Chapter 1: Introduction

1.1. Alzheimer's disease

The progressive loss of neurons and synapses, especially in the brain's cholinergic system, is the hallmark of Alzheimer's disease (AD), a multifactorial neurodegenerative illness that impairs memory and other cognitive abilities. [1].The symptoms of AD include cognitive, memory, language, and personal behavior impairments. [2]. These symptoms are brought on by neuronal injury that may affect neurons in other areas of the brain and the area of the brain responsible for cognitive function. As the illness worsens, behavioral abnormalities and psychological symptoms such as agitation, hallucinations, sadness, and delusions—collectively referred to as non-cognitive symptoms—develop. Although the precise molecular mechanisms causing the neurodegeneration in AD are still unknown, a wealth of evidence, including research published by our group, indicates that low concentrations of neurotransmitters, particularly acetylcholine (ACh), amyloid-beta (Aβ) aggregates, oxidative stress (OS), and metal concentrations all play a significant role in the neurodegeneration process. [3-8].

The initial description of AD was given by German psychiatrist Alois Alzheimer in 1907. Auguste, an Alzheimer's patient under research, had severe memory loss, false suspicions about her family, and progressively increasing psychological problems. [9]. Alzheimer observed aberrant deposits in and around nerve cells, as well as a severe shrinkage, during a histopathological analysis of her brain. He identified the two categories of brain lesions: neurofibrillary tangles and senile plaques. He determined that there was a unique cerebral cortex illness. [10].

The neurotransmitters acetylcholine (ACh) and butyrylcholine (BCh) are essential for memory and learning. The body of research and literature both clearly demonstrated the

critical function these neurotransmitters play in the a etiology of AD. [11]. ACh's levels and function are both known to decline with AD. Acetylcholinesterase (AChE) is responsible for the metabolism of ACh in the synaptic gap and for maintaining the dynamic equilibrium of ACh in the healthy brain. Therefore, in the treatment of AD, inhibition of AChE and BChE can successfully result in symptom alleviation.

The formation of Aβ plaques outside of neurons and neurofibrillary tau protein tangles (NFTs) inside of sick neurons are the pathological hallmarks of AD. Growing research in recent years has supported the theory that these indicators likely manifest several years before to the beginning of AD's cognitive symptoms.[12]. These plaques and neurofibrillary tangles are typically located in the hippocampus, amygdala, entorhinal cortex, and basal forebrain important for memory, learning, and emotional behaviors [13].

Reactive oxygen species (ROS) can arise as a result of both oxidative stress (OS) and elevated iron levels. These reactive oxygen species (ROS) prevent mitochondrial respiration and encourage the accumulation of amyloid plaques as extracellular neurofibrillary tangles and intracellular plaques. Age and genetics also play a significant part in the disease process, even if the precise cause of AD is unknown.[14].

1.2. Statistics of AD

In the US, AD ranks as the sixth most prevalent cause of mortality and the most common cause of dementia [15]. AD affects 10% of those over 65 In the United States. The mortality rate from heart disease, stroke, and HIV has declined dramatically over the past 20 years, according to 2020 AD facts and figures, yet there has been a reported 146.2% rise in fatalities from AD [16]. According to the WHO, 50 million individuals worldwide suffer from dementia, and the number is growing at an accelerated pace each year [17]. By 2050, the total cost of caring for everyone with AD and other dementias is predicted

to reach around $1.1 trillion. Perhaps the most well-known risk factor for Alzheimer's disease is age [15]. Every five years beyond the age of 65, the chance of getting the illness doubles. Individuals in their 80s, 90s, and beyond are more likely to suffer from AD. Because the number of elderly persons is increasing, these statistics are important. Determinable impairments in cognitive function in many domains, such as working and spatial memory, are observed in around 20–40% of healthy individuals aged 60–78. [19].Furthermore, it is predicted that low- and middle-income nations would account for almost 68% of the anticipated rise in the prevalence and burden of dementia linked to AD worldwide. AD imposes a heavy societal and financial burden; the estimated $1 trillion annual cost of dementia is expected to increase by more than double by 2030.

1.3. Pathophysiology of AD

1.3.1. Role of acetyl and butyrylcholines in AD

The brain uses neurotransmitters, which are highly ordered neurons that connect to create a neural network, to interact with one another [20]. Acetylcholine (ACh) is the neurotransmitter secreted by cholinergic neurons. One of the main neurotransmitters involved in memory and learning is ACh. Since cholinergic neurons make up the majority of the brain regions most impacted by neuronal loss in AD, restoring normal ACh levels has been proposed as a potential treatment for AD. [21, 22]. The neurotransmitters acetylcholine (ACh) and butyrylcholine (BCh) are essential for memory and learning. The corpus of study findings and literature both clearly demonstrated the critical function these neurotransmitters play in the etiology of AD. [23-26]. The cortical deficiency of cholinergic neurotransmission is one of the main contributing elements to the patient's typical cognitive impairment, according to the cholinergic AD theory. ACh's function and concentration are both known to decline in AD. Presynaptic nerve terminals produce acetyl coenzyme A (ACh) via condensation of choline and acetyl coenzyme A with co-

enzyme acetyltransferase (CAT). It is the enzyme that limits the pace at which ACh is synthesized. In order to further facilitate nerve-to-nerve communication, synthesized ACh is therefore kept in presynaptic vesicles. Later, it is released into the synaptic gap and acts on the postsynaptic target cell.

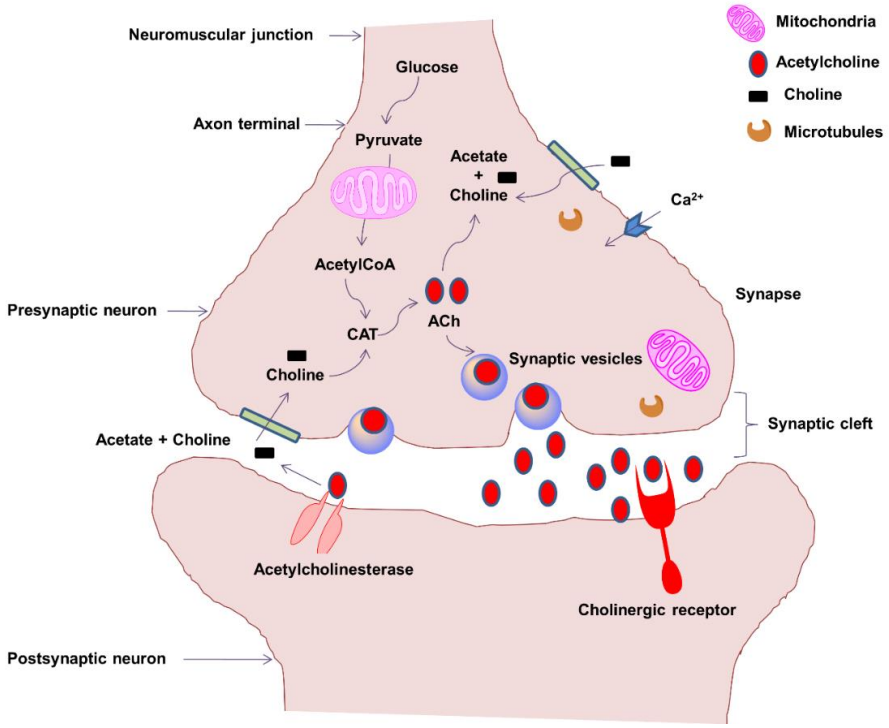

Figure 1.1. Diagrammatic representation of ACh synthesis, metabolism, and its mode of action.

Acetylcholinesterase (AChE) is responsible for the metabolism of ACh in the synaptic gap and for maintaining the dynamic equilibrium of ACh in the healthy brain (Figure 1.1). [27]. Through a charge relay mechanism involving hydrogen bonding between the glutamate carboxyl, the histidine imidazole (His447), and the hydroxyl of the serine (Ser203), the serine residue at the active site is made very nucleophilic. [28] (Figure 1.2). Choline is released from the active site and the serine residue is acylated as a result of the hydroxyl anion attacking the carbonyl carbon of ACh upon entering the active site. Subsequently, the serine residue undergoes de-acylation owing to nucleophilic assault by water molecules, which leads to the release of acetic acid and the renewal of the active site. [29]

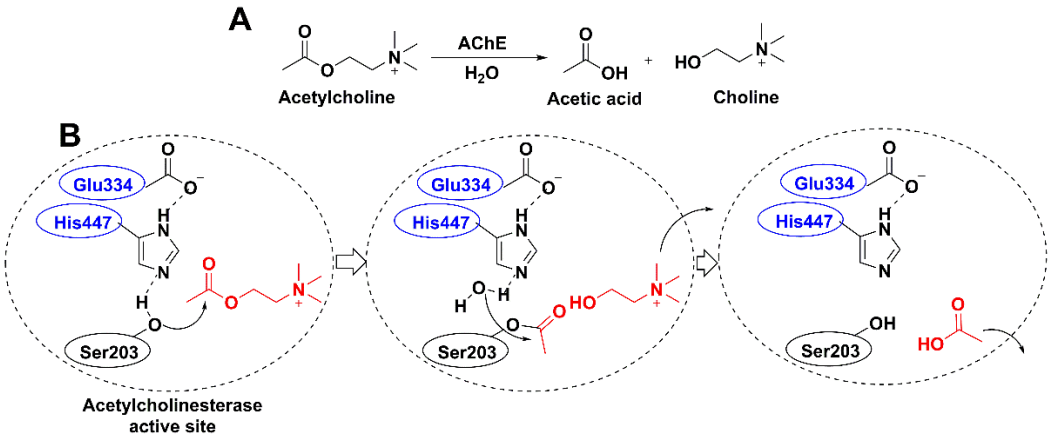

Figure 1.2. Mechanism of substrate cleavage through AChE (**A**&**B**).

1.3.2. Role of amyloid beta (Aβ) and tau proteins

The pathological hallmarks of AD are the formation of neurofibrillary tau protein tangles (NFTs) inside sick neurons and amyloid β (Aβ) plaques outside of neurons. Growing research in recent years has supported the theory that these indicators likely manifest several years before to the beginning of AD's cognitive symptoms. [12, 30] The entorhinal cortex, basal forebrain, hippocampal, and amygdala—regions involved in memory, learning, and emotional behaviors—are the primary locations of these plaques and neurofibrillary tangles. [13] The transmembrane glycoprotein, sometimes referred to as an amyloid precursor protein (APP; 695 amino acid residues), undergoes β-amyloidogenic processing that results in the production of hazardous Aβ peptides with varying lengths (39–42 AAs) by fragmentation. Aβ1-42 peptides have a higher degree of hydrophobicity and have a tendency to self-aggregate into soluble misfolded Aβ-aggregates (dimers and oligomers), protofibrils, fibrils, and insoluble fibrils (senile plaque). These aggregates are known to be neurotoxic. [31] The primary neurotoxic is thought to be the oligomeric isoforms. [32], It causes the activation of the innate immune system's pattern recognition receptors (PRRs) by interaction with many synaptic receptors (such as NMDAR, PRPc, FPRL1, RAGE, and P75NTR).By promoting different types of cellular stress such as oxidative stress, reducing glucose intake, and calcium

dyshomeostasis, the accumulation of Aβ aggregates inside the mitochondria, disrupting its normal functioning and upregulating Janus kinase (JNK), Cdk5, dual-specificity tyrosine-phosphorylation regulated kinase-1 A (Dyrk1A), and mitogen-activated protein kinase (p38MAPK) activity.[33]Moreover, it can increase the production of proinflammatory cytokines, including as tumour necrosis factor (TNF∝), interleukin 6 (IL-6), and interleukin-1β (IL-1β), and the consequent generation of reactive oxygen species (ROS).[34]. By producing a range of inflammatory factors, such as TNFα, monocyte chemotactic protein 1 (MCP-1), IL-6, and ROS, the activation of microglial cells mediated by Aβ causes a neuronal inflammatory response. This sets off a protracted reactive cycle in microglial cells that ultimately promotes neurotoxicity and results in the development of AD. [35, 36].

Increased levels of Aβ trigger a series of intricate processes, including as the production of proinflammatory cytokines and chemokines in astrocytes through the action of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells). This might trigger cellular damage to neurons or even cause astrocytes to produce too much Aβ. [37]. Numerous experimental findings from preclinical and in-vitro investigations have linked the production of proinflammatory cytokines. By increasing the activity of Cdk5, JNK, Dyrk1A, and p38MAPK, the ROS hasten the development of NFT and tau pathology. This is achieved by boosting the activity of tau kinases and inactivating phosphatases, which in turn exacerbates the hyperphosphorylation of tau proteins linked with axonal microtubules. [38-41]. These aberrant tau proteins are precursors to the production of straight, helical filaments (SFs) and paired helical filaments (PHFs), which destabilize microtubules by losing their ability to bind tubulin. NFTs are created when these filamentous tau proteins self-polymerize. [42].Though the fundamental causes of AD remain unknown despite great advancements in its pathogenesis, buildup of Aβ is thought

to be one of the early events that might set off a convoluted chain of events and the signaling mechanism implicated in tauopathies. Preclinical and prodromal phases, which usually last more than two decades, usually precede the 8–10 years that clinically evident dementia lasts. [13].

1.3.3. Role of metals in AD

Our bodies require trace quantities of certain metals, such iron, copper, and zinc, to function properly. The brain depends on the biometals zinc, copper, and iron for processes like neuroplasticity and cell signaling. [43]. On the other hand, too much exposure to free metals might be detrimental to human health. Aβ plaques, the deadly protein deposits that are the hallmark of Alzheimer's disease, have been discovered to have abnormally high quantities of the metals mentioned above. [44]. It is also thought that iron and copper have a role in oxidative stress.

Numerous biological processes, including as mitochondrial oxidation, cell division, neurotransmitter production, and dopamine (DA) metabolism, depend on iron. [45]. As we age, the amount of iron in our brains steadily rises. Although iron is necessary for the formation of neurons, too much iron can lead to a neuron's destruction.[46] Therefore, in order to prevent the harmful effects of iron, such as the production of reactive oxygen species (ROS), an ideal concentration of iron should always be maintained in cellular compartments. Under conditions of overload, the iron storage proteins ferritin and neuromelanin become saturated; hence, an increase in the labile iron pool causes neurodegeneration. [47].

In the hydrophilic N-terminal portion of the peptide, Aβ links to iron through three histidine residues and one tyrosine residue, helping to stabilize these iron ions. [48]. Additionally, research indicates that binding of Fe^{2+} ion to Aβ increases the β-sheet

richness of the peptide and decreases the peptide helix structure, suggesting that Fe2+ ion enhances the interaction between peptide-peptides to promote the formation of oligomers and fibrils from Aβ monomers. [49]. The main factor causing the production of ROS is the high binding affinity of Aβ for metals and its capacity to lower Fe2+. Aggregation and tau phosphorylation are also caused by iron. When hyperphosphorylated tau builds up in NFTs, antioxidant heme oxygenase-1 (HO-1) protein is induced. HO-1 is an antioxidant but also triggers the Fenton reaction by releasing Fe2+ (Figure 1.3). Iron excess prevents furin from being expressed, which supports β-secretase activation and the generation of Aβ via the amyloid pathway. [50] Iron appears to increase Aβ aggregation and decrease the toxicity of these aggregates. [51] (Figure 1.4).

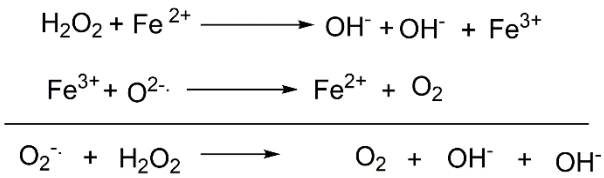

Figure 1.3. Fenton reaction by which H_2O_2 forms hydroxyl radical in iron rich environment.

Since disturbance of its homeostasis results in neurodegenerative illnesses such as AD, copper is an essential trace metal for developing the nervous system. Cu2+ ions have a stronger affinity for Aβ peptides and raise the proportion of β-sheet and α-helix structures in Aβ peptides, which in turn causes Aβ aggregation. [52] While Aβ's toxicity to cells is markedly increased when Cu2+ ions bind to it, Aβ fibril production is enhanced by increased quantities of Cu2+ ions. In the presence of reducing agents, H_2O_2 is produced when copper ions combine with Aβ fibrils. Aggregate shape shifts from fibrillar to amorphous when the ratio of Cu2+ to peptide rises, along with increased H_2O_2 levels and OH- radical generation. [53].

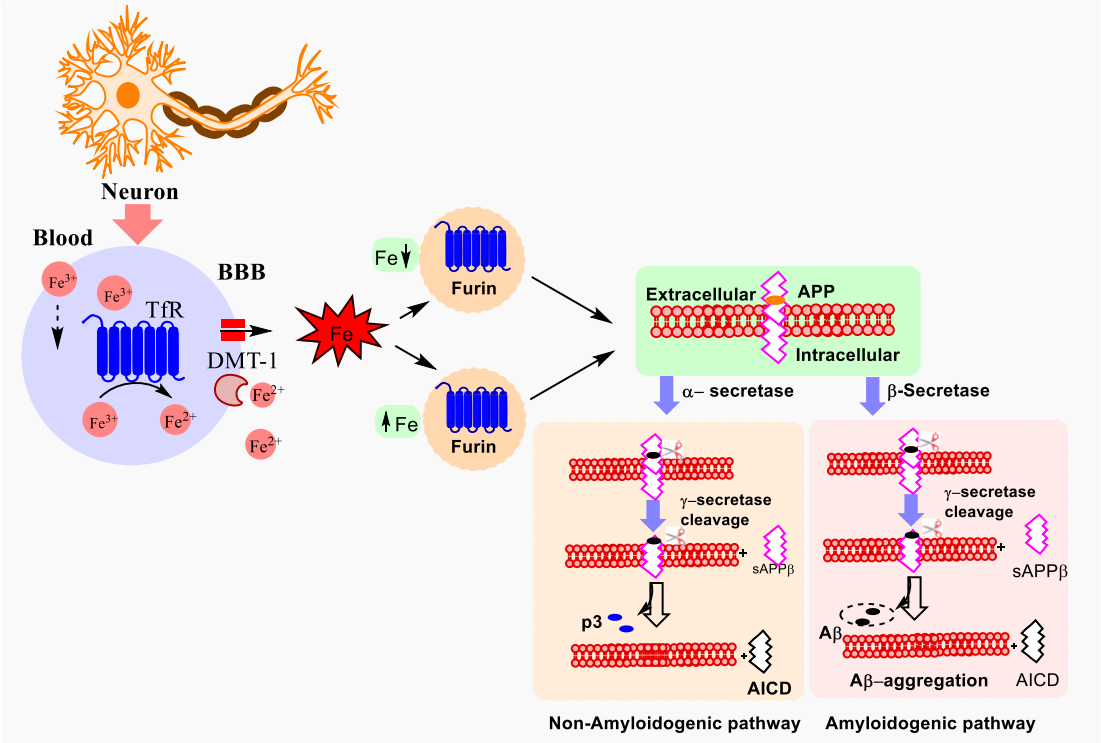

Figure 1.4. Schematic representation of the role of iron in AD.

1.3.4. Role of oxidative stress (OS) in AD

Dyshomeostasis between ROS/RNS and the antioxidant capacity of the cells to counteract them is known as oxidative stress. Elevated oxidative stress is one of the initial steps in the AD-related neurodegenerative process. An imbalance in the redox system of the mitochondria is the main source of ROS. [54]. The inner membrane's oxygen and electrons combine to form superoxide anions (O2.-). Other ROS forms, such as hydrogen peroxide (H_2O_2) and hydroxyl ions (OH-), are produced when these superoxide radicals undergo further reactions. However, reactive oxygen species (ROS) such as superoxide (O_2.-) and H_2O_2 combine with nitric oxide (NO) to produce peroxynitrites anion (RNS). [55, 56]. As seen in Figure 1.5, metals, particularly copper and iron, also act as mediators of oxidative stress. Free radicals are produced by iron through the Fenton reaction. Reactive species (ROS/RNS) overproduction impairs antioxidant action and causes toxicity through protein, DNA, and RNA oxidation, lipid peroxidation, and other

mechanisms. [57]. The most likely source of oxidised nucleotides is hydroxyl radicals produced when highly diffusible H_2O_2 combines with redox-active metals (Cu and Fe) (DNA and RNA) [58]. Essential genes such nucleoside guanosine can be transcriptionally and replicated as a result of oxidative damage to neuronal DNA and RNA. [59]

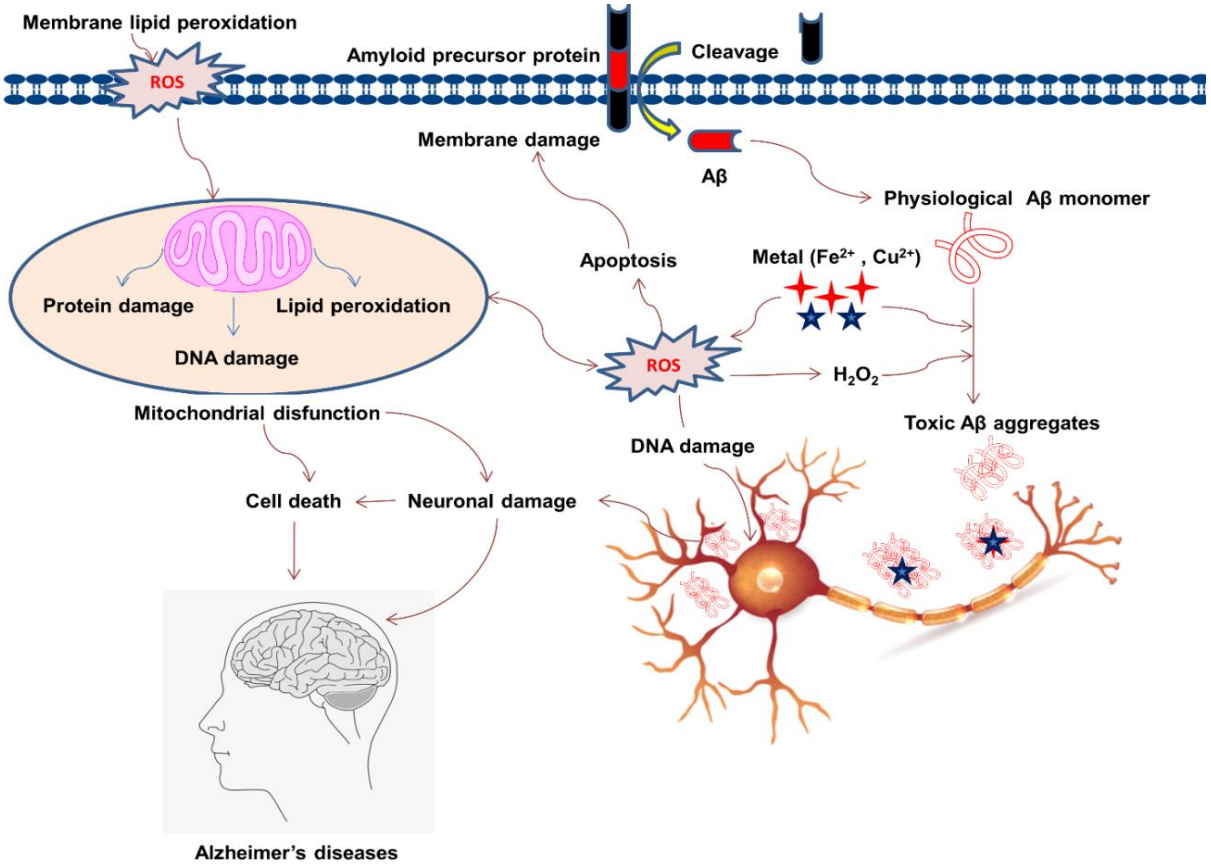

Figure 1.5. Diagrammatic representation indicating the role of ROS and metals in AD progression.

1.3.5. Role of β-secretase (BACE) in AD

A broad family of type 1 transmembrane proteins, which also includes the amyloid precursor protein-like (APPL) in *Drosophila* and the amyloid precursor protein-like (APLP1 and APLP2) in mammals, comprises the amyloid precursor protein (APP). [60]. Neurons create a significant quantity of APP, which is quickly metabolized. The

precise biological role of APP is still a matter of great debate in this field and lacks clear evidence. APP is endoproteolyzed to create Aβ. The α and β-secretases are two proteases that may hydrolyze APP on the cell surface. The cleavage that α-secretase initiates is not amyloidogenic, meaning that poisonous Aβ is not produced. [61]. While β-secretase initiates the cleavage that results in the N terminus of Aβ, a membrane-bound C-terminal fragment known as C99 is produced. [62]. The mature, deadly Aβ peptide is then produced when γ-secretase cleaves C99. On the other hand, β-secretase cleaves Aβ exactly at Asp+1 and Glu+11, suggesting that it is a site-specific protease. It is noteworthy that the formation of all Aβ forms, including the pathogenic Aβ42, would be reduced by therapeutic inhibition of β-secretase (Figure 1.6).

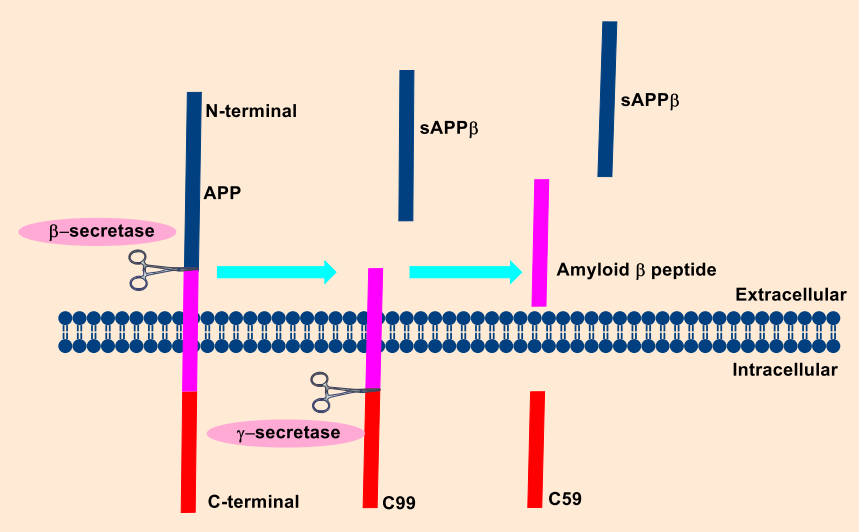

Figure 1.6. Diagrammatic representation indicating the role of β-secretase in Aβ aggregation.

1.4. Current drug targets for AD

AChE and the NMDA receptor are the two AD targets that are currently available. The enzyme known as acetylcholinesterase (AChE) is a serine protease that breaks down acetylcholine. [63]. It is therefore necessary for cholinergic neurotransmission. The many types of the active site include the anionic site, catalytic triad or esteratic site (ES), oxyanion hole, selectivity determinant acyl pocket, and peripheral anionic site (PAS).

[64]. Next, the brain also contains butyrylcholinesterase (BChE), an isomer of cholinesterase that is involved in ACh cleavage. According to recent research, brain-targeted BChE enhances animal cognitive function and reduces Aβ levels in transgenic mice. As the illness worsens, there is a significant shift in the levels of both BChE and AChE. Thus, the potential of both enzymes as neuroprotective and disease-modifying therapies for AD has been investigated.

1.4.1. Cholinesterases (ChEs)

The hydrolysis of ACh into choline and acetic acid is carried out by cholinesterases (ChEs). The literature reports on the two primary forms of ChEs: (i) butyrylcholinesterase (BChE) and (ii) acetylcholinesterase (AChE). [65].One of the important enzymes involved in synaptic transmission is ChE, which works by hydrolyzing the neurotransmitter ACh. Thus, improving ACh levels by blocking AChE activity may help AD patients' cognitive and mental functioning. Interestingly, the amount of AChE decreases by 90% in the latter stages of AD compared to the normal brain, raising serious concerns regarding its potential use as a target for therapeutic drugs in the later stages of AD. [25]. According to recent biological research, AChE inhibition alone is unable to raise ACh levels. As a result, ChE inhibitors have beneficial effects that endure for a shorter period of time—roughly one to three years—without affecting the course of the illness. [66]. Clinical research data mostly supports the notion that AChE inhibitors are ineffective for managing moderate-to-severe AD stages. [67-69].

In its normal state, AChE is a monomer with a MW of about 60,000. It is made up of 537 amino acid residues that are grouped into a mixed β-sheet with 12 strands and 14 α-helices around it. The hydrophobic active site of AChE is composed of the catalytic active site (CAS) and the peripheral anionic site (PAS), two subunits. CAS, which consists of Ser200, Glu327, and His440, is located at the bottom of a small canyon that is

approximately 20 Å long and 4.5 Å broad. The gorge is bordered by 14 aromatic residues. [29]The active site also includes a subsite (the "anionic subsite") near the bottom of the cavity that includes the Phe330 and Trp84, a necessary residue to interact with the quaternary ammonium group in the substrate (ACh) and other ligands through cation π-interaction. Approximately 20 Å distant from the active centre, the catalytic gorge entrance is home to the poorly characterized PAS or β-anionic site of AChE. The active comprising Tyr70, Asp72, Tyr121, Trp279, and Tyr334 undergoes conformational changes in the presence of a ligand. One of these residues, Trp279, is essential to the adhesive activity of AChE (Figure 1.7). As the first phase, the PAS momentarily binds to the substrate, increasing catalytic efficiency and trapping the substrate route to the active site. [70].

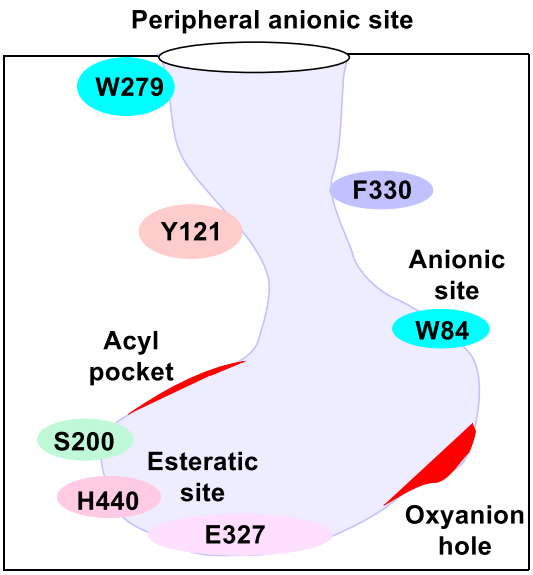

Figure 1.7. Schematic view of the active site of AChE. The bottom of the gorge is characterized by an anionic site, which contains esteratic site, acyl pocket, an oxyanion hole, and PAS is located 20 Å above the active site.

Conversely, BChE is an α-glycoprotein that is present in both the peripheral and central nervous systems. It is a nonspecific cholinesterase that also hydrolyzes aliphatic esters and pseudocholinesterase, also known as serum cholinesterase. [71]The residues Ser198, His438 and Glu325 make up the CAS of hBChE. Although the precise function of BChE

in AD is yet unknown, it can replace and compensate for the role of neuronal AChE in the progressive neurodegeneration seen in AD.[72] Therefore, in the treatment of AD, inhibition of AChE and BChE can successfully result in symptom alleviation. [73] Consequently, the successful treatment of AD may involve the use of dual and specific AChE/BChE inhibitors.

1.4.2. NMDA receptor in AD

Neurons have an ionotropic glutamate receptor and ion channel called N-methyl-D-aspartate receptor, or NMDAR (Figure 1.8). NMDA is essential for synaptic transmission, and learning and memory—which are essential for both neurotoxicity and nervous system function—are assumed to be based on synaptic plasticity. Excitotoxicity, which results in an increased Ca2+ influx and overactivation of the NMDA receptor, is a cause of neurodegenerative diseases, including AD. [74]. The primary issue with using NMDA receptor antagonists for neuroprotection is that normal neuronal function depends on the physiological functions of the NMDA receptor. [75].

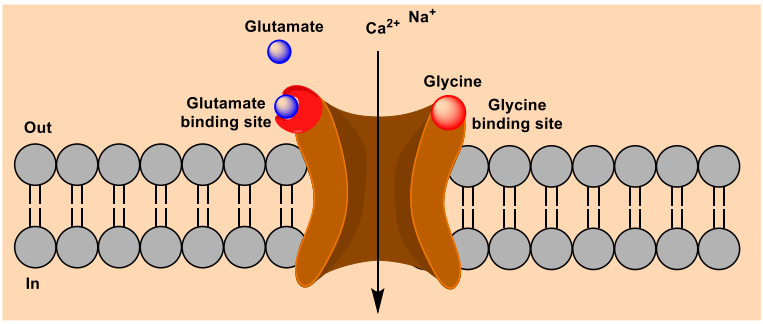

Figure 1.8. NMDA receptor complex as a therapeutic target in AD.