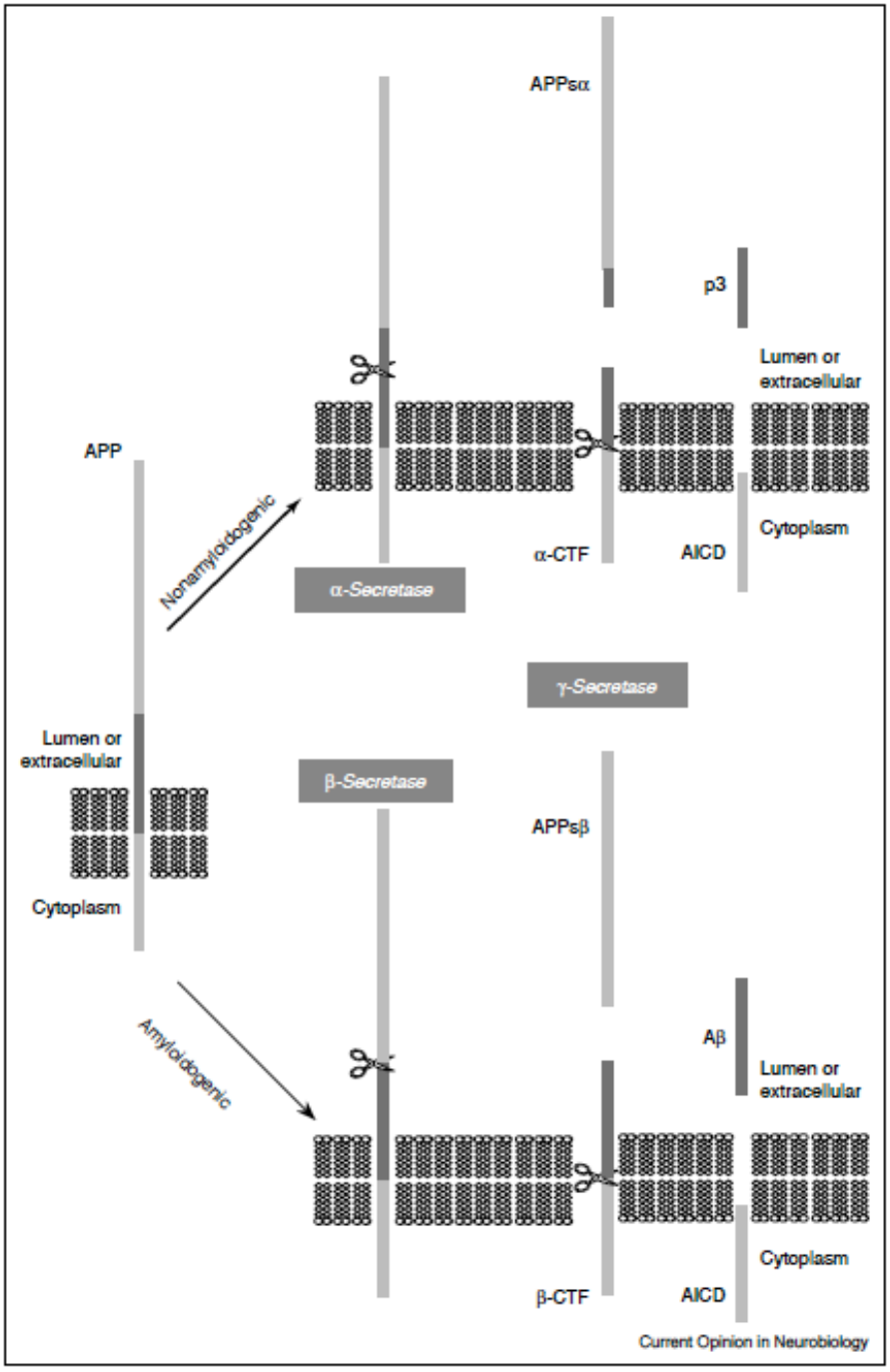

Figure 5. APP can be processed by way of two pathways. Both amyloidogenic and non-amyloidogenic pathways are possible in APP processing. β- and γ-secretase are involved in the amyloidogenic pathway whereas α- and γ-secretase are involved in the non-amyloidogenic pathway (Wilquet & Strooper, 2004).

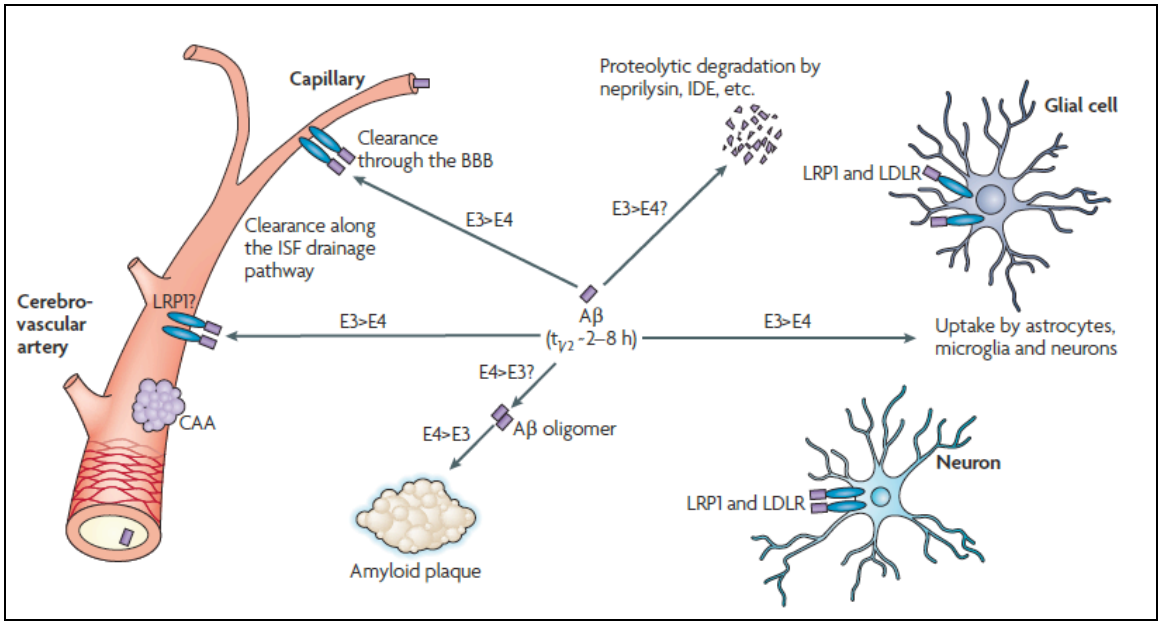

Figure 6. ApoE is also involved in Aβ clearance. The two major pathways in which ApoE clears Aβ deposits are receptor-mediated clearance and clearance by proteolytic degradation by endopeptidases. The different effects of ApoE E3 and E4 are noted. LDLR-related protein 1 (LRP1) is heavily involved in the receptor-mediated clearance pathway by binding to Aβ directly (Bu, 2009, p.7).

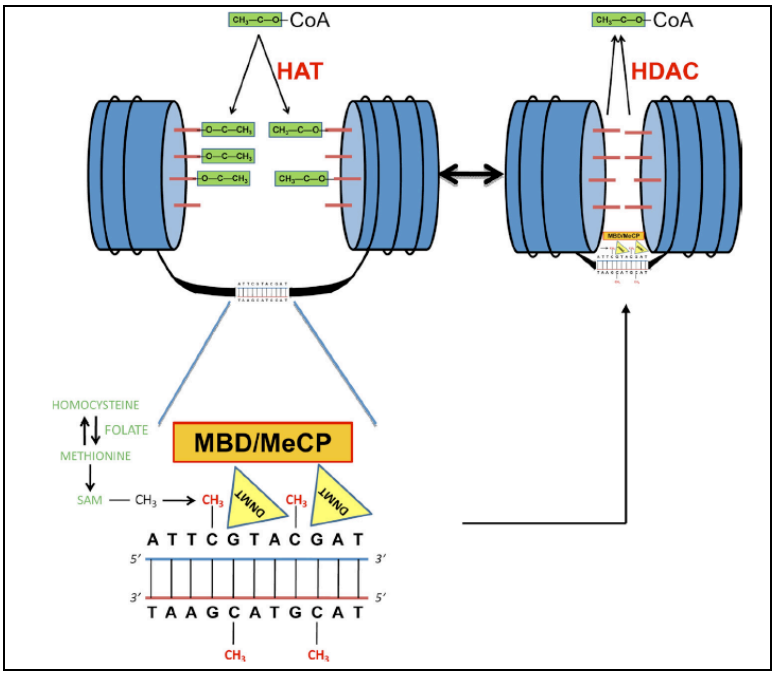

Figure 7. DNA methylation is an important means for altering gene expression. Chromatin, which is made of histones (blue cylinders) and DNA, is transcriptionally active in a relaxed state. Chromatin transitions to the relaxed state when acetyl groups (green blocks) are transferred from acetyl-coenzyme A to histone tails (red rods) by way of histone acetyltransferases (HATs). DNA methylation occurs at the cytosines of adjacent C-G/G-C dinucleotides by the action of DNA methyltransferases (DNMTs). Methyl groups originate from methyltetrahydrofolate in conjunction with the methionine/homocysteine cycle. CpG-methyl-binding-domain proteins (MBDs) and methylation complex proteins (MeCps), which attract histone deacetylases (HDACs), are involved in further inhibition of transcriptional access (Mastroeni et al., 2011, p. 1163).

References

Alzheimer Research Forum. (2010). Gene overview of all published AD-association studies for APOE_E2/3/4 [Data File]. Retrieved from http://www.alzgene.org/geneoverview.asp?geneid=83

Alzheimer's Association. 2012 Alzheimer's disease facts and figures. *Alzheimer's and Dementia: The Journal of the Alzheimer's Association*. March 2012; 8:131–168.

Balazs, R., Vernon, J., & Hardy, J. (2011). Epigenetic mechanisms in Alzheimer's Disease: progress but much to do. *Neurobiology of Aging, 32*(7), 1181-1187. doi: 10.1016/j.neurobiolaging.2011.02.024

Bollati, V., Galimberti, D., Pergoli, L., Dalla Valle, E., Barretta, F., Cortini, F., . . . Baccarelli, A. (2011). DNA methylation in repetitive elements and Alzheimer Disease. *Brain Behavior and Immunity, 25*(6), 1078-1083. doi: 10.1016/j.bbi.2011.01.017

Brody, H. (2011). Alzheimer's Disease. *Nature, 475*(7355), S1-S39.

Bu, G. (2009). Apolipoprotein E and its receptors in Alzheimer's Disease: pathways, pathogenesis and therapy. *Nature Reviews Neuroscience, 10*(5), 333-344. doi: 10.1038/nrn2620

Bu, G. (2012). ApoE and ApoE receptors in brain lipid metabolism and AD. *Molecular Neurodegeneration, 7*(Suppl 1), L10-L10. doi: 10.1186/1750-1326-7-S1-L10

Campbell, T. C., & Campbell, T. M., II. (2006). *The China study: the most comprehensive study of nutrition ever conducted and the startling implications for diet, weight loss and long-term health*. Dallas, Tex: BenBella Books.

Coppieters, N., & Dragunow, M. (2011). Epigenetics in Alzheimer's Disease: A focus on DNA modifications. *Current Pharmaceutical Design, 17*(31), 3398-3412.

Cramer, P., & Wolberger, C. (2011). Proteins: histones and chromatin. *Current Opinion in Structural Biology, 21*(6), 695-697. doi: 10.1016/j.sbi.2011.10.006

Dalvi, A. (2012). Alzheimer's Disease. *Disease-a-Month, 58*(12), 666-667. doi: 10.1016/j.disamonth.2012.08.008

Elshourbagy, N. A., Liao, W. S., Mahley, R. W., & Taylor, J. M. (1985). Apolipoprotein E mRNA is abundant in the brain and adrenals, as well as in the liver, and is present in other peripheral tissues of rats and marmosets. *Proceedings of the National Academy of Sciences of the United States of America, 82*(1), 203-207. doi: 10.1073/pnas.82.1.203

Fenili, D., & McLaurin, J. (2005). Cholesterol and ApoE: a target for Alzheimer's Disease therapeutics. *Current Drug Target -CNS & Neurological Disorders, 4*(5), 553-567. doi: 10.2174/156800705774322085

Ghebranious, N., Ivacic, L., Mallum, J., & Dokken, C. (2005). Detection of ApoE E2, E3 and E4 alleles using MALDI-TOF mass spectrometry and the homogeneous mass-extend technology. *Nucleic Acids Research, 33*(17), e149-e149. doi: 10.1093/nar/gni155

Hsieh, Y., & Chou, C. (2011). Structural and functional characterization of human Apolipoprotein E 72-166 peptides in both aqueous and lipid environments. *Journal of Biomedical Science, 18*(1), 1-9. doi: 10.1186/1423-0127-18-4

Koedam, E. L. G. E., Lauffer, V., van der Vlies, A. E., van der Flier, W. M., Scheltens, P., & Pijnenburg, Y. A. L. (2010). Early-versus late-onset Alzheimer's Disease: more than age alone. *Journal of Alzheimer's Disease : JAD, 19*(4), 1401-1408.

Ladd-Acosta, C., Feinberg, A. P., Pevsner, J., Sabunciyan, S., Yolken, R. H., Webster, M. J., . . . Potash, J. B. (2007). DNA methylation signatures within the human brain. *The American Journal of Human Genetics, 81*(6), 1304-1315. doi: 10.1086/524110

Leszek, J., Sochocka, M., & Gąsiorowski, K. (2012). Vascular factors and epigenetic modifications in the pathogenesis of Alzheimer's Disease. *Journal of the Neurological Sciences, 323*(1-2), 25-32. doi: 10.1016/j.jns.2012.09.010

Mahley, R. W., Weisgraber, K. H., & Huang, Y. (2009). Apolipoprotein E: structure determines function, from atherosclerosis to Alzheimer's Disease to AIDS. *Journal of Lipid Research, 50 Suppl*(Supplement), S183-S188. doi: 10.1194/jlr.R800069-JLR200

Mastroeni, D., Grover, A., Delvaux, E., Whiteside, C., Coleman, P. D., & Rogers, J. (2011). Epigenetic mechanisms in Alzheimer's Disease. *Neurobiology of Aging, 32*(7), 1161-1180. doi: 10.1016/j.neurobiolaging.2010.08.017

O'Brien, R. J., & Wong, P. C. (2011). Amyloid precursor protein processing and Alzheimer's Disease. *Annual Review of Neuroscience, 34*(1), 185-204. doi: 10.1146/annurev-neuro-061010-113613

Parihar, M. S., & Hemnani, T. (2004). Alzheimer’s Disease pathogenesis and therapeutic interventions. *Journal of Clinical Neuroscience, 11*(5), 456-467. doi: 10.1016/j.jocn.2003.12.007

Pfrieger, F. W. (2003). Cholesterol homeostasis and function in neurons of the central nervous system. *Cellular and Molecular Life Sciences : CMLS, 60*(6), 1158-1171. doi: 10.1007/s00018-003-3018-7

Potter, H., & Wisniewski, T. (2012). Apolipoprotein E: essential catalyst of the Alzheimer amyloid cascade. *International Journal of Alzheimer's Disease, 2012*, 1-9. doi: 10.1155/2012/489428

Singh, P. P., Singh, M., & Mastana, S. S. (2002). Genetic variation of apolipoproteins in North Indians. *Human Biology; an International Record of Research, 74*(5), 673-682. doi: 10.1353/hub.2002.0057

Steinberg, M. G. (2009). Pathogenic chromatin modifiers: their molecular action linking pathogenicity with genetic variability, epigenetic modifications and environmental factors in Alzheimer Disease. *Bioscience Hypotheses, 2*(3), 163-169. doi: 10.1016/j.bihy.2009.02.002

Verhey, F. R. J. (2009). Alois Alzheimer (1864-1915). *Journal of Neurology, 256*(3), 502-503. doi: 10.1007/s00415-009-0003-6

Wang, S., Oelze, B., & Schumacher, A. (2008). Age-specific epigenetic drift in late-onset Alzheimer's Disease. *PloS One, 3*(7), e2698. doi: 10.1371/journal.pone.0002698

Welsh-Bohmer, K. A., Plassman, B. L., & Hayden, K. M. (2010). Genetic and environmental contributions to cognitive decline in aging and Alzheimer's Disease. *Annual Review of Gerontology & Geriatrics, 30*, 81-114.

Wilquet, V., & Strooper, B. D. (2004). Amyloid-beta precursor protein processing in neurodegeneration. *Current Opinion in Neurobiology, 14*(5), 582-588. doi: 10.1016/j.conb.2004.08.001

World Health Organization. (2011). The top 10 causes of death [Date file]. Retrieved from http://www.who.int/mediacentre/factsheets/fs310/en/index.html

Xu, Q., Bernardo, A., Walker, D., Kanegawa, T., Mahley, R. W., & Huang, Y. (2006). Profile and regulation of Apolipoprotein E (ApoE) expression in the CNS in mice with targeting of green fluorescent protein gene to the ApoE locus. *Journal of Neuroscience, 26*(19), 4985-4994. doi: 10.1523/JNEUROSCI.5476-05.2006