Alzheimer's Disease: Possible Causes and New Theories for Diagnosis

Sachleen Kaur
(ORC-ID: 0009-0002-1466-816X)

Abstract:

The commonly accepted cause of Alzheimer's Disease (AD) is the accumulation of amyloid-β, a protein that is prone to aggregation. However, treatments that focus on reducing the production of this dangerous substance have been less effective than expected, leading to the idea that there may be other potential causes of AD. This review focuses on understanding these different causal factors, focusing on tau hyperphosphorylation, presenilin mutations, advanced glycation endproducts, acetylcholine levels, the APOE4 gene, and glutamate uptake. There are many more substances that play a role in Alzheimer's prognosis, and more research on causes of AD needs to be conducted to find the optimal treatment for this damaging disease.

Introduction:

Alzheimer's Disease (AD) is a neurodegenerative disease that impacts 1 in 9 Americans over the age of 65 [1]. As of today, there is no cure for AD, and the few treatments that do exist are subpar. In order for treatment to become more efficient, the causes of Alzheimer's must be found. Unfortunately, the exact cause is still unclear, and further discovery continues with many researchers arguing over which substance is the root cause of AD. Currently, there are two main products that are believed to cause the slow neurodegeneration seen in AD: amyloid-β and hyperphosphorylated tau. There are also many other substances that are less discussed but still contribute to the progression of Alzheimer's.

The Amyloid-β Hypothesis:

Originally, Alzheimer's Disease was believed to be caused by a buildup of amyloid-β (Aβ) in the brain. The Aβ protein is formed through two different processes that lead to one normal and another neurotoxic product. Aβ is part of the larger amyloid precursor protein (APP) that is membrane bound. Studies have shown that APP plays a role in copper and zinc binding, neurite extension, and general cell growth [2]. Enzymes known as secretases catalyze the proteolysis of APP through two different pathways, non-amyloidogenic and amyloidogenic.

APP has a larger extracellular N-terminus and a smaller intracellular C-terminus with a YENPTY domain which binds to adaptor proteins (Fig. 1a). In the benign non-amyloidogenic pathway, α-secretase splits APP in a way where the dangerous portion of the protein is cut in the middle, leading to the formation of nontoxic peptide Aβ40 consisting of 40 amino acids (Fig. 1b). In contrast, the non-amyloidogenic pathway occurs when he neurodegenerative product

Aβ42 (42 amino acids) is formed by APP being split by β-secretase in a way that releases the dangerous portion of the protein (Fig. 1c). Both pathways require the use of γ-secretase to complete proteolysis, generating soluble ectodomains and identical intracellular C-terminal fragments. Aβ42 is focused on for AD pathogenesis due to its high tendency to aggregate and form plaques [3].

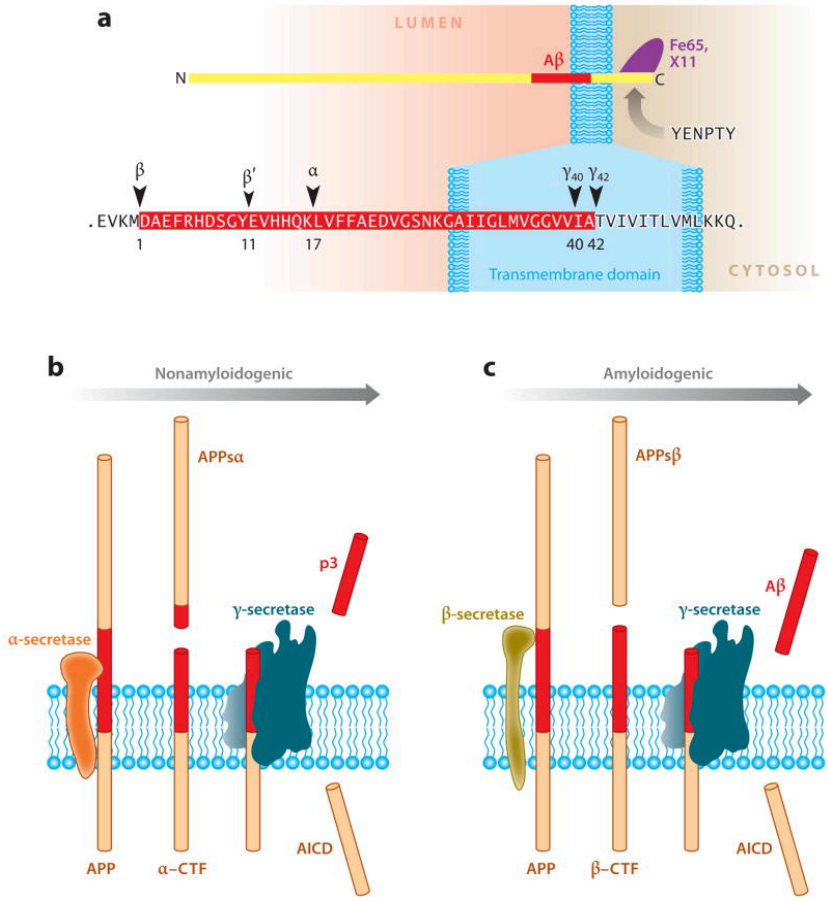

Figure 1: Amyloid precursor protein processing: (a) The regions of APP, a transmembrane protein. (b) Nonamyloidogenic technique uses α-secretase, splitting the dangerous region. (c) Amyloidogenic pathway uses β-secretase which exposes the Aβ portion of APP [2].

Aβ42 is believed to be the primary cause of Alzheimer's Disease. As more and more Aβ42 accumulates, oligomers and plaques form due to the nonpolar nature of the protein. The oligomers have been shown to bind to PirB (paired immunoglobulin-like receptor B) in mice, causing the deactivation of cofilin (an enzyme that splits actin). With cofilin deactivated, fewer microtubules are assembled, causing less dendritic spines to form and fewer synapses to occur. Information is transmitted by synapses between neurons, so Aβ42 appears to be a very strong cause of AD. Aβ42 also leads to neurodegeneration by blocking the extracellular Receptor for

Advanced Glycation Endproducts (RAGE) in microglia, which causes inflammation and oxidative stress. Oxidative stress leads to the inactivation of enzymes through protein oxidation, leading to mitochondrial damage. Eventually, not enough ATP can be produced to sustain the cell, causing cell death [4]. This loss of cells explains the difference in size noted when comparing a healthy brain with an Alzheimer's brain (Fig. 2).

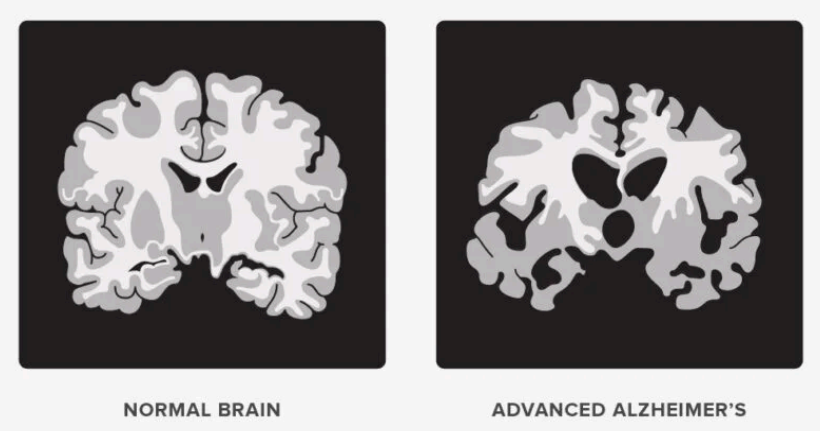

Figure 2: A side-by-side comparison of a healthy brain versus a brain with Alzheimer's Disease showing shrinkage of the cortex and hippocampus and increased size of ventricles [5].

Limitations of the Amyloid-β Hypothesis:

Even though Aβ42 seems to be the leading cause of AD, medications that inhibit the production of Aβ42 are not as effective as in theory. The drug verubecestat formulated by Merck and Co. (MK-8931) allosterically inhibits β-secretase, causing decreased Aβ42 production [6]. Patients who were treated with verubecestat experienced severe side effects such as extreme weight loss and suicidal ideation, leading to the drug being discontinued as a possible treatment [7]. Similarly, Eli Lilly recently released a new drug for Alzheimer's called donanemab. Donanemab is an IgG1 antibody that flags Aβ42 plaques so the immune system can remove them. This new medication has shown to have positive effects on decreasing amyloid concentration, but it causes side effects such as cerebral edema and death [8]. The treatments that exist for Alzheimer's based on amyloid plaques show much harm and sometimes do not work. Hence, scientists and researchers believe that Aβ accumulation and other factors lead to the progression of the disease.

Role of Tau Hyperphosphorylation:

One of the other components that plays a significant role in AD is tau hyperphosphorylation. Regular tau protein binds to microtubules, maintaining cell structure and

allowing for internal transport. High amounts of Aβ upregulate the enzymes that phosphorylate tau, such as cdk5 [9]. This leads to more tau being phosphorylated which decreases microtubule interactions, weakens the cytoskeleton, and impedes axonal transport and mitochondrial function [10].

This hyperphosphorylated tau then accumulates in dendritic spines, causing certain receptors to stop functioning and leading to synaptic loss. It also forms neurofibrillary tangles, which block cell functions and cause apoptosis [11].

The Presenilin Hypothesis and Familial Alzheimer's Disease:

Familial AD can be traced back to a genetic mutation in presenilin in the PSEN1 or PSEN2 gene [12]. Presenilin is a subunit of γ-secretase, an enzyme that aids in the splitting of APP. When presenilin is mutated, the production of Aβ42 increases while that of Aβ40 decreases. According to the amyloid hypothesis, increased concentrations of Aβ42 should lead to neurodegeneration, but mice that overproduced Aβ42 did not show any signs of neurodegeneration while mice with mutated presenilin had memory loss [13]. This study, as well as several others, reveal that increased Aβ42 levels are not the only cause of AD. The presenilin hypothesis (Fig. 3) adds on to the amyloid-β idea, but does not state the Aβ42 to Aβ40 ratio as the cause of Alzheimer's. Instead, it is believed that the lack of other important activities that PSEN1 controls are the cause of severe neurodegeneration [14].

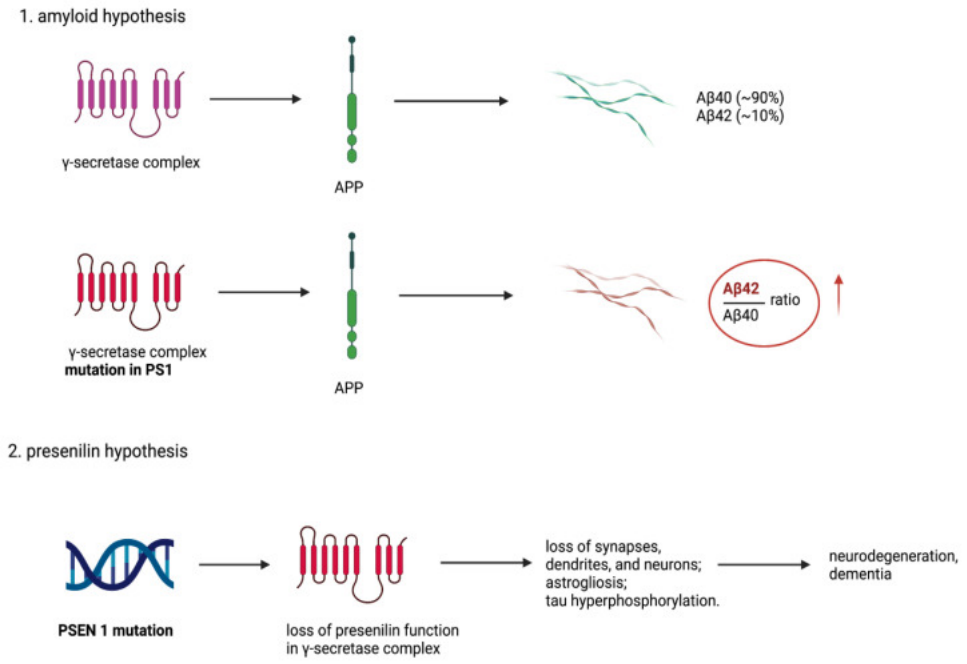

Figure 3: Comparison of the Amyloid hypothesis and the Presenilin hypothesis [14].

Advanced Glycation Endproducts:

Several other less discussed factors play a role in the progression of this disease, one of which is increased advanced glycation endproducts. Advanced glycation endproducts (AGEs) are toxic protein aggregates that form in non enzymatic pathways when reduced sugars and amino acids react during oxidative stress (Fig. 4). In AD, high rates of protein oxidation and lipid peroxidation cause oxidative stress [15].

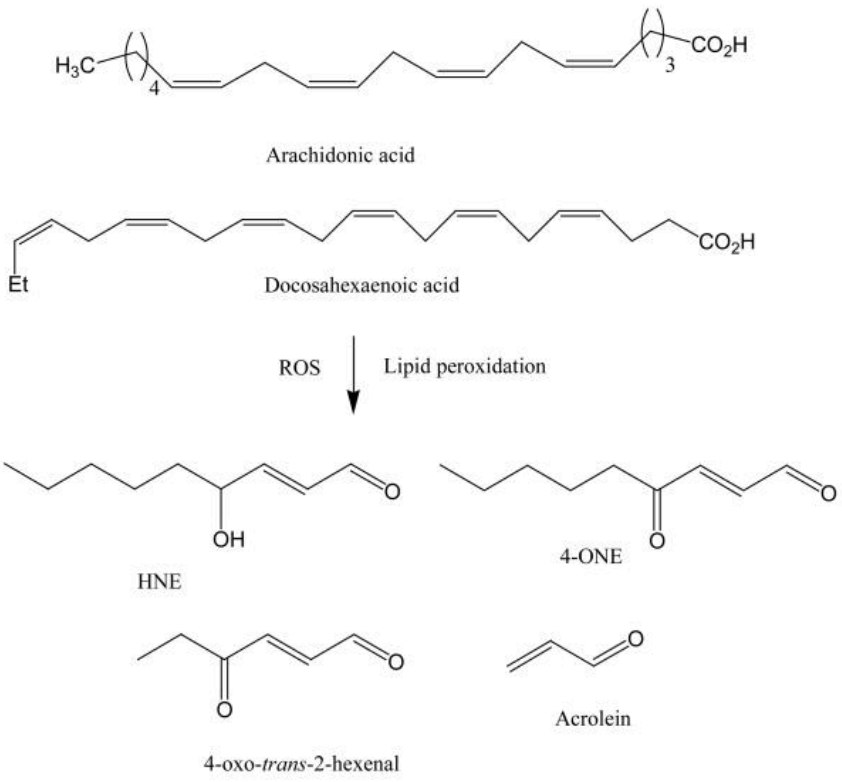

Figure 4: A scheme of lipid peroxidation. Reactive Oxygen Species (ROS) react with polyunsaturated fatty acids, leading to the creation of dangerous γ-hydroxy and γ-keto aldehydes. These products go on to bind to proteins, making them inactive [4].

When reduced sugars and amino acids react during oxidative stress, toxic protein aggregates known as advanced glycation endproducts (AGEs) are created [16]. As these products are strong oxidizers, they cause adverse effects in and out of the cell. When AGEs interact with extracellular receptors, a cascade is activated, producing inflammatory cytokines and reactive oxygen species and causing high oxidative stress. The balance of protons in the mitochondria's inner membrane is extremely important to its function, so oxidative stress leads to mitochondrial dysfunction, increased Aβ42 aggregations, and cell death [17]. This form of apoptosis is indirectly caused by Aβ42 and other diseases related to AGEs such as diabetes.

Lipid Peroxidation in Regards to the APOE4 Gene:

Interestingly, cell death caused by lipid peroxidation may play a role in the progression of familial AD. Researchers have found that the gene APOE4, a variant of the apolipoprotein E (APOE) gene, increases the risk of developing Alzheimer's. APOE is a protein that is involved in lipid metabolism by carrying fats through the bloodstream, and having two copies of the APOE4 gene means lipids will quickly accumulate in cells [18]. Increased lipid levels means increased lipid peroxidation (Fig. 4), leading to more AGEs, higher oxidative stress, and more frequent cell death. This may explain why those with the APOE4 gene have a 65% chance of developing AD by age 85 [19].

The Role of Acetylcholine:

Studies have shown that those with Alzheimer's have lower levels of acetylcholine (ACh), a neurotransmitter with roles in learning and cognition. This can be explained by the decrease in cholinergic neurons in the basal forebrain which is typical of AD. Enzyme acetylcholinesterase (AChE) breaks down ACh, leading to less information transfer and lowering cognition. As seen in Figure 5, when Ca^{2+} enters the cell during a synapse, ACh is released, binding to ACh receptors on the postsynaptic neuron. ACh is simultaneously degraded by acetylcholinesterase (AChE). Thus, less ACh binds with receptors, leading to less information being transferred. AChE inhibitors are used as medication today to combat this issue [20].

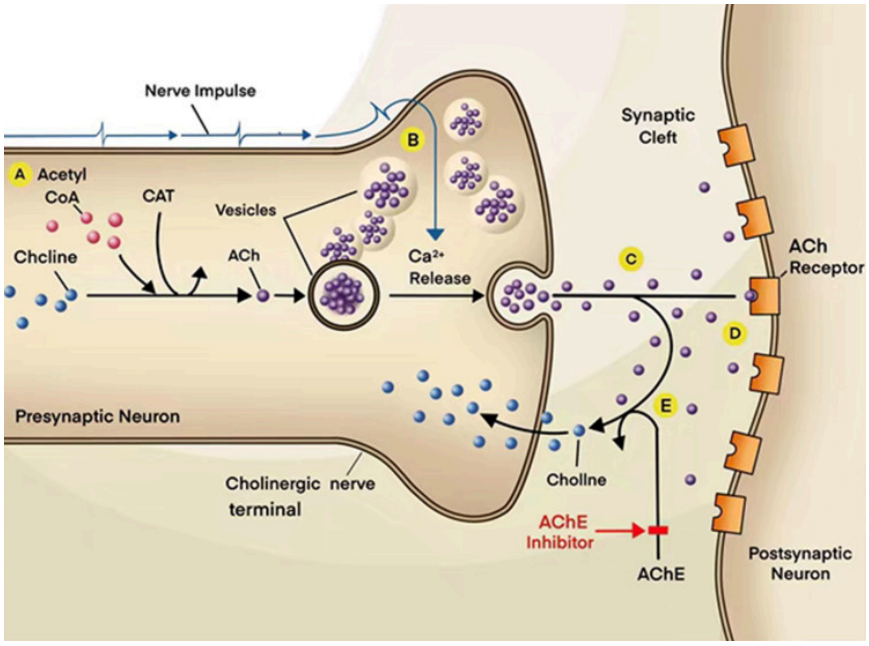

Figure 5: A healthy synapse with the neurotransmitter acetylcholine (ACh) [20].

AChE also binds to PS1 which inhibits the activity of γ-secretase. This leads to decreased production of amyloid-β, slowing down the progression of AD. Interestingly, AChE activity is restricted in patients with Alzheimer's, leading to less γ-secretase restriction and increased amyloid-β formation. This explains the weak benefits that AChE inhibitors display [21].

Interaction of Glutamate with Presenilin:

The roles of other neurotransmitters are also impacted in those with AD. Glutamate is a neurotransmitter that controls 95% of excitatory signaling, and 95% of glutamate uptake is done by one protein: excitatory amino acid transporter 2 (EAAT2) [12], also known as GLT-1. In a study done on human embryo cells and AD brain tissue, the PSEN1 and GLT-1 relationship was elucidated. In those with sporadic AD, PSEN1 has a "closed" conformation similar to that seen in familial aD, decreasing its interaction with GLT-1. It was also seen that increased PSEN1 caused more GLT-1 channels in the cell membrane [22]. This follows the presenilin hypothesis, as mutated PSEN1 will harm the PSEN1/GLT-1 interaction and decrease the amount of GLT-1 channels. This will then cause less glutamate uptake, decreasing the amount of information transferred and leading to cognitive decline. These results point to a new form of treatment for Alzheimer's: increase γ-secretase, which is exactly the opposite of modern day medication.

Conclusion:

Altogether, it can be understood that there is not just one single cause of Alzheimer's Disease; instead, many different factors together lead to neurodegeneration. Increased Aβ42 to Aβ40 ratio was believed to be the main cause of AD due to the production of plaques, but medications that tried to lower this did not have favorable results. Tau hyperphosphorylation is also a very important part of the progression of Alzheimer's due to the neurofibrillary tangles that it forms. Other proteins and lipids can also misform and lose their function due to oxidation, known as advanced glycation end products. New ideas are emerging to explain AD, one of which focuses on PSEN1, a subunit of γ-secretase. PSEN1 has a large impact on the uptake of glutamate, one of the neurotransmitters required for synapses. Another neurotransmitter, acetylcholine, is also involved in the progression of AD. Even with all the factors discussed, many more still impact neurodegeneration. To finally find a cure for Alzheimer's Disease, more research must be conducted to find the true cause.

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