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# Molecular Mechanisms of Alzheimer's Disease

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### Abstract

Alzheimer's disease was first discovered in 1906 by Alois Alzheimer. It is a neurodegenerative disease characterized by the buildup of toxic amyloid plaque and intracellular neurofibrillary tangles, which results in the progressive loss of cognitive function and memory. Since its discovery, the disease has become a growing health concern, particularly in the developed world, where the ageing demographics have contributed to an increase in its prevalence and incidence. The earliest research into the disease focused on neurochemical analyses and resulted in the formulation of the cholinergic hypothesis. The mechanism of disease was explained as the degeneration of the cholinergic system and a reduction in acetylcholine. While much data supports this hypothesis, it fails to explain the accumulation of amyloid plaque, a hallmark of the disease. Analysis of the genetic factors in familial Alzheimer's disease, and the discovery of the higher risk for Alzheimer's disease amongst individuals with Down's syndrome led to the more comprehensive amyloid cascade hypothesis. The failure of both amyloid centric drugs and cholinesterase inhibitors to have a significant impact on disease progression has caused some to have rejected both these hypotheses to focus on other possible causes. However, there is undoubtedly a wealth of data in support of both the cholinergic hypothesis and the amyloid cascade hypothesis. Understanding the functional relationship between the cholinergic system and the formation of beta amyloid plaques may lead to a greater understanding of the mechanism of disease and provide a target for more effective therapy.


### Introduction

Alzheimer's disease was first identified by Alois Alzheimer in 1906. However not until the 1970's did it become a major and significant area of research. Since that time much has been discovered about the mechanisms of the disease, however the precise biological processes in the disease are mostly unknown and the large variance in its progression amongst patients with the disease needs to be better understood.

Alzheimer's disease is a neurodegenerative disease that effects memory, behavior and eventually leads to death within an average of 8 years of diagnosis, the last three of which are typically spent with full time care or in an institution.

The changing demographics worldwide and with the baby boom generation reaching ages 70 and beyond has led Alzheimer's disease to become one of the biggest healthcare concerns in the developed world. This greater prevalence and incidence of Alzheimer's disease, the largest cause of dementia, has created a huge burden on society.

Millions of Americans have Alzheimer's disease and other forms of dementias. An estimated 5.4 million Americans of all ages had Alzheimer's disease in 2016. One in nine people aged 65 years or older and about one third (32%) of people aged 85 and older have the disease (Herbert et al., 2013).

The future projections for Alzheimer's disease are equally bleak. Approximately 476,000 people will develop the disease in the United States in 2016, with the numbers increasing dramatically with age. There will be 63,000 new cases among people aged 65 to 74 and 241,000 new cases among people aged 85 and older. Because of the ageing demographics in the United States, these numbers are projected to double by 2050 (Herbert et al., 2001).

Alzheimer's disease is one of the leading causes of both mortality and morbidity in the United States. It is currently the sixth leading cause of death for those 65 years and older. According to data from the National Center for Health Statistics of the Centers for Disease Control and Prevention (CDC), 84,767 people died from it in 2013. These numbers only consider those who have cause of death listed as Alzheimer's disease on their death certificate. Death certificates for individuals with Alzheimer's disease often list other acute conditions, such as pneumonia, as the primary cause of death and therefore death due to Alzheimer's is most likely underreported.

The cost of the disease is very substantial, both in terms of the value of the unpaid caregiving for Alzheimer's disease which has been estimated at $221.3 billion in 2013, and the total payments made which in 2016 was estimated at $236 billion. Overall the cost of healthcare, long term care and hospice care for individuals with the disease makes it one of the costliest chronic diseases to society (Hurd et al., 2013).

Alzheimer's disease is characterized by a gradually worsening ability to remember new information. This occurs because the first neurons to be damaged and destroyed are in the area of the brain responsible for creating new memories. As the disease causes the destruction of neurons in other regions of the brain, symptoms worsen and individuals experience other difficulties such as challenges in problem solving and planning, confusion with time and place, decreased and poor judgement, and withdrawal from social activities and work.

There are about 100 billion neurons in the adult healthy brain. During the development of Alzheimer's disease, the connections and synapses between neurons is hindered and the overall number of neurons decrease. The destruction of neurons and the disruption of the cellular neuronal circuits lead to many of

the symptoms. The brains of people with advanced Alzheimer's disease show inflammation, dramatic shrinkage from cell loss and widespread debris from dead and destroyed neurons. Some of these brain changes can begin 20 years prior to the onset of symptoms for the disease (Villemagne et al., 2013).

No simple test currently exists for diagnosis of Alzheimer's disease, rather an individual's physician together with the help of a neurologist will use a variety of methods to assist in a diagnosis. These include obtaining a family history and medical history of the patient which may include psychiatric, cognitive and behavioral histories. In addition, diagnosis is often aided by conducting cognitive tests and physical and neurological examinations. Finally, a physician will typically use blood tests and brain imaging to rule out other possible causes of behavioral and memory changes such as tumor formation or nutrient deficiencies.

Much research has focused on a potential precursor to Alzheimer's disease known as Mild Cognitive Impairment (MCI). An individual with Mild Cognitive Impairment will experience mild but measurable changes in thinking abilities that are noticeable to friends and families of the affected person but that do not affect the person's ability to carry out normal everyday functions and activities (Roberts & Knopman, 2013). It is estimated that about 20% of people over the age of 65 have Mild Cognitive Impairment. Recent studies have shown that an average of 32% of people with MCI will develop Alzheimer's disease within 5 years. However, some with MCI will see their cognitive decline stabilize, and in some cases, they may even return to normal cognition (Ward et al., 2013).

In a small percent of those diagnosed with Alzheimer's disease the development of the disease can be attributed to genetic mutations. Three genes have been implicated in its development. These are the genes which encode for the amyloid precursor protein (APP), the genes for presenilin-1 and for presenilin-2. Mutations in both the APP gene and the presenilin-1 gene result in guaranteed development of the disease, while a mutation in the presenilin-2 gene leads to a 95% chance of its development. Individuals with mutations in any of these three genes will usually develop symptoms as young as age 30, unlike the vast majority of Alzheimer's cases, which are late onset where symptoms typically develop at age 65 and over (Bekris et al., 2010).

People with Down syndrome are born with an additional copy of chromosome 21 and have a greater risk of developing all forms of dementia including Alzheimer's disease. Studies have found that more than 75% of people with Down Syndrome aged 65 and over have Alzheimer's. While the exact relationship between Down Syndrome and Alzheimer's is not entirely clear, one possible explanation might be that the gene that codes for amyloid precursor protein is located on chromosome 21 and the additional copy of this gene increases the likelihood of the development of the plaques associated with the amyloid precursor protein. By age 40 most people with Down Syndrome have high levels of beta amyloid plaques in their brains, a marker for Alzheimer's disease (Lott & Dierssen, 2010).

Besides for the genetic factors in Alzheimer's disease much research has been conducted on the effects of environmental and modifiable risk factors. Studies have shown that regular physical activity, and management of cardiovascular risk factors such as obesity, smoking and high blood pressure reduce the risk of the development of Alzheimer's disease and dementia. There is also evidence that a healthy lifestyle and diet as well as continued engagement in learning can prevent cognitive decline in old age (Baumgart et al., 2015).

Current treatment options for Alzheimer's disease are limited. None of the pharmacological treatments available cures or stops the damage the disease causes to the brain. The six drugs that have thus far been approved by the U.S Food and Drug Administration (FDA) focus primarily on increasing the levels of neurotransmitter present in the brain. While this helps deal with the symptoms of Alzheimer's disease such as memory loss and reduced cognitive capacity, these drugs don't deal with the underlying issues in the disease, and their effectiveness is limited to the early stages of the disease.

This paper attempts to review some of the complex molecular mechanisms involved in Alzheimer's disease. It will explore the current research on the molecular mechanisms in Alzheimer's disease to better understand the development of the disease and the wide variance in disease progression, and explain possible areas of future research in the development of more effective therapeutic agents.

## Methods

This study was performed through the analysis of various original and peer reviewed articles which were accessed using databases such as the Touro Database, PubMed, and Google Scholar. The research collected in this study was used to understand the molecular processes in Alzheimer's disease, and to evaluate the various hypotheses postulated in the formation of the disease.

## Discussion

Since the systematic biochemical analysis of patients with Alzheimer's began in the early 1970's much knowledge has been acquired concerning the possible mechanisms responsible for the disease. The earliest research into neurochemical abnormalities that are present in Alzheimer's disease led to the formulation of the cholinergic hypothesis.

## Cholinergic Hypothesis

Acetylcholine was the first neurotransmitter to be identified and is the neurotransmitter used in all cholinergic neurons. It is fundamental in both the central nervous system (CNS) and the peripheral nervous system (PNS).

Successful neurotransmission of acetylcholine is dependent on proteins needed for its synthesis, transport, degradation and reuptake. Acetylcholine synthesis takes place in the cytoplasm of cholinergic cells. The enzyme, choline acetyltransferase (ChAT) catalyzes the combination reaction of dietary choline and Acetyl-CoA, generated by the mitochondria, to form the product acetylcholine. Three forms of the ChAT enzyme have been found in humans. Formation of acetylcholine is followed by its transfer to the synaptic vesicles before release. This step is enabled by the vesicular acetylcholine transporter (VAChT). When the cholinergic neurons are depolarized, acetylcholine is released by exocytosis into the synaptic cleft. On the post synaptic neuron, acetylcholine can activate both muscarinic and nicotinic receptors. Nicotinic ACh receptors are ion gated channels which are selective for cations including sodium, potassium, and calcium. Nicotinic receptors are made up from a combination of five different subunits. The large variance in the properties and functions of the different nicotinic receptors is a direct result of the many different possible combinations of subunits that form each receptor. In the PNS the activation of these receptors results in the transmission of the signal to ganglion cells and the innervation of muscles and glands. In the CNS the role of nicotinic receptors is regulatory rather than purely the transmission of excitatory or inhibitory signals. In the PNS the nicotinic receptors are mostly located on the post synaptic neuronal membrane, where they are activated by acetylcholine and facilitate the transmission of signals. However, in the CNS the receptors are mostly located on the pre-synaptic neuronal membrane where their activation regulates the release of acetylcholine and other neurotransmitters into the synapse. The activation of these receptors results in an increase in calcium levels in the presynaptic neuron, which is a crucial step in the exocytosis of neurotransmitters such as GABA, glutamate, dopamine and serotonin into the synapse. Muscarinic receptors are G-protein coupled receptors, and unlike the nicotinic receptors, their role is largely in the transmission of signals. Five isoforms of the receptor have so far been identified. M1, M3 and M5 are excitatory receptors and their activation results in the formation of second messengers by phospholipase C which results in closure of K+ channels enabling the greater depolarization of the cell and the transmission of signal down the axon. M2 and M4 are inhibitory and their activation has the opposite effect. They result in the inhibition of adenylyl cyclase which leads to lower levels of cyclic adenosine monophosphate (cAMP) and promotes the inhibition of [Ca]++ channels diminishing cell excitability.

In the synaptic cleft acetylcholine is broken down to its components Acetyl-CoA and choline by the enzyme acetylcholinesterase (AChE). AChE is one of the most kinetically effective enzymes. It is able to catalyze the breakdown of 5000 molecules of acetylcholine per second. The choline transporter, CHT1, facilitates the uptake of choline, that is produced by the breakdown of acetylcholine by AChE, into the presynaptic neuron. This transporter is also the source of the choline used in acetylcholine synthesis and it therefore plays a crucial role in the recycling of acetylcholine (Fig. 1, Ferreira Vieira et al., 2016).

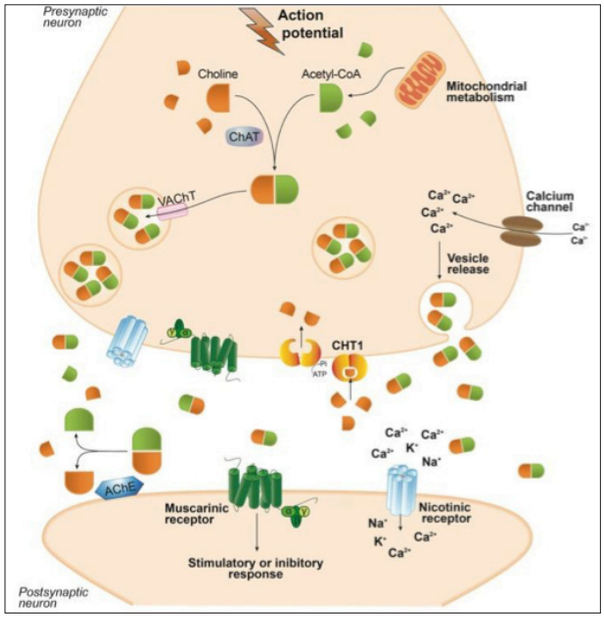


*Figure 1 Schematic representation of biological aspects involving acetylcholine neurotransmission. (Ferreira Vieira et al., 2016)*

Early research into Alzheimer's disease showed reduced activity of choline acetyltransferase in the amygdala, hippocampus and cortex of patients with the disease. In one such study, the levels of the enzyme responsible for acetylcholine synthesis in patients with the disease was found to be lower than 10% of the normal activity seen in the control group. Similarly, the levels of acetylcholinesterase in the same brain regions were markedly lower in patients with Alzheimer's disease (Davies & Maloney 1976).

Further studies showed that in addition to the decreased activity of the enzymes responsible for acetylcholine synthesis, the levels of acetylcholine uptake by neurons in the frontal cortex and in the hippocampus, were significantly lower in patients with Alzheimer's disease. In the frontal cortex, the transport of choline into synaptosomes was reduced by 50% and an even greater reduction of 80% was seen in the hippocampus. Further evidence of the loss of cholinergic function in the uptake of

choline in Alzheimer's disease is seen in the lower densities of the presynaptic high affinity choline uptake carrier (HACU) in both the cortex and hippocampus (Rylett et al., 1983; Pascual et al., 1991).

Other studies have shown there to be a reduction in the number of nicotinic and muscarinic M2 acetylcholine receptors in Alzheimer's disease brains and there is evidence for the disruption of the M1 receptors and their G-proteins which effects the second messenger systems and transmission of signals (Whitehouse et al., 1988).

The cholinergic hypothesis of Alzheimer's disease was also evidenced by the reduction in the amount of acetylcholine released from neurons in the brains of affected patients. An experiment conducted on brain tissue of both controls and patients with the disease collected with short post mortem delay, showed a significant reduction in tritium (3H-acetylcholine) release during potassium stimulation, as compared with the controls (Nilsson et al., 1986).

In addition, the loss of cholinergic neurons as a result of the neurodegeneration caused by Alzheimer's has further implicated the cholinergic system in the etiology of the disease. In particular, the selective degeneration of neurons in the nucleus basalis of Meynert, a significant source of neurons which are fundamental in the cholinergic innervation of the cerebral cortex, explains the reduction in the levels of acetylcholine in individuals with Alzheimer's disease (Whitehouse et al., 1982).

It has also been demonstrated that the cholinergic system plays a role in memory and in learning. The loss of the neurons in the nucleus basalis of Meynert has been correlated with the impaired memory and cognitive abilities seen in patients with Alzheimer's disease. The effects on the cholinergic system has also been shown to result in the many behavioral and psychological symptoms seen in individuals with the disease. Emotional processing deficits associated with Alzheimer's disease may be caused by loss of cholinergic function in the areas of the amygdala and frontal cortex. Apathy and depression as well as disturbance in sleep cycle are symptoms commonly found in patients with Alzheimer's disease. It has been postulated that the degeneration of cholinergic neurons and the resulting loss of regulation of neurotransmitters, including dopamine and serotonin, is responsible for the many psychiatric symptoms observed. Moreover, cholinesterase inhibitors, the primary focus of the current pharmacologic agents available for the treatment of Alzheimer's disease have shown to improve many of the psychiatric symptoms. Clinical trials conducted on affected patients, reported reduced delusions, stress, apathy and depression in the treated group (Ferreira et al., 2016; Pinto et al., 2011).

There is much research that shows the link between the cholinergic system and Alzheimer's disease. The neurodegeneration of neuronal cells critical in the cholinergic system, the reduction in the enzymes responsible for the synthesis of acetylcholine, and lower levels of proteins that facilitate choline uptake in neuronal cells that is seen in the brains of patients with Alzheimer's disease, together with the role that the cholinergic system plays in memory and learning have contributed to the formulation of the cholinergic hypothesis. However there remains some inconsistencies in this hypothesis that suggest the role of the cholinergic system in the disease needs to be better understood.

Although cholinergic loss seems to correlate with cognitive impairment, other factors such as loss of synapses and pyramidal cells may also be responsible for the cognitive decline. Additionally, some patients with Alzheimer's disease do not show the large decreases in ChAT activity that would be expected. Moreover, patients with inherited olivopontocerebellar activity have levels of ChAT which are reduced to similar levels as those seen in Alzheimer's patients yet they don't experience the decline in cognition and memory that would be expected.

An expectation of the cholinergic hypothesis would be that drugs that restore cholinergic function to the brain regions effected by Alzheimer's disease would improve and reverse the cognitive symptoms seen in the disease. Five drugs have been approved by the FDA for Alzheimer's disease. Four of these drugs are cholinesterase inhibitors and one is a receptor antagonist. The cholinesterase inhibitors have shown limited success in clinical trials and are mostly able to merely delay the progression of the disease. The failure of the cholinesterase inhibitors to cure Alzheimer's disease was seen by some as the strongest evidence against the cholinergic hypothesis.

Many researchers have continued to explore the role of neurotransmission in the disease because of the acetylcholine dysfunction seen in the brains of Alzheimer's disease patients. However, the inconsistencies with the cholinergic hypothesis have resulted in a shift in the focus of much of the more recent research towards two of the hallmarks of the disease, the buildup of beta amyloid plaques in the extracellular space, and the intracellular formation of neurofibrillary tangles (Craig et al., 2011; Francis et al., 1999).

## Amyloid Cascade Hypothsis

The origins of the Amyloid Cascade Hypothesis lie in the sequencing of the Aβ extracted from cerebral blood vessels and the brain parenchyma of Alzheimer's disease patients. The identification of the Aβ sequence led to the sequencing of the amyloid precursor protein gene (APP). This gene located on chromosome 21 encodes the holoprotein that is cleaved first by

the β-amyloid cleaving enzyme and then by γ-secretase to form the Aβ peptide. (Masters et al., 1985; Kang et al., 1987; Hussain et al., 1999). The essence of the amyloid cascade hypothesis is that the increased production or decreased clearance of the Aβ peptides causes the disease. Aggregation of the hydrophobic Aβ40 and Aβ42 peptides results in the formation of insoluble plaque which triggers a cascade of changes ultimately resulting in cell death and the symptoms of the disease.

Human APP belongs to a family of type I transmembrane glycoproteins that also includes the similar amyloid precursor like proteins 1 and 2 (APLP1 and APLP2). These proteins are functionally the same as the APP but they lack the Aβ sequence. The APP gene is highly conserved and several alternatively spliced isoforms of APP have been identified in humans. Invertebrates such as the fruit fly D. melanogaster and the worm C. elegans contain paralogs of the amyloid precursor protein, amyloid protein precursor-like (APPL) and APP-like 1, (APL-1). The zebrafish genome encodes two variants of APP, Appa and Appb. All of these proteins share domains that are highly conserved in both the large extracellular domains and in the shorter cytoplasmic domain (Nicolas & Hassan, 2014).

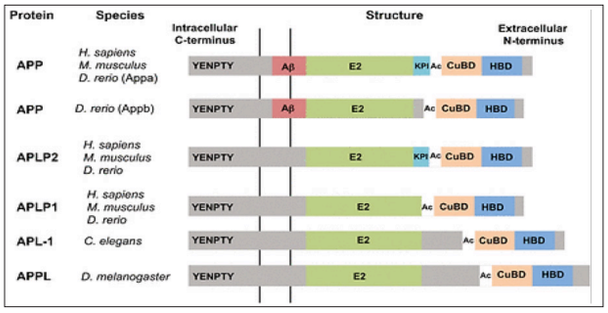


*Figure 2 The domain structure of APP family members in model organisms. All APP homologs contain the extracellular domains E2, an acidic domain (Ac), a copper binding domain (CuBD), and a heparin binding domain (HBD). A kunitz protease inhibitor domain (KPI) is found only in the APP and APLP-2 forms of the protein and the Aβ sequence is only present in APP. The most highly conserved domain across species is the intracellular YENPTY domain (Nicolas & Hassan, 2014).*

APP can undergo two types of processing depending on the secretases that cleave it. In the non-amyloidogenic pathway APP is cleaved within the Aβ sequence by α-secretase forming the sAPPα extracellular protein and the membrane bound αAPP-CT. The membrane bound protein is then cleaved by γ-secretase forming P3 peptide and amyloid precursor protein intracellular domain (AICD).

In the amyloidogenic pathway, APP is first cleaved by β-secretase forming the soluble sAPPβ protein and the C-terminal membrane bound fragment βAPP-CTF. The subsequent cleavage of the βAPP-CTF fragment by γ-secretase forms the Aβ peptide and the amyloid precursor intracellular domain (ACID). The release of the Aβ peptide by γ-secretase is thought to be fundamental to Alzheimer's disease pathology, and the aggregation of these insoluble and toxic protein fragments results in the formation of the senile plaque that is a hallmark of the disease. In the earliest and most direct elucidation of the amyloid cascade

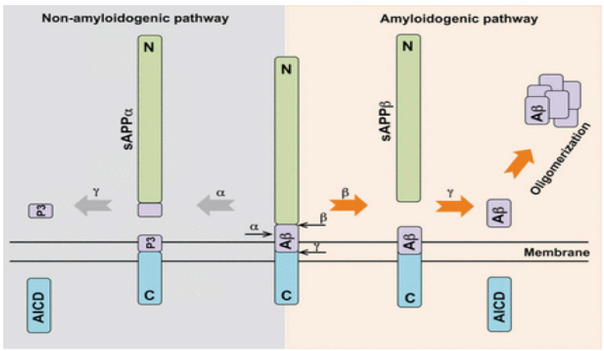


*Figure 3 proteolytic processing of APP (Nicolas & Hassan, 2014).*

hypothesis the aggregation of the Aβ peptide was explained as critical event in the development of Alzheimer's disease. The accumulation of the peptide was seen as both a cause of cell death and as a crucial step in the hyper-phosphorylation of Tau proteins leading to the formation of intracellular neurofibrillary tangles (Hardy & Higgins, 1992).

Critical evidence for the ACH comes from human genetics. The observation that the Aβ peptide deposited in elderly Down's syndrome patients, was the same as that found in patients with Alzheimer's suggested that a gene on chromosome 21 was central to the development of the disease (Glenner & Wong, 1984a). Later studies on a family with a history of early onset Alzheimer's provided further evidence for a genetic link in the disease, and was the basis for much of the research that followed. These studies revealed a missense mutation in the APP gene that resulted in a V717I amino acid substitution in the protein product. The position of the substitution, just upstream from the carboxyl terminal cleavage site of the Aβ peptide, provided further evidence for the role of Aβ peptide in the etiology of the disease (Goate et al., 1991).

The link between mutations in the presenilin 1 and presenilin 2 genes, that encode a part of the γ-secretase mutiprotein complex, and the development of the disease has likewise provided support for the amyloid cascade hypothesis.

There are now hundreds of mutations to the PSEN-1, PSEN-2 and APP genes that are known to cause early onset familial AD (FAD). These mutations effect the formation and accumulation of amyloid plaque in several ways. Some of these mutations result in

the extension of the C-terminal side of the Aβ peptide, others increase the overall ratio of the longer less soluble Aβ peptides to the shorter more soluble forms, and some are directly responsible for an increase in the aggregatory properties of the protein.

In addition to the effects of these three genes on familial Alzheimer's disease, there are also genes that are connected to sporadic Alzheimer's disease (SAD). There are three major alleles of the APOE gene in the human population (Nickerson et al., 2000). These are APOE2, APOE3 and APOE4. A heterozygous APOE4 carrier has a four-fold increased risk for the disease as compared with the homozygous APOE3 genotype. The homozygous APOE4 genotype has an even more drastic 12 fold increase in risk. Conversely, a carrier of the APOE2 gene has a reduced risk of the disease (Verghase et al., 2011). The ApoE protein is believed to have a role in the deposition of the amyloid plaques. Furthermore, there are specific mutations in the APP gene that result in the protection against Alzheimer's disease, with the evidence suggesting that they disrupt the ability to form the Aβ peptides.

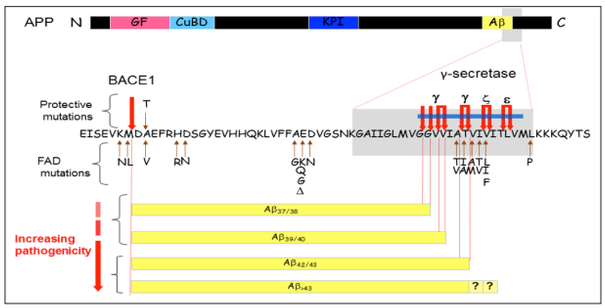


*Figure 4 Amyloid precursor proteins. The diagram shows both the β- secretase and γ-secretase cleavage sites and protective and pathogenic mutations (Karran & De Strooper, 2016).*

While many of the above studies have shown a link between the genes involved in the formation of the Aβ peptides and the development of the disease, like the cholinergic hypothesis there remain some inconsistencies and unanswered questions. These questions have led many to believe that rather than being the central mechanism in the development of Alzheimer's disease, the formation of amyloid plaque may just be one of several factors that contribute to its development.

Much to the frustration of the proponents of the ACH, the amyloidocentric drugs have failed to produce the results that would have been expected according to the hypothesis. The phase three clinical trials of 6 of these drugs have provided disappointing results. Triampirosate, a small molecule drug that prevents the aggregation of Aβ peptide showed no significant effect on cognition and memory during the clinical trials. Similarly no significant effect on primary outcomes were seen in the trials of Tarenflurbil, a γ-secretase modulator that reduces the ratio of aggregatory Aβ peptide to the shorter more soluble form. The only limited success in this class of drug for the treatment of Alzheimer's disease, was Solanezumab, a monoclonal antibody directed at the amyloid plaque protein. In its phase three clinical trials there was a significant improvement in cognitive abilities. In an extension trial, these positive effects on cognition were sustained over a two-year period raising hope and providing some evidence for a disease modifying effect (Karran & De Strooper, 2016).

A central tenet of the amyloid cascade hypothesis is that the accumulation of the Aβ peptides causes both cell death and the intracellular neurofibrillary tangles. Tissue studies in vitro have shown the toxic nature of Aβ peptide and Aβ42. Many early studies showed that the addition of the peptide to cell culture resulted in neuronal death and apoptosis as well as synaptic and dendritic loss. In vivo studies produced similar results, when these deleterious effects were seen in mouse brains injected with the peptide. However, the relationship between the formation of amyloid plaque and the tangles remains unclear. The aggregation of plaque initially takes place in the frontal cortex, before spreading throughout the cortex as the disease progresses. Neurofibrillary tangles first appear in the limbic system, in the hippocampus and dentate gyrus. This spatial discrepancy together with further studies that have shown that the tangles appear before the accumulation of plaque, and are more closely correlated with disease progression and severity have contributed to the re-evaluation of the ACH (Braak & Braak, 1998).

An analysis conducted to determine the correlation between cognitive abilities and some of the neurochemical and structural measurements in 15 patients with Alzheimer's disease further questions the relationship between amyloid plaque and cognitive impairment. In this study, there were only weak correlations between plaque and tangles and performance on psychometric tests. In contrast, the density of the cortical synapses was found to be strongly correlated with the psychological assessments of the patients (Terry et al., 1991). Mouse models of Alzheimer's disease have shown the accumulation of plaques without the observed cognitive impairments. In addition, neuroimaging analysis have shown the presence of plaques in cognitively healthy people.

The amyloid cascade hypothesis dominates the field of Alzheimer's research as the most complete and evidenced hypothesis about the causes of the disease. However, it is not without its flaws, which has led to some reassessing the hypothesis and to others dismissing its significance altogether. The genetic link to Alzheimer's disease was considered the central evidence for this hypothesis. Mutations in APP, PSEN1 and

PSEN2 are associated with familial Alzheimer's disease and the EPOE4 allele is a risk factor for sporadic AD. The fact that these genes are involved in the processing of APP and the formation of Aβ peptide has provided strong evidence for the role of amyloid plaque in disease progression. The mutations associated with APP are all located in the proximity of the cleavage sites of Aβ peptide, suggesting that the plaque formed by the peptide is central to the development of the disease. However, the mutations in the presenilin genes, which encode γ-secretase, occur throughout the protein, and not just the sites that are involved with Aβ formation. This has led to the belief that defective APP processing may in fact be the actual cause of the disease, and that the formation of the Aβ peptide and plaques might just be a secondary effect. This belief is supported by the weak correlation between plaque formation and density and cognition. While the ACH is the best defined and most widely accepted view, the data both for and against this hypothesis is significant. Many still strongly support the ACH and others have dismissed it entirely to focus on other hypotheses.

## Alternative Hypotheses

Analysis of the relationship between mitochondrial function and Alzheimer's disease has resulted in the proposition of the mitochondrial cascade hypothesis (MCH). The fundamental principle of the MCH is that Alzheimer's disease is a usual if not inevitable consequence of ageing. Support for this hypothesis comes from the undoubted evidence of mitochondrial damage in the brains of patients with the disease. Mitochondria are critical in the regulation of cell death and mutations in mitochondrial DNA and oxidative stress both contribute to ageing and neurodegenerative diseases (Lin & Beal, 2006). Studies using flourodeoxyglucose positron emission tomography (PET) imaging, as a measure of oxygen uptake, showed deficits in the brains of Alzheimer's disease patients (Jack et al., 2012). There is also strong evidence of free radical damage in AD brains (Sonnen et al., 2008). These findings suggest that dysfunctional and altered mitochondria is central in the disease. Experimental evidence for the MCH comes from studies conducted on cybrid cells- cells that contains mitochondria from a different cell. Studies on cybrid cells which were induced to take up mitochondrial DNA from platelets of patients with Alzheimer's disease, showed increased production of beta amyloid peptide (Khan et al., 2000). This data provides support for the MCH, by showing that mitochondrial deficiency is the cause of the amyloid plaques and central to disease development. Despite this data, the MCH fails to explain the full array of Alzheimer's disease pathology. In addition, genome wide association studies have failed to show links between mitochondrial genes and proteins and the disease. The cybrid experiments show that mitochondrial dysregulation in Alzheimer's disease leads to increases in beta amyloid production. However, they do not account for the mutations in APP and PSEN which increase the ratio of insoluble Aβ peptide to the smaller soluble form, or the mutations that result in an increase in aggregatory properties.

Another hypothesis that deserves mention is the metabolism hypothesis. According to this hypothesis, the underlying cause of AD is hypometabolism of glucose in the brain. The basis of this hypothesis was experiments on a rat model injected with streptozotocin, which effects insulin production, and resulted in decreased glucose metabolism in the brain, and learning and memory impairments (Lannert & Hoyer 1998). Further studies showed that the insulin signaling pathway is significantly depressed in many brain regions in Alzheimer's disease (Steen et al., 2005). Clinical experiments also have linked insulin with Aβ peptide buildup. Incretin mimetics injected in mice resulted in significant reductions in Aβ plaque load (Mcclean & Hölscher, 2014), and there is also data that suggests that Aβ oligomers disrupt the insulin pathway leading to an increase in glycogen synthase kinase 3β (GSK 3β), a Tau kinase, and the formation of neurofibrillary tangles (Morgen & Frölich, 2015). As with the mitochondrial hypothesis, no data from genome wide association studies have confirmed this link between Alzheimer's disease and insulin signaling. However, this hypothesis does provide several targets for possible therapeutic intervention.

A more generalized proposition is the cell cycle re-entry hypothesis. This view examines Alzheimer's disease from the perspective of age related DNA damage. Neurons are post mitotic and therefore must maintain integrity long term. Brain cells have a very high energy requirement and therefore are particularly susceptible to DNA damage. Mitogen kinases have increased expression in the brains of Alzheimer's disease patients, and have been posited to stimulate the neurodegenerative pathways and effect cell repair mechanisms (Arendt et al., 1995). Later studies on differentiated neurons that were infected with the oncogenes, c-myc and ras resulted in DNA duplication and hyperphosphorylated and unusually folded Tau proteins, similar to those observed in Alzheimer's disease. However, the disrupted DNA repair mechanisms does not adequately explain the formation of Aβ plaques, and as before there is little support for this hypothesis from genome wide association studies.

The observation that the Alzheimer's disease brain has a much-reduced capillary and vascular network has led to the formulation of the vascular hypothesis. There is much evidence linking the brain's vascular network and the disease.

Research has found that hypertension and diabetes, which both have vascular effects, significantly increase the risk of the development of the disease (Prince et al., 2014). In one study, it was discovered that the formation of 85% of the amyloid plaques in AD brains were either centered or proximal to vasculature (Kumar & Singh et al., 2005). A study of several different forms of dementia including AD revealed substantial reductions in microvasculature (Buee et al., 1994). Although there appears to be a credible link between the disease and the vasculature, it is not entirely clear if AD causes damage to the vasculature or if a vascular insufficiency promotes deposition of Aβ plaque (Karran & De Strooper, 2016).

One final hypothesis to consider is the Aβ oligomer hypothesis (AβOH). This theory is a variation of the original ACH. The main traction behind this theory is that it provides possible explanation as to why the density and amount of amyloid plaques does not correlate with the symptoms of AD. According to the AβOH the oligomers act at a distance from the plaques and mediate their effects. There is a large amount of data in support of this view. These include the treatment of cells with Aβ oligomers to induce neuronal death, the impact of the oligomers on insulin and nicotinic receptors, and the injection of these oligomers in rat brains which induced impaired memory and cognition. The AβOH would also explain why amyloidocentric drugs have had little success (Karran & De Strooper, 2016).

The cholinergic hypothesis and the amyloid cascade hypothesis clearly are supported by the strongest evidence. The earliest experiments demonstrating the reduction in cholinergic function provided the basis for much of the later research into Alzheimer's disease. Studies have consistently shown reductions in the levels of enzymes responsible for acetylcholine synthesis, and loss of receptor activity disrupting its uptake into neurons. This together with the degeneration of cholinergic neurons has provided the basis for this hypothesis. The genetic studies into familial Alzheimer's disease and the link between the disease and Down's syndrome laid the path for the formulation of the ACH which is the most comprehensive hypothesis in Alzheimer's disease. The cholinergic hypothesis fails to explain the mutations in APP in familial Alzheimer's disease, the formation of the Aβ peptide, amyloid plaques and neurofibrillary tangles. The Amyloid cascade hypothesis too has its inconsistencies and unanswered questions. However, rather than rejecting the undeniable data in support of each of these hypothesis, understanding the links and interactions between the two may provide a clearer picture of the mechanism of disease and provide a target for more effective therapy.

The connection between the cholinergic system and APP processing has been established. In one study, human embryonic kidney (HEK) cell lines were transfected with muscarinic acetylcholine receptors. As mentioned earlier there are five isoforms of muscarinic receptors; M1, M2, M3, M4 and M5. Cells transfected with M1 and M3 receptors showed significant increase in the release of APPα the product in the non-amyloidogenic pathway, when exposed to Carbachol a muscarinic receptor agonist. These cells also released lower levels of Aβ (Nitsch et al., 1992). Similar effects were seen in rat cerebral cortex slices that were exposed to M1 receptor agonists (Pittel et al., 1996). In addition to the effect the cholinergic system has on APP processing, Aβ peptides appear to modulate cholinergic function. Picomolar and nanomolar concentration of the beta amyloid peptide inhibit acetylcholine release from neurons in studies conducted on rat hippocampus and cortex sections. The exact mechanism by which the Aβ adversely effects acetylcholine release remains unclear. However, it appears that the Aβ peptides inhibit uptake of choline in the presynaptic neurons. The activity of choline acetyltransferase (ChAT) appears to be unaffected, while high affinity choline uptake is decreased by 20 minutes after incubation with Aβ (Kar et al., 2004). Further studies on rat septal neurons found that Aβ42 reduces the levels of acetylcholine by inhibiting the activity of the enzyme pyruvate dehydrogenase (PDH). The inhibition of pyruvate dehydrogenase results in a reduction in the biosynthesis of Acetyl-CoA, which is critical for acetylcholine synthesis. Aβ peptides have also been shown to disrupt cellular signaling and secondary messengers activated by muscarinic receptors.

Aside from the disruption to the various cellular processes Aβ exposure is also toxic to neurons in the long term. Differentiated cholinergic cell lines were the most susceptible to the toxic effects of beta amyloid, while GABAergic and serotonergic cells were more resistant. As stated above Aβ peptide appears to effect choline uptake in neurons. Under conditions of choline depletion, cells synthesize acetylcholine using membrane phosphatidylcholine. Therefore, a severe choline depletion may result in regulated membrane turnover being disrupted and eventually lead to cell death.

Aβ has also been shown to have a role in the hyper-phosphorylation of Tau. Hyper- phosphorylated Tau can no longer associate with microtubules, effecting both cell structure and vital mechanisms of transport. In cholinergic cell lines, aggregated Aβ peptide induced the phosphorylation of Tau proteins. The exact mechanism is unclear but it is most likely a result of an increase in kinase activity as Aβ peptides have been shown to increase the activity of both MAP kinase and GSK3β.

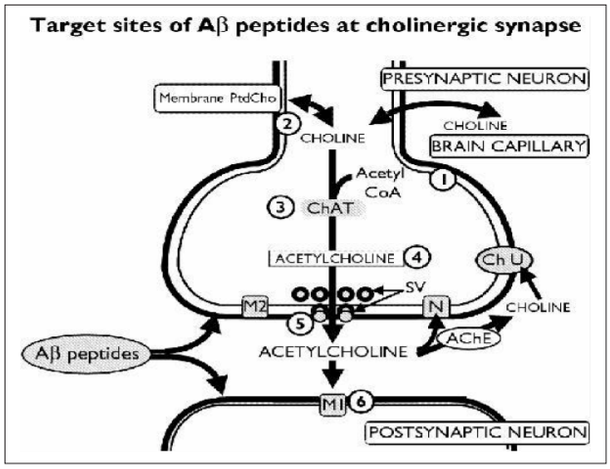


*Figure 5 Targets of Aβ that modulate cholinergic transmission: (1) Aβ reduces activity of pyruvate dehydrogenase, an enzyme that generates acetyl coenzyme A (CoA) from pyruvate; (2) Aβ reduces high-affinity uptake of choline; (3) long-term or high-dose exposure to Aβ reduces activity of the choline acetyltransferase (ChAT) enzyme; (4) Aβ reduces acetylcholine (ACh) content; (5) Aβ reduces ACh release from synaptic vesicles (SV); (6) Aβ impairs muscarinic M1-like signalling. AChE = acetylcholinesterase, Ch U = site of choline uptake, M2 = presynaptic muscarinic M2 receptor, N = presynaptic nicotinic receptor, PtdCho = phosphatidylcholine. (Kar et al., 2004).*

## Conclusion

With the wealth of evidence in support of both the cholinergic hypothesis and the amyloid cascade hypothesis, an attempt at fully understanding the mechanism of disease will require better understanding of the link between Aβ peptides and the cholinergic system. The cholinergic system has been shown to play a significant role in the regulation of APP processing. Reciprocally, studies have found that the accumulation of Aβ peptides disrupts the muscarinic and nicotinic receptors on cholinergic neurons while also having a downstream effect on the signaling pathway and secondary messengers. In addition, Aβ inhibits synthesis of acetylcholine in a variety of ways and cholinergic neurons are particularly susceptible to the toxic effects of Aβ peptide. Genetics provides the basis of support for the amyloid cascade hypothesis, and while the amyloid plaque density may not correlate well with cognitive impairment in Alzheimer's disease, its interactions with the cholinergic system may be responsible for the symptoms of AD. The exact mechanism of that interaction needs to be better elucidated, as that understanding provides the greatest potential for more effective and targeted therapy.

## References

Arendt, T., Holzer, M., Großmann, A., Zedlick, D., & Bru¨ckner, M. (1995). Increased expression and subcellular translocation of the mitogen activated protein kinase kinase and mitogen-activated protein kinase in Alzheimer's disease. Neuroscience, 68(1), 5-18. doi:10.1016/0306-4522(95)00146-a

Baumgart, M., Snyder, H. M., Carrillo, M. C., Fazio, S., Kim, H., & Johns, H. (2015). Summary of the evidence on modifiable risk factors for cognitive decline and dementia: A population-based perspective. Alzheimer's & Dementia, 11(6), 718-726. doi:10.1016/j.jalz.2015.05.016

Bekris, L. M., Yu, C., Bird, T. D., & Tsuang, D. W. (2010). Review Article: Genetics of Alzheimer Disease. Journal of Geriatric Psychiatry and Neurology, 23(4), 213-227. doi:10.1177/0891988710383571

Braak, H., & Braak, E. (1998). Evolution of neuronal changes in the course of Alzheimer's disease. Journal of Neural Transmission. Supplementa Ageing and Dementia, 127-140. doi:10.1007/978-3-7091-6467-9_11

Craig, L. A., Hong, N. S., & Mcdonald, R. J. (2011). Revisiting the cholinergic hypothesis in the development of Alzheimer's disease. Neuroscience & Biobehavioral Reviews, 35(6), 1397-1409. doi:10.1016/j.neubiorev.2011.03.001

Davies, P. (1976). Selective Loss Of Central Cholinergic Neurons In Alzheimer's Disease. The Lancet, 308(8000), 1403. doi:10.1016/s0140-6736(76)91936-x

Ferreira-Vieira, T. H., Guimaraes, I. M., Silva, F. R., & Ribeiro, F. M. (2016). Alzheimer's disease: Targeting the Cholinergic System. Current Neuropharmacology CN, 14(1), 101-115. doi:10.2174/1570159x13666150716165726

Francis, P. T., Palmer, A. M., Snape, M., & Wilcock, G. K. (1999). The cholinergic hypothesis of Alzheimer's disease: A review of progress. Journal of Neurology, Neurosurgery & Psychiatry, 66(2), 137-147. doi:10.1136/jnnp.66.2.137

Glenner, G. G., & Wong, C. W. (1984). Alzheimer's disease and Down's syndrome: Sharing of a unique cerebrovascular amyloid fibril protein. Biochemical and Biophysical Research Communications, 122(3), 1131-1135. doi:10.1016/0006-291x(84)91209-9

Goate, A. (n.d.). Segregation of a missense mutation in the amyloid β-protein precursor gene with familial Alzheimer's disease. Alzheimer: 100 Years and Beyond Research and Perspectives in Alzheimer's Disease, 157-161. doi:10.1007/978-3-540-37652-1_16

Hardy, J., & Higgins, G. (1992). Alzheimer's disease: The amyloid cascade hypothesis. Science, 256(5054), 184-185. doi:10.1126/science.1566067

Hebert, L. E., Beckett, L. A., Scherr, P. A., & Evans, D. A. (2001). Annual Incidence of Alzheimer Disease in the United States Projected to the Years 2000 Through 2050. Alzheimer Disease and Associated Disorders, 15(4), 169-173. doi:10.1097/00002093-200110000-00002

Hebert, L. E., Weuve, J., Scherr, P. A., & Evans, D. A. (2013). Alzheimer disease in the United States (2010-2050) estimated using the 2010 census. Neurology, 80(19), 1778-1783. doi:10.1212/wnl.0b013e31828726f5

Hurd, M. D., Martorell, P., Delavande, A., Mullen, K. J., & Langa, K. M. (2013). Monetary Costs of Dementia in the United

States. New England Journal of Medicine, 368(14), 1326-1334. doi:10.1056/nejmsa1204629

Hussain, I., Powell, D., Howlett, D. R., Tew, D. G., Meek, T. D., Chapman, C., . . . Christie, G. (1999). Identification of a Novel Aspartic Protease (Asp 2) as β-Secretase. Molecular and Cellular Neuroscience, 14(6), 419-427. doi:10.1006/mcne.1999.0811

Jack, C. R., Vemuri, P., Wiste, H. J., Weigand, S. D., Lesnick, T. G., Lowe, V., . . . For The Alzheimer's Disease Neuroimaging Initiative. (2012). Shapes of the Trajectories of 5 Major Biomarkers of Alzheimer Disease. Archives of Neurology, 69(7). doi:10.1001/archneurol.2011.3405

Kang, J., Lemaire, H., Unterbeck, A., Salbaum, J., Masters, C., Grzeschik, K., . . . M??ller-Hill, B. (1987). The precursor of Alzheimer??s disease amyloid A4 protein resembles a cell-surface receptor. Alzheimer Disease & Associated Disorders, 1(3), 206-207. doi:10.1097/00002093-198701030-00032

Kar, S., Slowikowski, S., Westaway, D., and Mount, H., (2004). Interactions between β-amyloid and central cholinergic neurons: implications for Alzheimer's disease. J Psychiatry Neurosci. 29(6): 427–441.

Karran, E., & Strooper, B. D. (2016). The amyloid cascade hypothesis: Are we poised for success or failure? Journal of Neurochemistry, 139, 237-252. doi:10.1111/jnc.13632

Khan, S. M., Cassarino, D. S., Abramova, N. N., Keeney, P. M., Borland, M. K., Trimmer, P. A., . . . Bennett, J. P. (2000). Alzheimer's disease cybrids replicate β-amyloid abnormalities through cell death pathways. Annals of Neurology, 48(2), 148-155. doi:10.1002/1531-8249(200008)48:23.3.co;2-z

Lin, M. T., & Beal, M. F. (2006). Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature, 443(7113), 787-795. doi:10.1038/nature05292

Lott, I. T., & Dierssen, M. (2010). Cognitive deficits and associated neurological complications in individuals with Down's syndrome. The Lancet Neurology, 9(6), 623-633. doi:10.1016/s1474-4422(10)70112-5

Masters, C. L., Simms, G., Weinman, N. A., Multhaup, G., Mcdonald, B. L., & Beyreuther, K. (1985). Amyloid plaque core protein in Alzheimer disease and Down syndrome. Proceedings of the National Academy of Sciences, 82(12), 4245-4249. doi:10.1073/pnas.82.12.4245

Mcclean, P. L., & Hölscher, C. (2014). Liraglutide can reverse memory impairment, synaptic loss and reduce plaque load in aged APP/PS1 mice, a model of Alzheimer's disease. Neuropharmacology, 76, 57-67. doi:10.1016/j.neuropharm.2013.08.005

Morgen, K., & Frölich, L. (2015). The metabolism hypothesis of Alzheimer's disease: From the concept of central insulin resistance and associated consequences to insulin therapy. Journal of Neural Transmission, 122(4), 499-504. doi:10.1007/s00702-015-1377-5

National Center for Health Statistics. Deaths: Final Data for 2012. National Vital Statistics Report, Volume 63, Number 9. Hyattsville, MD; 2014. Available at: http://www.cdc.gov/nchs/data/nvsr/nvsr63/ nvsr63_09.pdf.

Nicolas, M., and B. A. Hassan. "Amyloid Precursor Protein and Neural Development." Development 141.13 (2014): 2543-548. Web.

Nickerson, D. A. (2000). Sequence Diversity and Large-Scale Typing of SNPs in the Human Apolipoprotein E Gene. Genome Research, 10(10), 1532-1545. doi:10.1101/gr.146900

Nilsson, L., Nordberg, A., Hardy, J., Wester, P., & Winblad, B. (1986). Physostigmine restores3H-acetylcholine efflux from Alzheimer brain slices to normal level. Journal of Neural Transmission, 67(3-4), 275-285. doi:10.1007/bf01243353

Nitsch, R., Slack, B., Wurtman, R., & Growdon, J. (1992). Release of Alzheimer amyloid precursor derivatives stimulated by activation of muscarinic acetylcholine receptors. Science, 258(5080), 304-307. doi:10.1126/science.1411529

Pascual, J., Fontán, A., Zarranz, J. J., Berciano, J., Flórez, J., & Pazos, A. (1991). High-affinity choline uptake carrier in Alzheimer's disease: Implications for the cholinergic hypothesis of dementia. Brain Research, 552(1), 170-174. doi:10.1016/0006-8993(91)90676-m

Pinto, T., Lanctôt, K. L., & Herrmann, N. (2011). Revisiting the cholinergic hypothesis of behavioral and psychological symptoms in dementia of the Alzheimer's type. Ageing Research Reviews. doi:10.1016/j.arr.2011.01.003

Pittel, Z., Heldman, E., Barg, J., Haring, R., & Fisher, A. (1996). Muscarinic control of amyloid precursor protein secretion in rat cerebral cortex and cerebellum. Brain Research, 742(1-2), 299-304. doi:10.1016/s0006-8993(96)01031-1

Roberts, R., & Knopman, D. S. (2013). Classification and Epidemiology of MCI. Clinics in Geriatric Medicine, 29(4), 753-772. doi:10.1016/j.cger.2013.07.003

Rylett, R., Ball, M., & Colhoun, E. (1983). Evidence for high affinity choline transport in synaptosomes prepared from hippocampus and neocortex of patients with Alzheimer's disease. Brain Research, 289(1-2), 169-175. doi:10.1016/0006-8993(83)90017-3

Sonnen, J. A., Breitner, J. C., Lovell, M. A., Markesbery, W. R., Quinn, J. F., & Montine, T. J. (2008). Free radical-mediated damage to brain in Alzheimer's disease and its transgenic mouse models. Free Radical Biology and Medicine, 45(3), 219-230. doi:10.1016/j.freeradbiomed.2008.04.022

Terry, R. D., Masliah, E., Salmon, D. P., Butters, N., Deteresa, R., Hill, R., . . . Katzman, R. (1991). Physical basis of cognitive alterations in alzheimer's disease: Synapse loss is the major correlate of cognitive impairment. Annals of Neurology, 30(4), 572-580. doi:10.1002/ana.410300410

Verghese PB1, Castellano JM, Holtzman DM. (2011). Apolipoprotein E in Alzheimer's disease and other neurological disorders. Lancet Neurol. Mar;10(3):241-52. doi: 10.1016/S1474-4422(10)70325-2.

Villemagne, V. L., Burnham, S., Bourgeat, P., Brown, B., Ellis, K. A., Salvado, O., . . . Masters, C. L. (2013). Amyloid β deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: A prospective cohort study. The Lancet Neurology, 12(4), 357-367. doi:10.1016/s1474-4422(13)70044-9

Ward, A., Tardiff, S., Dye, C., & Arrighi, H. M. (2013). Rate of Conversion from Prodromal Alzheimer's Disease to Alzheimer's Dementia: A Systematic Review of the Literature. Dementia and Geriatric Cognitive Disorders Extra, 3(1), 320-332. doi:10.1159/000354370

Whitehouse, P. J., Martino, A. M., Marcus, K. A., Zweig, R. M., Singer, H. S., Price, D. L., & Kellar, K. J. (1988). Reductions in Acetylcholine and Nicotine Binding in Several Degenerative Diseases. Archives of Neurology, 45(7), 722-724. doi:10.1001/archneur.1988.00520310028012

Whitehouse, P., Price, D., Struble, R., Clark, A., Coyle, J., & Delon, M. (1982). Alzheimer's disease and senile dementia: Loss of neurons in the basal forebrain. Science, 215(4537), 1237-1239. doi:10.1126/science.7058341