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The amyloid hypothesis of Alzheimer's Disease and its current treatment.

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Abstract

Alzheimer's disease (AD), a progressive neurodegenerative disorder, is the commonest cause of dementia in the elderly population worldwide. It is a multifactorial disorder, characterized by extracellular β-amyloid peptide (Aβ) deposits, leading to the formation of senile plaques; and intracellular neurofibrillary tangles, composed of hyperphosphorylated Tau protein. There exist competing hypotheses regarding the causes of AD and their significance in AD progression. Therefore, this bibliographical review focuses on detailing the Aβ hypothesis thus explaining the generation of Aβ peptides from amyloid precursor protein (APP), and its accumulation leading to the formation of amyloid plaques. Over the last two decades, Aβ proteins have been extensively studied, not only for their role in the progression of the disorder, but also as a potential target for novel therapeutic strategies. This study also highlights several therapeutic approaches including the inhibition of β- and γ-secretase, the enhancement of Aβ clearance, the regulation of Aβ production; as well as, vaccination and immunization therapies. Many such approaches have reached clinical trials, yet they were abandoned due to low efficacy and side effects on AD patients. Currently the available treatments are focused on the cholinergic hypothesis of AD, and includes acetylcholinesterase inhibitors and N-methyl D-aspartate receptor antagonists. These drugs decelerate the progression of AD and offer symptom-relief to the patients. However, these drugs fail to achieve a definite cure. Thus, the enormous challenges AD poses to researchers is the urgent need to develop innovative therapeutics strategies.

Aβ	Amyloid-β peptide
Ab	Antibody
ACE	Angiotensin-Converting Enzyme
ACh	Acetylcholine
AChEIs	Cholinesterase Ihibitors
AD	Alzheimer's Disease
AICD	Amyloid Intracellular Domain
APP	Amyloid Precursor Protein
APPL	Amyloid Precursor protein-like
ARIA	Amyloid Related Imaging Abnormalities
BACE	β-site APP-cleavage Enzymes
ChAT	Choline Acetyltransferase
CME	Clathrin-mediated endocytosis
CNS	Central Nervous System
CSF	Cerebrospinal fluid
CuBD	Cooper-binding domain
ER	Endoplasmic Reticulum
FAD	Familial Alzheimer's Disease
HBD	Heparin-binding domain
IDE	Insulin dependent enzyme
KPI	Kunitz protease inhibitor
MMP	Matrix Metalloproteinase
Nct	Nicastrin
NFTs	Neurofibrillary Tangles
NSAIDs	Non-steroidal anti-inflammatory drugs
PHF	Paired Helical Filaments
PS	Presinilin gene
PTM	Post translational modifications

RAGE	Receptor for andanced glycation end product
RIP	Regulated intramembrane Proteolysis
SPs	Seline Plaques
SREBPs	Sterol Regulatory Element Binding Proteins
TMD	Transmembrane domain

1. Introduction

Alzheimer's disease (AD) is acknowledged as a multifarious neurodegenerative disorder which accounts as the most common form of mental failure in late adult life (De Strooper & Karran, 2016). AD progresses symptomatically from mild to severe and has a place amongst the eight top most health complications worldwide (Cornutiu, 2015). Currently, AD is considered for more than 80% of dementia cases in elderly population, 50% of people who are 85 year or older are afflicted with AD (Avramopoulos, 2009). It is estimated that about 200,000 young AD patients (younger than 65 years old) comprise the early onset AD population whereas approximately 5 million people are 65 years old or older who comprise the late onset AD population.

1.1 Historical Perspective of AD

A Bavarian psychiatrist, named Alois Alzheimer, was the first that defined this clinicopathological syndrome. In 1907, Auguste Deter, a 50-year-old woman, patient of Alois suffered with several cardinal characteristics of AD, such as memory impairment and general cognitive decline (Stelzmann et al., 1995). Alois identified in Deter's autopsy, the presence of two distinctive histopathological features in her disease-damaged brain which are considered as the pathological hallmarks of AD (O'Brien and Wong, 2011). Those brain lesions were; (a) large numbers of extracellular "amyloid" plaques (Seline plaques, SPs or neurotic plaques) and (b) aggregates of intraneuronal neurofibrillary tangles (NFTs) (Ballatore et al., 2007). However, Alois could not distinguish whether those two brain features were causative or markers of AD (**Figure 1**).

Notably, later in 1984 Glenner and Wong reported that neurotic plaques, are large extracellular deposits composed of hydrophobic 40 to 42 amino acid peptides, called amyloid-β peptides (Aβ) (Glenner and Wong, 1984). Those peptides are derived through the proteolytic cleavage of the amyloid precursor protein (APP) (Hardy, 1997). The second type of lesion of Alois reported, were the NFTs which occurs intracellularly and are composed with twisted filaments of the cytoskeletal Tau protein (Medeiros et al., 2010). After Aloi's observations he suggested that Deter's case was a rare cause of dementia, until the late 1960s with the work of Blessed, Tomlinson and Roth (Blessed et al., 1968). Those three researchers found out that there is an interplay between SPs in elderly brains thus was accepted as the commonest basis of senile dementia.

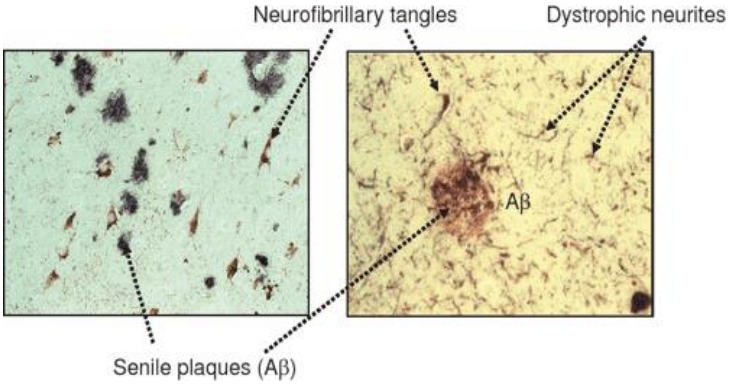

Figure 1 The two hallmarks of Alzheimer's Disease. *Senile plaques (known also as neurotic plaques) are deposits of Aβ, extracellularly of brain cells. Neurofibrillary tangles are aggregates of abnormal hyperphosphorylated Tau protein. These two lesions in an Alzheimer's brain results in many dystrophic neurites.*

Adapted from: Skaper, 2012

In terms of histopathological progression, both lesions that were previously referred lead to the loss of both neurons and synapses in specific areas of the brain. Those pathological hallmarks of AD results to the progressive neuronal degeneration of both cerebral and limbic cortices, reactive gliosis and deposition in the brain parenchyma of amyloid aggregates (or plaques) closely associated with dystrophic neurons. The two hallmarks of AD can be located initially in specific areas of the brain and then spread according to the evolution of the symptoms of the disease (Figure 2) (Jouanne et al., 2017).

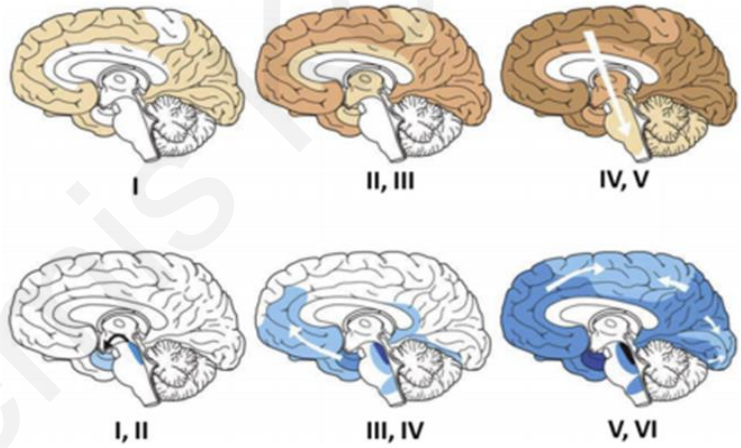

Figure 2 The spread of lesions during Alzheimer's Disease. The five-step describes the progressive spread of Aβ peptide deposits from the neocortex to the intranasal region and the hippocampus, reaching the subcortical structures, the brain stem and finally the cerebellum. At the beginning NFTs target the endotracheal cortex (phases I and II), gradually expanding into the hippocampal region (stages III and IV) and the neocortex (stages V and VI).

Adapted from: Jouanne et al., 2017

1.2 Symptoms

The disorder is clinically characterized with insidious onset and irreversible progression that makes many of the patients requiring constant care. The main signs of AD is the loss of memory and progressive deterioration of mental functions (Castro et al., 2010). Nowadays, during the progression of AD there are several features that are observed in most AD patients. At the early and middle stages of AD the alertness, motoric and sensory functions are preserved and remain intact. Additionally, among the symptoms of the disorder is spatial disorientation, gradual deterioration of mental capacity and behavioral alterations which includes paranoia, delusions or loss of social appropriateness (De Strooper & Karran, 2016). Also a characteristic symptom is the progressive decline in language function. As the disorder progress to later phases, in every patient we identify a decline of motor functions, for example gait and coordination.

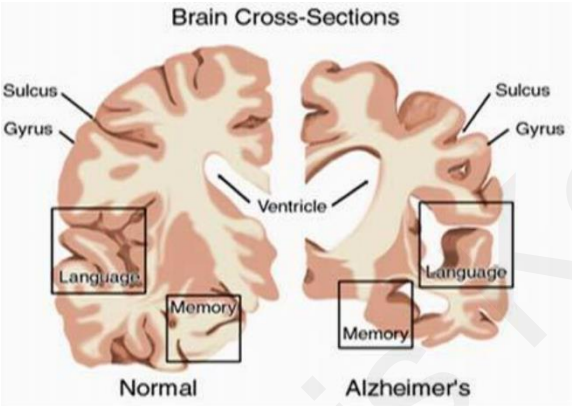

Figure 3 Comparison of a healthy brain and an AD-damage brain. *During AD it is clear the shrinkage of brain tissue. AD is characterized by regional atrophy of the cerebral cortex (sulcus) and enlarged of the ventricles. The levels of brain substance in the folds of the brain (gyri) is elevated and the spaces in those brain folds (sulci) are grossly widened. Thus, lead to a loss of cognitive function such as mood changes, behavioral symptoms and language deterioration. Damage to hippocampus, a region responsible for memory formation, results to short term memory loss.*

Adapted from: Castro et al., 2010

1.3 Subtypes of AD

AD is considered as a multifactorial disorder, with environmental and genetic factors for its pathogenesis. Epidemiological studies suggested that risk factors for AD development include age, head injuries, cardiovascular disease and the gender (females have a greater risk to develop the disorder). In 1991, according to phenotypic analyses and the search of genetic linkages highlighted that AD can be inherited in an autosomal-dominant fashion and it is considered as the familial AD (FAD) (Munter et al., 2010). FAD is very rare, with early onset of symptoms and in most cases it is occurred due to genetic background (O'Brien & Wong, 2011). Mainly is caused by mutations in three genes; the presinilin gene (PS1) located on chromosome 14, presenilin 2 gene (PS2) on chromosome 1, and APP localized on chromosome 21. Mutations on the gene of APP are missense

mutations which serve to pass AD via autosomal-dominant fashion. These lesions are present in and around the Aβ region of the APP (Hardy, 2006). In familial AD the clinical manifestations are generally similar or almost indistinguishable from non-familial cases. Thus, according to the phenotypic similarity indicates that the mechanisms and more specific the mutations in the APP and presenilin genes in familial form of AD is likely to be relevant to the pathogenesis of non-familial AD.

1.4 Mutations in AD

According to studies, missense mutations not only in APP but also in presenilins, the proteins of the γ-secretase complex, can alter Aβ generation, degradation and/or aggregation (Tsubuki et al., 2003). To date have been identified a large number of mutations which are observed in AD people with the symptom onset before the age of 65 (early-onset FAD) (Masters et al., 2015). Have been identified 32 APP, 179 PSEN1 and 14PSEN1 mutations which are connected with the FAD. Although the more important genetic cause of AD is the occurrence of mutations specifically in the gene of APP. Those mutations affect the proteolytic processing of APP at the three secretase cleavage sites. Most mutations are found either before the β-cleavage site, after the α-secretase cleavage site or to the COOH-terminal to the γ-cleavage site (Selkoe, 2001).

The result of missense mutations in the gene encoding APP, is the alteration of APP γ-secretase cleavage in order to increase the production of the less soluble and amyloidogenic Aβ42 in contrast to Aβ40 (Shen and Kelleher, 2006). Aβ42 is a primary component of amyloid plaques which are deposited in AD brains in both sporadic and familial form of AD. As mentioned before, mutations in the gene of APP are found around the three secretase cleavage sites (Wilquet and Strooper, 2004). Mutations near the β- and γ-secretase cleavage sites result to an increase Aβ42 species. In the case of missense mutations close to β-secretase cleavage site results in β-site proteolysis and thus to the increased level of both Aβ40 and Aβ42 (Citron et al., 1992; Cai et al., 1993). On the other hand, mutations close to γ-site elevate the production of Aβ42 (Suzuki et al., 1994). Researchers took into consideration all these evidences and suggested the involvement of Aβ in the pathogenesis of AD.

The overexpression of APP is a second way of AD predisposition. As mentioned before, APP is located on chromosome 21, thus in cases of trisomy 21 (Down's syndrome) results to elevated gene dosage (Selkoe, 2001). The prolonged APP overexpression due to duplication of

chromosome 21 or translocation of trisomy 21, that part of 21q which contains the gene of APP, leads in overproduction of both Aβ40 and Aβ42. This contributes to the early formation of Aβ42 diffuse plaques and therefore the individual has an early-onset AD (Lemere et al., 1996).

1.5 Competing hypotheses of AD

At this point according to biochemical and histopathological evidence several competing hypotheses have been suggested regarding the causes of the disorder. Some of the descriptive hypothesis of AD include the cholinergic hypothesis, amyloid hypothesis, Tau hypothesis, inflammatory hypothesis, oxidative stress hypothesis, excitotoxicity, ApoE hypothesis, etc (Sharma et al., 2019). At the same time all of these hypothesis are also the pathological hallmarks of AD. Among the competing hypothesis only two of them stood out, the Aβ and Tau protein hypothesis which are the most crucial components the molecular pathogenesis of AD (NFTs and SPs) (Liu et al., 2019). However, even the big range of evidence that support many of these hypothesis, the main causation of AD remains unclear. Therefore, in the pharmaceutical industry there is not currently available any efficient treatment or therapy for prevention.

1.5.1 Cholinergic Hypothesis

In 1976, Peter Davies and A.J. F. Maloney were the two first researchers who suggested the Cholinergic hypothesis (Davies, 1976). These hypothesis raised through the observations of the post-mortem brains of AD patients and the activity of crucial enzymes which implicate in the synthesis of neurotransmitters, such as acetylcholine (ACh).

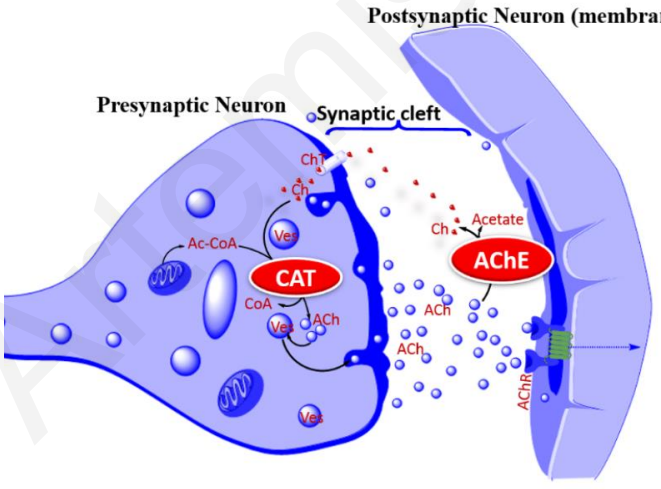

Figure 4 Synthesis of ACh through the action of ChAT. *The catalytic activity of CAT in order to synthesize ACh requires choline, acetyl-CoA and adenosine triphosphate (ATP). ACh is released into the synaptic cleft and interacts with presynaptic nicotinic (N), muscarinic type 2 (M2) and muscarinic type 1 (M1) receptors. In the case of AD acetylcholinesterase (AChE) takes part and cleaves acetylcholine into choline and acetate. Last step, is the uptake of the released choline by the presynaptic neuron.*

Adapted from: Habtemariam, 2019

A characteristic investigation was the incredible low concentration of choline acetyltransferase (ChAT) in the brain of AD patients (Perry et al., 1977) (**Figure 4**). In depth, AD brains have reduced

activity of ChAT in the amygdala, hippocampus and cortex (H. Ferreira-Vieira et al., 2016). At this point it is very important to mention that reduced levels of ChAT specifically in hippocampal and neocortical regions of the brain are the regions where neurodegeneration occurs during AD (Perry et al., 1977). In those brain regions the levels of ACh, which is synthesized through the action of ChAT, was also extremely low at synapses (Fotiou et al., 2015). ACh is a neurotransmitter in the brain with a major role in the neuromodulation of memory, learning and cognitive functions (Lane et al., 2005). Therefore, this hypothesis propose that cholinergic neurons are affected in the early phase of the disorder accompanied with cholinergic function impairment which are part of the ACh synthesis in basal forebrain and thus leading into cognitive dysfunctions. This evidence has also been observed in some other neurodegenerative disorders such as Parkinson's Disease and in psychiatric disorders like depression (Selkoe, 2001).

1.5.2 Tau hypothesis

Microtubule-associated proteins (MAPs) contribute to the stabilization of microtubules. Some of these are MAP1A, MAP1B, MAP2 found in mature neurons. The interaction of MAPs with microtubules is regulated by their phosphorylation state via the action of kinesin. A member of this family is the Tau protein which is located in the axons of neurons where it contributes to the axial transport of signaling molecules and cellular components, stabilizing the microtubules (**Figure 5Figure 5**) (Goedert et al., 2006).

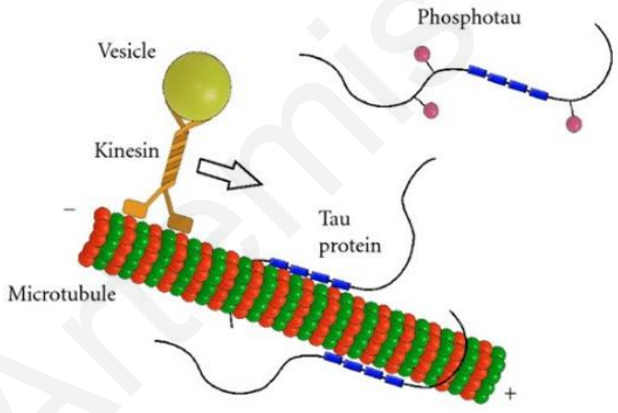

Figure 5 Normal function of Tau protein*. Tau protein stabilizes the microtubules via four / three tubulin binding sites (blue). Tau phosphorylation (pink) regulates its binding to the microtubules and may affect axial transport. Tau protein can inhibit the axial transport of vesicles along the microtubules which is achieved through kinesin.*

Adapted from: Kolarova et al., 2012

One of AD hallmarks is the intracellular NFTs, which is the second type of lesion of Alois reports. NFTs are composed of Tau protein and are formed by the aggregation of paired helical filaments (PHFs) (Braak et al., 1999). Normally Tau is located on the microtubules of neurons and plays a crucial role in the network of neuronal microtubules and for the preservation of their morphology

(Hardy, 1997). The equilibrium balance of Tau protein is controlled by Tau phosphorylation which in turn is controlled by kinases and phosphatases (Merlini & Bellotti, 2003). Moreover, the binding and release of Tau from the microtubules, according to the level of phosphorylation and dephosphorylation, respectively, is necessary for efficient axial transport (Buée et al., 2010). Thus, every normal function of Tau is regulated by post translational modifications (PTMs), which can alter its stereotyping and affect Tau's affinity for microtubules (Nizynski et al., 2017).

In the case of hyperphosphorylation or abnormal phosphorylation, results in the altered structure of Tau and contributes to its pathological functions, such as its ability to self-assemble into neural fibers found in neurodegenerative diseases, like AD (Ebneth et al., 1998). Therefore, when Tau protein is hyperphosphorylated leads to its release from the microtubules and thus microtubules are depolymerized and they form aggregates(**Figure 6Figure 6**). The aggregation leads to a change in the conformation of the secondary structure of the molecule resulting to the formation of PHFs which induce the formation of NFTs. Thus, neurons with non-functional microtubules lose their signaling capacity and undergo apoptosis (Medeiros et al., 2010).

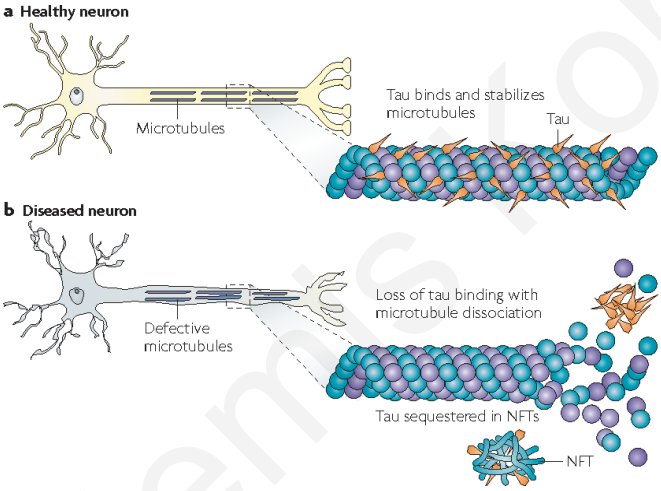

Figure 6 Formation of neurofibrillar tangles (NFTs). *(a) The normal binding of Tau protein and how stabilizes microtubules in healthy neurons. (b) In the case of diseased neuron during AD, hyperphosphorylation of Tau protein causes its release from the microtubules and the disassembly of the microtubule network. This causes the formation of helical filaments (PHFs) which in turn lead to the formation of neurofibrillar filaments (NFTs).*

Adapted from: Brunden et al., 2009

1.5.3 Amyloid beta (Aβ) hypothesis

The most persuasive hypothesis was first described in 1991 by John Hardy and David Allsop (Hardy & Allsop, 1991). Back then they identified a mutation in the gene of APP which is located on the chromosome 21q21.3 and they proposed that APP mismetabolism and Aβ deposition are the very first events in AD (Ling et al., 2003).

APP normally is located on the surface of neurons and involves in the development and repair of the neuron after damage. The release of Aβ from the APP occurs after the action of β- and γ-secretases (Figure 7). Those enzymes truncate the peptide on both amino-terminus and carboxyl-terminus of the Aβ sequence, respectively (Zheng & Koo, 2006). After the processing of APP, the mutant APP forms a monomer called Aβ which accumulates outside the neuron leading to the formation of amyloid plaques (**Figure 7**). These plaques can be deposited between neurons by blocking neuronal signaling resulting in altered brain function (Robakis, 2016). Many circumstantial evidence suggests that these proteolytic cleavage is essential to the pathogenesis of the AD (Selkoe, 2001). Thus, a better understanding of the mechanistic transition of soluble Aβ(solution) to neurotoxic Aβ(amyloid) is crucial for elucidation of the molecular causation of AD.

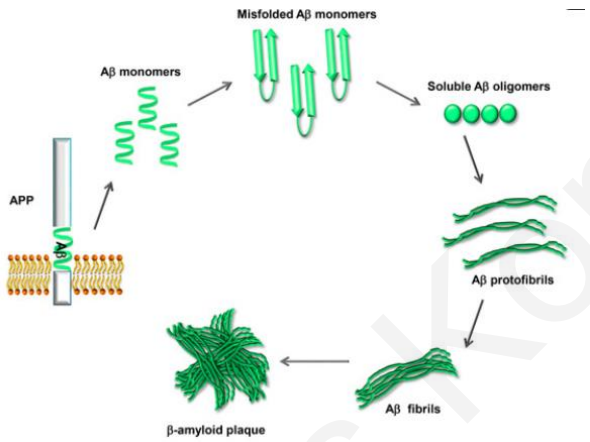

Figure 7 Formation of Aβ plaques. *Aβ plaques are generated via the proteolytic cleavage of APP by the action of β- and γ-secretase thus producing a 40-42 amino acid peptide, called Aβ. Then Aβ oligomers accumulate resulting to the formation of Aβ deposits.*

Adapted from: Mantile & Prisco, 202)

1.6 The structure of Amyloid Precursor Protein (APP)

APP belongs to the family of conserved type 1 membrane proteins that includes the amyloid precursor-like proteins, such as APLP1 and APLP2 in mammals and the amyloid precursor protein-like (APPL) in Drosophila (Muller & Zheng 2012). APP is a single-pass transmembrane protein with a large extracellular domain and is expressed ubiquitously (Ling et al., 2003). The gene of human APP is located on the long arm of chromosome 21 and contains 18 exons with a size of about 240 kb (Sondag & Combs, 2006). Notably among the other APP-related genes, only APP is able to generate amyloidogenic fragments. Exons 16 and 17 contain the region encoding the Aβ sequence composed of 40- and 43-amino acid residues that extend from the ectodomain into the transmembrane domain of APP. Additionally, the APP protein contains a huge ectodomain, which includes a cysteine-rich globular domain (E1), an acidic domain, a helix-rich

domain (E2) and a region of Aβ sequence which is continued into the transmembrane domain (Lichtenthaler et al., 2011). Additionally, the cytoplasmic domain of the protein contains the C-terminus which is a region with multiple phosphorylation sites and last a YENPTY sorting motif. YENPTY motif is crucial in the regulation of intracellular events regarding the activity of APP (Ando et al., 2001).

APP splice variants exist by alternative splicing, generating 8 isoforms, the most common encode transcripts are; the 695 amino acid isoform expressed predominantly in the central nervous system (CNS), the 751 and 770 amino acid isoforms (Weidemann et al., 1989) (**Figure 8a**). Those isoforms differ by the absence (APP-695) or presence (APP-751 and APP-770) of the exon 7 which encodes a serine protease inhibitor (called Kunitz proteinase inhibitor (KPI)) domain which is located toward the NH2 terminus of the APP protein (**Figure 8b**)(Sinha et al., 1990; Konig et al., 1992). Further, KPI-containing APP forms are more amyloidogenic and their spread increases in diseased AD brains (Rohan de Silva et al., 1997). Thus APP751 and APP770 are more amyloidogenic.

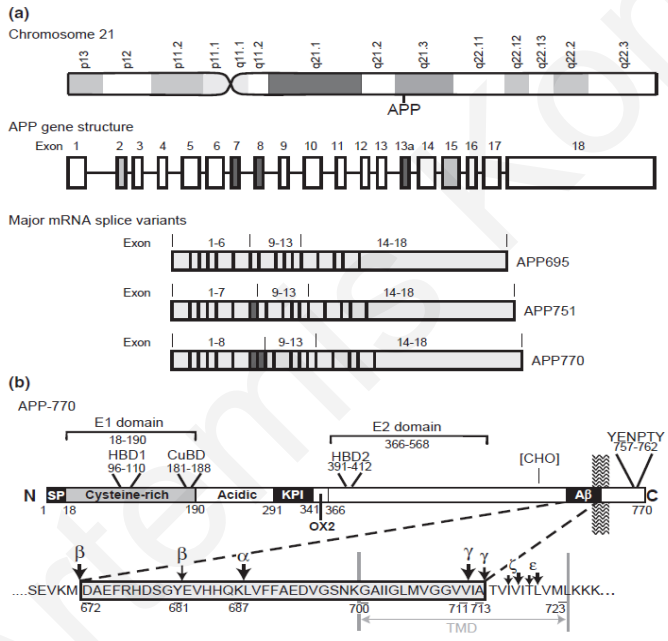

Figure 8 Schematic illustration of APP structure in gene, mRNA and protein level. (a) *The gene of APP is located on chromosome 21q21.3 and consists 18 exons. Through the differential mRNA splicing of the Exon 7 and 8 (dark grey) results to the expression of 695, 751 and 770 amino-acid variants.* ***(b) Protein structure of APP.*** *APP contains an N terminal signal peptide (SP) and the E1 domain which contains a heparin-binding domain (HBD1), and the copper-binding domain (CuBD). Further APP contains the E2 domain with a second heparin-binding domain (HBD2). Specifically, two isoforms, APP751 and APP770, consist a Kunitz protease inhibitor (KPI) and an Ox-2 antigen domain. Additionally, within the E2 and Aβ region there are two potential N-linked glycosylation sites (CHO). The Aβ region is illustrated along with the various secretase cleavage sites through the action of α-, β- and γ-secretase. Last, APP has its intracellular C-terminal domain which contains a YENPTY sorting motif. TMD, transmembrane domain.*

1.7 Trafficking of APP and its post-translational modifications

APP is widely produced in neurons and processed very rabidly. APP is generated and modified in the endoplasmic reticulum and Golgi apparatus. The second step is the migration of APP to the axon where it is delivered by fast axonal transport to synaptic terminals. Alternatively, APP is transported directly to an endosomal compartment. Clathrin-mediated endocytosis (CME) contribute in both steps. Following step is the non-amyloidogenic pathway where APP is metabolized by the action of α-secretase which results to the generation and release of sAPPα. Although a proportion of sAPPα is retrieved into endosomes. Then, the amyloidogenic processing occurs and the endosome recycles into the cell surface having as a result the production of Aβ and sAPPβ (**Figure 9**).

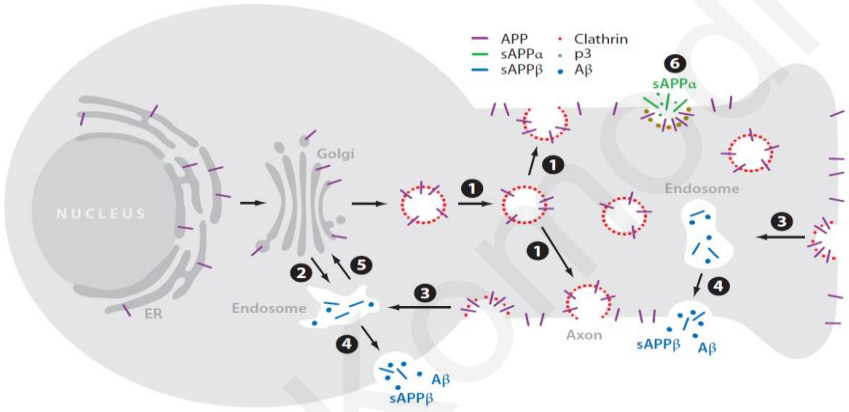

Figure 9 Diagram of intracellular APP trafficking. *Nascent synthesized APP (purple) migrates from the Golgi to the axon cell surface (step 1) or into an endosomal compartment (step 2). After the internalization into the cell surface, where secretases are present, the non-amyloidogenic processing occurs. Thus, a proportion of APP is processed via α-secretase (step 6) leading to the production of sAPPα fragment, which is released (green), and a part of it is reinternalized into endosomes (step 3), where the generation of Aβ is occurred (blue). Following event is the amyloidogenic proteolysis where the endosome recycles to the cell surface (setp 4) resulting to the release of both Aβ and sAPPβ (blue). The migration from the endosomes to the Golgi apparatus before the APP cleavage can also occur, mediated via retromers (step 5).*

Adapted from: O'Brien & Wong, 2011

After the expression of APP, the translated APP undergoes post-translational modifications which includes sulphation, glycosylation and phosphorylation (Selkoe, 2001). Thus following its modification in the Golgi, APP can be post-translationally processed ("maturation") via the action of specific enzymes known as secretases. Secretases have the ability to cleave APP in order to generate a number of smaller fragments. A closer look of APP processing will be discussed later.

1.8 Processing of APP and its routes

APP, the integral membrane protein, can be proteolyzed on the cell surface. AD pathogenesis is directly connected with the event of APP proteolytic processing which is occurred through the action of β-secretase, γ-secretase, and caspases. The processing of APP generates Aβ peptide and carboxyl-terminal fragments (CTF) of APP (Checler, 1995; Suh, 1997; Selkoe, 1999). There are various pathways for APP proteolysis but some of them have as a result the production of Aβ peptide.

1.8.1 α-secretase

α-secretase is a member of the metalloproteases family called ADAM. APP processing via the α-secretase does not yield Aβ and thus is considered as the nonamyloidogenic pathway. These pathway of APP cleavage is a major and ubiquitous pathway of APP metabolism in most cells. α-secretase cleaves within the Aβ domain between Lys16 and Leu17 and more specific cleaves the C-terminal side of residue 16 of the Aβ sequence (residues 687 and 688 of APP) (Esch et al., 1990). Thus, it produces soluble sAPPα, an 83-residue COOH-terminal fragment (CT83). CT83 act as a substrate for γ-secretase which performs a different type of cleavage within the transmembrane region. As a result, γ-secretase generates a 3-kDa non-pathogenic peptide called p3 and a CT57-59 fragment, from the CT83 fragment. p3 peptide is released into the extracellular milieu (**Figure 10B**). Has been highlighted that phorbol esters activate protein kinase C (PKC) which leads to the up regulation of non-amyloidogenic cleavage. Some other reagents in order to elevate the α-cleavage pathway include testosterone, estrogen, growth factors and many neurotransmitters.

1.8.2 β-secretase

A second mechanism, is the alternative amyloidogenic pathway which is enriched in neurons and brain. In these route of proteolytic processing, APP extracellular domain can be processed via the action of β-secretase. These enzyme is a membrane-bound protein with high homology to soluble aspartyl protease, called the β-site APP-cleavage enzymes (BACE). Have been identified two enzymes responsible for β-cleavage, BACE1 and BACE2. However, only BACE1 is the major β-secretase in the brain thus it is highly linked with AD pathology. Have been identified that BACE-2 is highly expressed in kidney, heart and placenta, proposing that it may be crucial in highly vascularized systemic tissues (Farzan et al., 2000). BACE1 generates the NH2 terminus of Aβ peptide and can cleave APP between Tyr10 and Glu11 in order to produce a soluble form of APP

(APPsβ) and a 99-residue COOH-terminal fragment (CT99) which remains anchored to the membrane. Like CT88 (previously mentioned), in the same way, subsequent proteolysis of CT99 fragment which act as a substrate for γ-secretase, cleaves APP and yield the 4-kDa Aβ and a cytoplasmic polypeptide CT57–59 [termed amyloid intracellular domain (AICD)]. Thus, Aβ is released into the extracellular milieu and AICD into the cytoplasm (**Figure 10C**). The generated Aβ is a soluble cellular metabolite which have two predominant forms with a different COOH-termini each of them. Those forms are Aβ40 and Aβ42. Aβ aggregation is the key in the causation of AD, however current evidence proposes that soluble Aβ oligomers, the ones that are the precursors for the fibril formation, are the neurotoxic elements. Soluble Aβ are the species that causes neurodegeneration and then dementia.

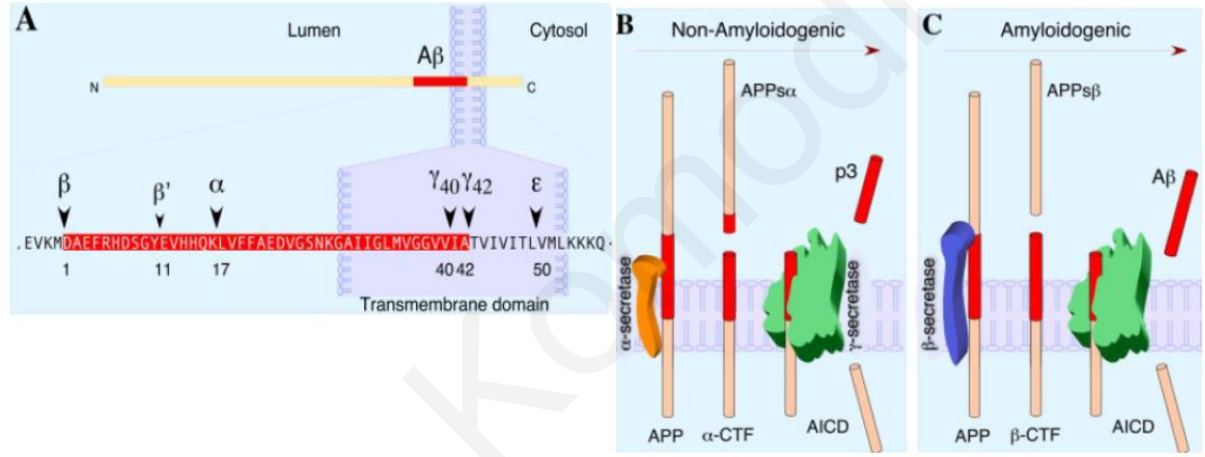

Figure 10 Schematic representation of APP proteolytic processing via amyloidogenic and non-amyloidogenic pathways by the secretases. *(A) APP structure which contains the Aϐ domain (shaded in red). Also the figure illustrates the cleavage sites via the action of the three secretases with numbering from the N terminus of Aϐ. (B) Nonamyloidogenic pathway involves the first cleavage of APP by α-secretase at peptide bond 16-17 producing APPsα and α- carboxy terminal fragment (α-CTF), precluding Aϐ generation. The membrane-bound a- CTF is cleaved via the action of γ-secretase within the plasma membrane. Thus leads to the release of p3 and APP intracellular domain (AICD). (C) Amyloidogenic pathway, includes the first cleavage of APP by ϐ-secretase resulting to the production of APPsϐ and ϐ-CTF. Then γ-cleavage of the ϐ-CTF fragment releases the full length Aϐ and AICD.*

Adapted from: Thinakaran & Koo, 2008

1.8.3 γ-secretase

γ-secretase catalyzes the secondary cleavage of APP. These enzyme has pharmacological features of an aspartyl protease and loose sequence specificity for its substrate. This features of γ-secretase became from mutations in APP near the γ-secretase site which allows the production of Aβ in affected cells (Maruyama et al., 1996; Tischer and Cordell, 1996; Lichtenthaler et al., 1997, 1999; Wolfe et al., 1999a–c) and seem to be a multiprotein complex. Thus, γ-secretase is a complex of

four integral membrane proteins; presenilin 1 (PS1) or presenilin 2 (PS2) which are candidates for its catalytic components. Further γ-secretase consists nicastrin (Nct), which is a type I transmembrane glycoprotein; APh-1 and Pen-2, which are multipass transmembrane protein (He et al., 2010). All these proteins are forming a complex which is necessary for sequential intramembranous proteolysis of various transmembrane proteins. The function of γ-secretase is to cleave the transmembrane domain of the carboxy terminal fragments of APP.

After either α- or β-secretase releases their remaining carboxyl-terminal fragments of APP they undergo a final transmembrane cleavage via γ-secretase. Both CT83 and CT99 undergo proteolysis within their plasma membrane region— it is called regulated intramembrane proteolysis (RIP)— and the intracellular part migrates to the nucleus where it may alter the transcription of target genes. Further proteins that undergo these type of process, RIP, include not only APP but also Notch, a receptor involved in fate decisions during embryonic development and the sterol regulatory element binding proteins (SREBPs), which are transmembrane proteins of the endoplasmic reticulum (ER) that controls lipid metabolism (Brown et al., 2000).

γ-secretase cleaves at multiple sites and its position of cut within the transmembrane domain of APP is crucial. Because of the multiple cleavage sites, γ-secretase yields Aβ peptides that differ in length from 38 to 43 residues. In the case of cleavage at residue 712-713 results to short Aβ, otherwise a cut after residue 714 leads in a long Aβ. The cleavage through γ-secretase is very heterogeneous. A large amount of the produced Aβ species are full-length which are considered as 40-residue peptides (Aβ40). However, a small proportion of them are 42-residue COOH-terminal variants (Aβ42). Aβ42 is more prone for fibril formation than Aβ40 because of its larger size and is more hydrophobic (Jarrett et al., 1993). Even if Aβ42 is a minor form, it is the major Aβ identified in cerebral plaques of AD patients (Iwatsubo et al., 1994).

1.9 Treatment of Alzheimer's disease.

In order to modify AD, novel strategies have been designed. Therefore major drug development is focused to the Aβ based therapeutics, which is a key to treat or prevent this disease in the near future. This paper highlights not only the currently approved treatment but also some developed drugs which failed during clinical trials. So far there is no cure for Alzheimer's disease but there are drugs that can reduce patient's clinical symptoms. Drugs that have been developed for treatment cannot be used due to side effects and most of the trials were announced for termination.

2. Main Part

2.1 How APP and Aβ facilitates neurodegeneration?

It is widely known that APP and specifically its toxic metabolite, Aβ, is a key player in AD pathology. As mentioned before, soluble, small Aβ species are cytotoxic to neurons (Klein et al. 2001; Walsh and Selkoe, 2004). Thus, the remaining question is, how oligomeric Aβ induces synaptic dysfunction and neurodegeneration? Kirouac et al., demonstrated that both APP and Aβ42 are able to modulate neuronal intracellular signaling, leading in APP and tau hyperphosphorylation, thus resulting to neuronal dysfunction and degeneration.

According to a previous study, they have used stable isotope labeling by amino acids in cell culture (SILAC)-based proteomic analysis and identified that APP enhanced the expression of proteins responsible in cellular morphology, assembly, organization and cell cycle (Chaput et al. 2012). According to their results they reported enhanced expression of Ras and phosphorylation and activation of ERK in B103 rat neuroblastoma cells expressing APP-695. Thus, they concluded that APP triggers Ras/mitogen-activated protein kinase (MAPK) signaling cascade and enhanced cell proliferation (Chaput et al. 2012). Kirouac et al., to confirm that APP has its fundamental role upstream of Ras-ERK signaling cascade they used RNAi approach in order to downregulate APP in B103-695 cells. According to their findings, APP downregulation results to decreased Ras expression as well as decline in ERK phosphorylation. These findings indicate that APP is considered as an upstream positive regulator which activates the Ras/MAPK signaling cascade in neuronal cell lines. Further they illustrated that B103 cells expressing APP enhance both expression and activation of Ras due to its ability to be in active form by the exchange of GDP to GTP. According to other studies focused on postmortem brains Ras-MAPK cascade is considered as an early driver of AD pathology (Arendt et al. 1995; Gärtner et al. 1995, 1999) (**Figure 11Figure 11**).

Amyloid deposition is a one of the crucial hallmarks of AD pathology development. Many clinical trials targeting on Aβ elimination failed, thus they hypothesized that there are additional APP-dependent mechanisms involved in AD pathogenesis. According to immunocytochemistry Ras and P-ERK are found in neurons proximal to tangles and plaques in human AD brains (Trojanowski et al. 1993; Hyman et al. 1994; Arendt et al. 1995; Ferrer et al. 2001). Kirouac et al., beyond the reported enhanced signaling of neurons they also demonstrated that APP has a crucial

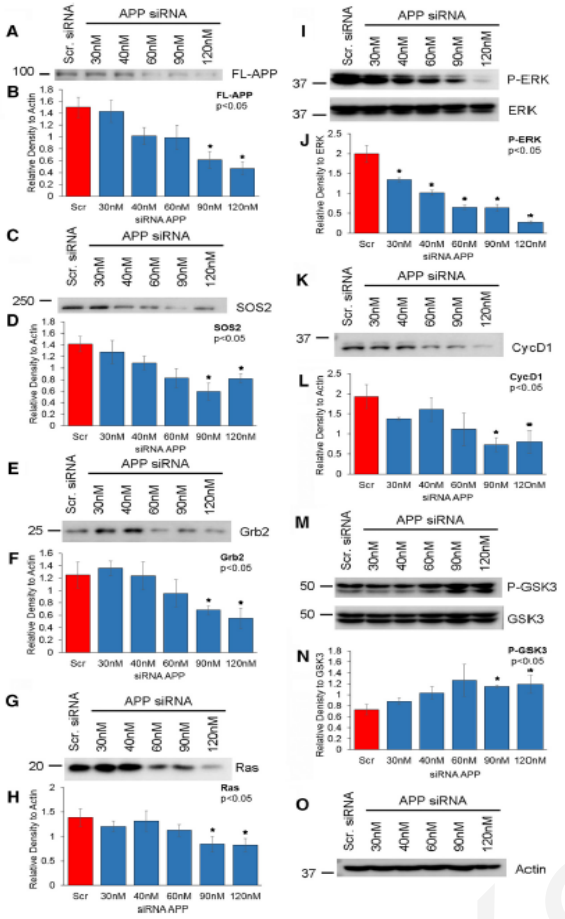

Figure 11 APP as an upstream regulator of Ras/MAPK pathway.(A,B) *B103-695 cells treated at different APP siRNA concentrations revealed decrease in APP levels compared with control siRNA transfected cells.* ***(C,D)*** *At siRNA concentrations, 90nm, transfected cells exhibited decrease in son of sevenless 2 (SOS2) which is an effector protein that triggers the exchange of GDP to GTP leading to the activation of Ras, Grb2* ***(E F)****, Ras* ***(G,H)****, and cyclin D1* ***(K,L)*** *were also observed.* ***(I,J)****ERK activation, was indicated by phosphorylation, revealed significant decrease even at the lowest APP siRNA concentrations****. (I, bottom panel)*** *The total ERK levels were not altered by APP downregulation.* ***(O)*** *Actin was used as a loading control.* ***(M, N)*** *Also phosphorylation of GSK3 was increased in APP knockdown cells whereas the total levels of GSK-3 were not affected. Due to specific phosphorylations which are already known to inhibit the GSK-3 activity, the observed increase in phosphorylation after the APP downregulation illustrates that APP or a metabolite of it can regulate GSK-3 activity.*

Adapted from: Kirouac et al., 2017

role in the activation of Ras-MAPK signaling pathway in an Aβ-dependent manner (**Figure 11Figure 12**). They identified that Ras-ERK signaling cascade, in Aβ treated cells, triggers the hyperphosphorylation of APP and Tau protein, the two abundant hallmarks in AD brains. Thus, increased Aβ levels in the brain involves to the high expression of Ras and phosphorylation of both APP and tau, an observation consistent with others' observations (Busciglio et al. 1995; Le et al. 1997; Geula et al. 1998; Takashima et al. 1998; Götz et al. 2001). However, the inhibition of Ras-MAPK activation leads not only to the absence of APP and tau hyperphosphorylation but also to neuronal cell cycle entry. According to these evidence Aβ has a fundamental role for proliferative signaling in neurodegeneration during AD. These finding demonstrated a positive feedback by which Aβ species induces APP-dependent neurodegeneration. Their study in AD brains enabled us to understand the importance of the aberrant APP or Aβ-dependent signaling in order to prevent the deregulation of cell cycle and neurodegeneration during AD.

As mentioned, APP can be cleaved through the action of β-secretase, this event is triggered by the APP phosphorylation specifically at threonine 668 (Thr668) in its cytoplasmic region (Lee et al.

2003; Chang et al. 2006). The responsible components for APP phosphorylation are Kinases such as JNK, GSK-3, extracellular signal–regulated kinase (ERK), cdk4, cdk5, and cdc2 (Suzuki et al.

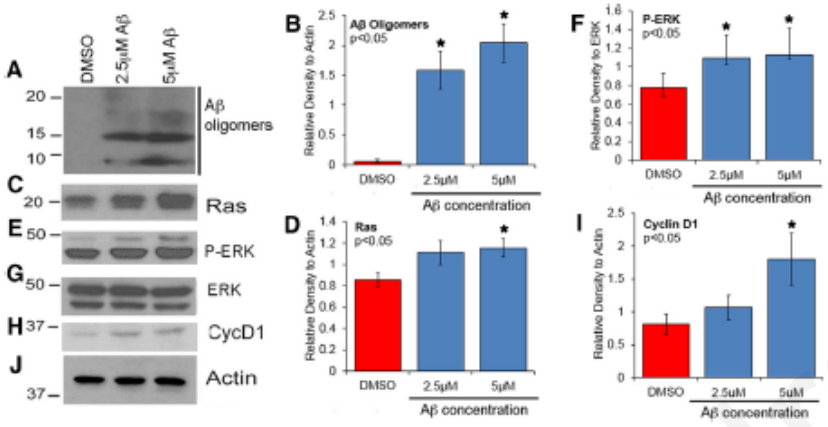

Figure 12 Oligomeric Aβ42 alters the expression of Ras and Cyclin D1 and activation ERK in neurons. (A,B) *Preparation of small oligomeric Aβ.* ***(C,D)*** *Ras levels were increased after 5μM Aβ treatment.* ***(E,F)*** *P-ERK levels were also increased at 2.5 and 5 μM Aβ concentrations.* ***(G)*** *Total ERK levels which remained the same. Thus, suggesting that Aβ mediates the Ras-MAPK signaling pathway in neurons.* ***(H,I)*** *At 2.5 and 5μM Aβ treatment the levels of cyclin D1 were increased and more significant increase was observed at 5μM Aβ concentration.* ***(J)*** *Actin antibody used to examine the protein load on the blot.*

Adapted from: Kirouac et al., 2017

1994; Muresan and Muresan, 2005; Judge et al. 2011). AD studies, revealed that compromised neurons exhibit not only activation of the prementioned kinases but also abnormal expression of cyclins D, B and E (Busser et al. 1998; Raina et al. 1999). Studies on AD brains reported high levels of APP phosphorylation at Thr668 (Lee et al. 2003), however it is unclear whether this phosphorylation is caused by abnormal cell cycle activation.

According to studies, not only P-ERK implicates in tau phosphorylation (Tanaka et al. 1998; Reynolds et al. 2000), but also tau is phosphorylated by other kinases such as cyclin-dependent kinases (Kobayashi et al. 1993), and most notably GSK-3 (Utton et al. 1997). Further, have been demonstrated that APP involves in the regulation of GSK-3 activity. The APP intracellular domain (AICD) interacts with GSK-3β and induce its activity (Zhou et al. 2012). In their research Kirouac et al., identified that after APP knockdown, the phosphorylation of GSK-3 is increased.

The activation of Ras-MAPK signaling cascade through Aβ in neurons and taking into account that this cascade is known to induce AP-1– dependent transcription of cyclin D1 (Balmanno and Cook, 1999), they sought to determine whether cyclin D1 expression was triggered upon Aβ

treatment (**Figure 12Figure 12**). Their study revealed that Aβ also induces the expression and nuclear accumulation of cyclin D1 in neurons. High levels of cyclin D1 in the nucleus results in cell cycle activation and G1/S progression, thus they suggested that a similar mechanism is induced via Aβ in neurons, forcing the neurons to enter the cell cycle. However, neurons are unable to undergo division due to lack of functional cell cycle machinery and they become vulnerable.

In addition, the phosphorylation of APP at Thr668 was increased in late AD patients, illustrating a role in the activation of Ras- MAPK cascade and GSK-3 in induction of phosphorylation. Further, Aβ induced not only Ras-ERK activation, but also GSK-3 activation, cyclin D1 expression, and nuclear translocation, with phosphorylation of both tau and APP (Thr668). The evidence that Aβ enhances APP phosphorylation is novel, and the aberrant activation of the Ras-MAPK signaling cascade and GSK-3 activity by Aβ-APP signaling is crucial for APP and tau phosphorylation, impaired axonal transport, and microtubule destabilization found in AD.

To sum up, concerted signaling by APP and Aβ is required for aberrant neuronal cell cycle entry. According to evidence Aβ toxicity is dependent on the presence of APP (Shaked et al. 2006; Sola Vigo et al. 2009), and APP has been proposed to have a role of a possible receptor for Aβ (Lorenzo et al. 2000; Kedikian et al. 2010). Further, the cytoplasmic domain, YENPTY motif, of APP serves as a docking sight for various adaptor proteins, such as, Shc and Grb2. Those adaptor proteins recruit the guanine nucleotide exchange factor, SOS2, in order to activate Ras-MAPK pathway and intracellular signaling (Nizzari et al. 2007, 2012). Based on their observations they hypothesized that Aβ acts via APP to enhance downstream signaling through the activation of Ras-MAPK pathway to trigger hyperphosphorylation of tau and APP (**Figure 13**). Thus, APP phosphorylation induces its proteolysis via the action of BACE to generate Aβ and is associated with the centrosomes, proposing a role for APP in aberrant cell cycle signaling in brain regions. All these data lead us to the conclusion that APP plays a crucial role in the promotion of neurodegeneration, via the activation of both Ras-ERK signaling pathway and GSK-3. Its involvement with these signaling pathways prevents cell cycle reentry and neurodegeneration in AD.

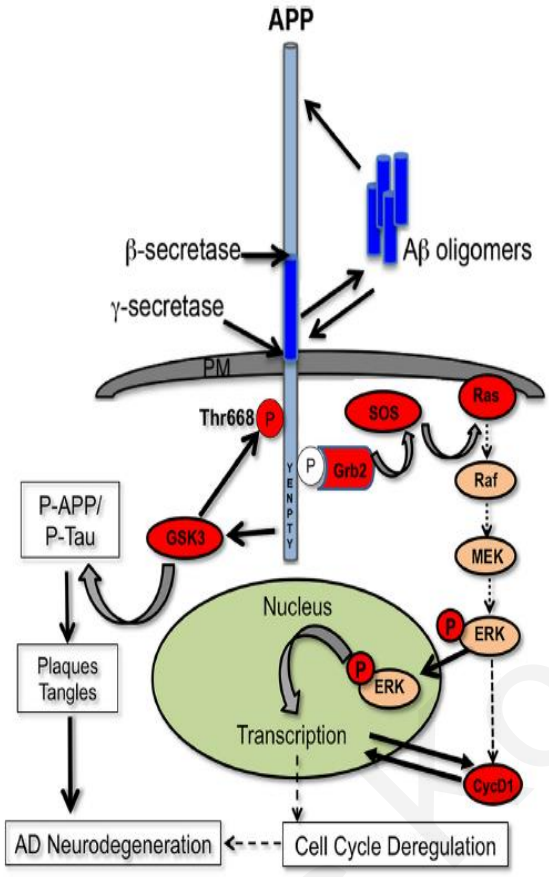

Figure 13 Intracellular signaling by APP and Aβ*. AD is characterized with high APP cleavage and Aβ production or low Aβ clearance. According to the results of Kirouac et al., altered expression of APP and Aβ production would promote Aβ-APP signaling and activation of Ras-MAPK signaling cascade, promoting the proliferative signal and neuronal cell cycle entry. Growth factor signaling could enhance interaction of APP intracellular domain (AICD) with adaptor proteins, like Grb2, and recruitment of many MAPK signaling components. Further, APP involves in GSK3 activation. Thus, activation of both ERK and GSK-3 could result to the pathogenic APP and tau phosphorylation. The phosphorylation of APP at Thr668 leads in pathogenic proteolysis of APP and increased production of Aβ, which induce the formation of neuritic plaque and neurofibrillary tangle, neurodegeneration, and neuronal death which are the hallmarks of AD.*

Adapted from: Kirouac et al., 2017

2.2 Transgenic Mice as AD models

Transgenic mice have been an invaluable tool, enabling the decoding of various gene functions in vivo as well as serving as disease models. To evaluate different hypotheses for potential therapeutics. Until now, there are multiple lines of transgenic (Tg) mice that carry the two hallmarks of AD; Aβ deposits and neuritic plaques in their brains. The following sections will review some APP Tg mice.

2.2.1 APPswe Transgenic Mice (Tg2576)

Hsiao et al. (1995) was the first that inserted the human APP695 isoform containing the double mutation (Lys670→Asn, Met671→Leu) into a hamster prion protein promoter in order to overexpress the human APP695swe mutation. The aged mice brains, were found to have elevated

levels of Aβ40 and Aβ42, resulting to Aβ deposits in specific brain regions such as; the amygdala, the cortex and the hippocampus. Many of these mice exhibited spontaneous death, behavioral changes and memory loss. Beyond these characteristics, mice showed low levels of hyperphosphorylated Tau protein and inflammatory changes. Despite these features, those mice models did not form neurofibrillary tangles or any neuronal loss in Cortical Area 1 (CA1). In addition, AD mice did not show brain atrophy as it is characterized in AD, concluding that these mice were incomplete models (Hsiao et al., 1996).

2.2.2 Amyloid Precursor Protein V717F Transgenic Mice (PDAPP Mice).

For the expression of a human APP gene that encodes the APP-V717F, which is highly linked to FAD, the platelet-derived growth factor β-chain promoter was required with the use of an outbred strain of mice (Games et al., 1995). As was mentioned before, the human APP has various isoforms such as; 695-, 751-, and 775-amino acid residues, depending on the presence or absence of exon 7 or 8 encoding the Kuniz inhibitor domain. Those mice models were constructed to contain these APP isoforms and exhibited in their brains neuritic plaques. In addition, they were characterized with synaptic impairment and high number of both astrocytes and microglia accumulated in and around the amyloid plaques. It was also reported that they exhibited failure in object-recognition, spatial-recognition, working memory (Dodart et al., 2000), and impairment of synaptic signal transmission which is a precursor step for Aβ deposition (Hsia et al., 1999). However, mice did not exhibit formation of neurofibrillary tangles (Irizarry et al., 1997).

2.3 Therapeutic strategies of AD

The understanding of APP processing mechanisms constitutes a key step for the identification of areas to target. This is a step forward for drug development in order to treat and prevent AD. During the progression of AD there is an imbalance between the production and clearance of Aβ protein, thus there are many therapeutic approaches that have been suggested until now. There are various strategies for AD which focuses on the Aβ hypothesis, and target the Aβ protein. Those approaches are divided into categories; inhibition of both β- and γ-secretases, used to inhibit the generation of Aβ neurotoxic species (Selkoe & Schenk, 2003); anti-aggregation drugs, used to inhibit Aβ aggregation; immunotherapy; and protease activity-regulating drugs, used for the initiation of Aβ clearance (Golde, 2014).

A currently approved therapy is via the modulation of secretase enzymes which are responsible for the proteolytic cleavage of APP at multiple sites in order to form Aβ fragments (Leissring et al., 2002). α-secretase has not been targeted, because it cleaves APP in the Aβ region without producing the Aβ protein. In contrast, targeting of β- and γ-secretases leads to the inhibition of Aβ formation and consequently interrupts AD progression (Vassar et al., 2009).

2.3.1 Inhibition of β-secretase

A significant therapeutic target of APP processing is the enzyme β-secretase, this approach suggests the development of β-secretase inhibitors (Arun K & Brindisi, 2012) which have been found to be effective in AD mouse models (Selkoe & Schenk, 2003). β-secretase a type I transmembrane protein, has an aspartic protease with active-site aspartyl residues which is considered as the catalytic domain of the enzyme. In addition, this enzyme contains a substrate-binding site which interacts with approximately 11 substrate residues (Turner et al. 2001, 2005). At this site the preference of the amino acid which binds to it is broad leading to the conclusion that various side chains of peptidic inhibitors can interact (Lee et al., 2010). This preference can be taken into account to develop inhibitors with lipophilicity which is important for their penetration into the membrane (Ohno et al., 2004).

BACE, a β-secretase enzyme, is widely identified in the brain and it is necessary for APP processing leading to the formation of Aβ fragments (Vassar et al., 1999). There are two BACE isoforms, BACE-1 and -2 (Farzan et al., 2000), more specific only BACE-1 is responsible for the cleavage of APP and the generation of Aβ peptides in the brain. BACE1 inhibitor, is the most attractive therapeutic target over the years because decelerates the progression of AD at an early stage by halting Aβ processing at the beginning of APP cleavage (Cai et al., 2001). No BACE1 inhibitor have successfully passed clinical trials, thus there are remaining problems which have to be considered for the designing of potential BACE- 1 inhibitors. According to evidence, BACE1 null mice exhibited few phenotypical abnormalities, proposing that inhibition of this β-secretase could be clinically achievable but with some mechanistic side effects (Nishitomi et al., 2006).

Evidence reported that knocking out the gene of BACE1 in mice models, exhibited eliminate levels of cerebral Aβ and consequently Aβ deposits and no neurological abnormalities. Thus, Ohno et al., to determine the therapeutic potential of BACE1 inhibitors they crossed BACE1$^{-/-}$ mice with transgenic mice which overexpressed the human APP695 isoform with the Swedish mutation (Tg2576^{+}). Therefore, they observed reduction of Aβ protein by BACE1 deletion which

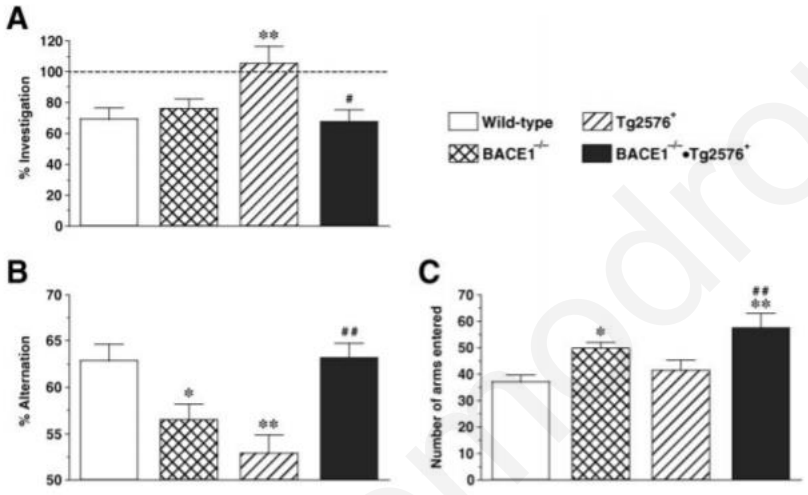

Figure 14 BACE1 null transgenic mice exhibit memory deficits. (A) Results on social recognition memory in mice with a 3 hr intertrial delay (n=10-20). Only Th2575 group of mice dies but exhibit reduction in spontaneous investigation to a familiar juvenile, thus is significantly impaired in these case. (B) Test for spatial working memory (n-12-21). In the case of Tg2576+ group of mice had low alteration. However, the three other groups were moderately but significantly impaired. (C) They reported the exploratory activity of mice (n=12-21). BACE$^{-/-}$ and BACE1$^{-/-}$.Tg2576^{+} groups of mice explored more than the group of wild mice.

Adopted from: Ohno et al., 2004

prevented memory and learning impairment in the Tg2576 AD mice models. In the case of bigenic mice BACE1$^{-/-}$. Tg2576^{+} they reported that APP mutant remains overexpressed. Thus concluded that excess Aβ than APP overexpression is responsible for the cognitive abnormalities identified in Tg2576 mice (Figure 14) (Ohno et al., 2004).

Additionally, a crucial conflict is the penetration of BACE-1 inhibitors via the blood-brain barrier (BBB) for the APP cleavage in the endosomes of neurons (Menting and CLaasen, 2014). Therefore, was urgent to develop smaller BACE1 inhibitors as they could easily pass the BBB. Further, most of the clinical trials focusing on smaller molecular inhibitors of BACE-1 were

discontinued. Those inhibitors of BACE-1 are; verubecestat (MK-8931) and azeliragon which raised the questions against this route of AD treatment (Hawkes, 2017).

Villarreal et al. (2017), tried to prove that treatment with verubecestat can affect and suppress the amyloid accumulation in transgenic mice. They treated mice with verubecestat and the controls had the anti-Aβ antibody analog of bapineuzumab (3D6) which were considered as the positive control for amyloid related imaging abnormalities (ARIA); such as vasogenic edema (ARIA-E) and microhemorrhage (ARIA-H) induction. It was shown that verubecestat effectively reduced Aβ40 and Aβ42 levels by up to 90% and 62-68% in plasma and cerebrospinal fluid (CSF), respectively in individuals with AD (Figure 15). According to the stereological analysis of both cortex and hippocampus plaque load revealed a significant reduction of Aβ immunoreactivity as

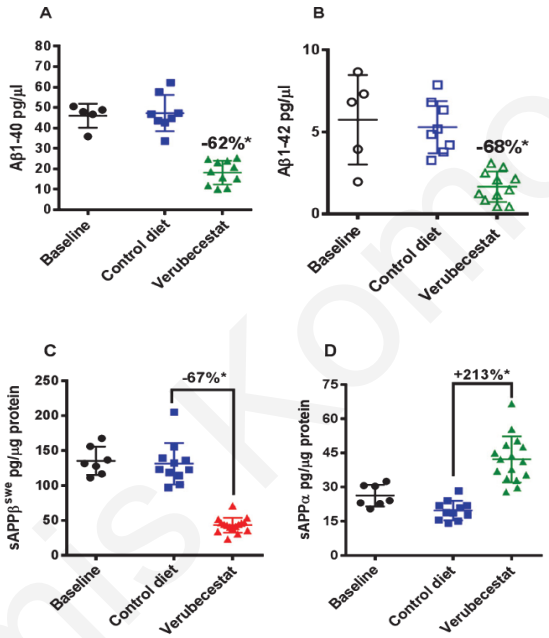

Figure 15 CSF Aβ40, Aβ42, sAPPβ and sAPPγ in Tg2576 mice at baseline, after a 12-week treatment with control vehicle diet or verubecestat diet.

Adapted from: Villarreal et al., 2017

well as decreased number of plaques in verubecestat-treated animals in contrast with the control group (Figure 16Figure 15). Also, the reduced levels of ARIA in verubecestat-treated mice were associated with a significant decline of the accumulated CNS amyloid pathology and Aβ peptides in the brain of AD models. However, verubecestat failed in its phase III clinical trial to improve cognitive decline in mild-to-moderate AD patients due to side effects. Therefore, requires more

research for the development of smaller molecular inhibitors of BACE-1 as they could easily pass the BBB.

Beyond the previously mentioned inhibitors there are several other BACE1 inhibitors. Lanabecestat (AZD3293, AstraZeneca/Eli Lilly) it is considered as a BACE1 inhibitor that

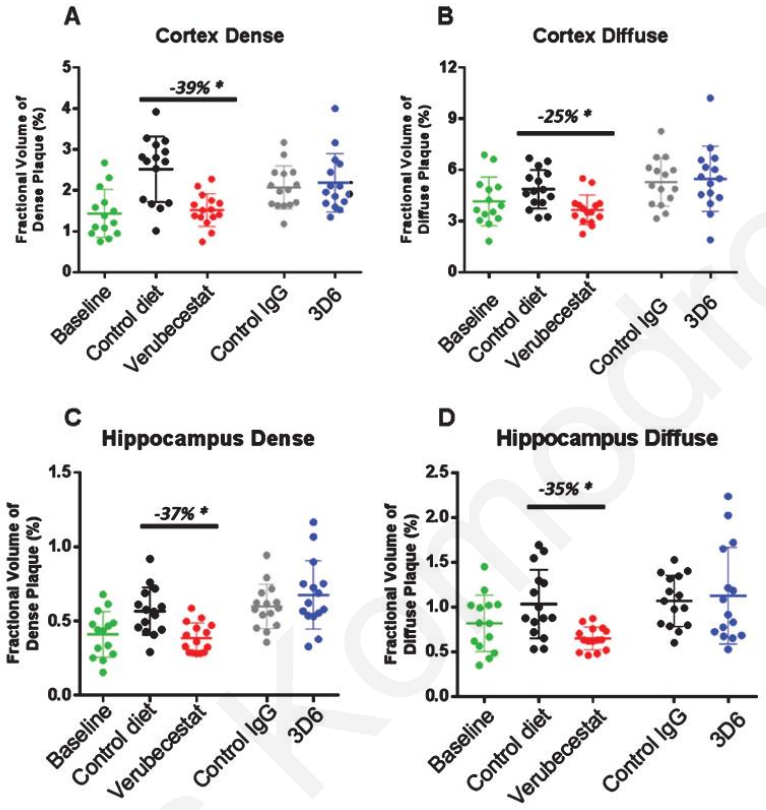

Figure 16 Stereological quantification of both dense and diffuse Aβ deposits in both cortex and hippocampus. (A),(B) Fractional volume of Aβ deposits of cortex and (C),(D) of hippocampus which were visually determined as dense or diffuse.

C-IgG, isotype control antibody-treated mice

Adapted from: Villarreal et al., 2017

declines CSF Aβ levels by up to 75%. However, on June 12, 2018, phase II/III trials of these drug were discontinued because of its low efficacy. Additionally, Atabecestat (JNJ-54861911, Janssen) was found in its phase I trial to induce a significant reduction in Aβ levels by up to 95%. Although due to its high efficacy, on May 17, 2018 was discontinued. Umibecestat, it is also a small-molecule inhibitor of aspartyl protease and BACE-1 which is ingested orally. These inhibitor was developed to interact with the amyloid cascade to inhibit Aβ production. Additionally, the umibecestat was also discontinued in phase II/III trials on July 11, 2019 due to worsening of the

cognitive function. The last BACE1 inhibitor, Elenbecestat (E2609, Eisai), was tested in prodromal or mild AD patients where it was reported reduced CSF Aβ levels in a dose-dependent manner by up to 80% (Wolfe, 2008).

2.3.2 Inhibition of γ-secretase

Another treatment strategy is the inhibition of γ-secretase. As mentioned previously, γ-secretase is an enzyme necessary for the final proteolytic cleavage of APP, resulting to the generation of Aβ peptides (Dovey et al., 2001). γ-secretase involves also in the cleavage of Notch. The way that γ-secretase disrupts the AD pathogenesis is highly connected with its involvement in Notch signaling pathway (van Es et al., 2005). Notch receptor involves in crucial events of cellular growth and development. The disruption of Notch pathway may result to intolerable toxic reactions linked to gastric, lymphatic, skin, and immune system (Grill and Cummings, 2010). Thus, it is important to regulate these events while developing γ-secretase inhibitors. Semagacestat (Henley et al., 2009) and DAPT (Morohashi et al., 2006) are two of the γ-secretase inhibitors which are into the late phase of clinical trials. Although those two inhibitors failed due to severe toxic reactions (Kelleher and Shen, 2010). These adverse events were induced due to Notch signaling pathway disruption thus, the new therapeutic strategy is termed as Notch sparing modulator of γ-secretase (Mayer et al., 2008). Further, sulfones and sulfonamide derivatives have been suggested to possess better performance than straight inhibitors. The major conflict in developing a novel therapeutic strategy of γ-secretase inhibitor is the lack of crystal structure availability (Holloway et al., 2009).

In addition, stabilizers of γ-secretase like chaperones requires further investigation. Chaperones have the ability to stabilize the interaction between enzyme and amyloid peptides leading to the production of less toxic shorter fragments (Andrew et al., 2016). Moreover, there is a need to design γ-secretase inhibitors with potential intracellular effects. Overall, spectral characterization methods would be necessary to measure the structure of γ-secretase with high resolution and precision.

2.3.3 Anti-aggregation drugs

Preventing Aβ oligomerisation is considered as a potential therapeutic strategy for AD. Aβ monomers are involved in the generation of oligomers thus, causing the production of severe toxic reactions in neuronal cells. A great interest was turned to the designing of smaller molecules that

interact with Aβ-Aβ interactions and preventig their aggregation (Brahmachari et al., 2017; Choi et al., 2010). To date researchers failed to develop a potential aggregation inhibitor. An example of these type of inhibitor is Homotaurine, a synthetic organic compound which binds to the Aβ protein preventing the generation of neurotoxic aggregates (Aisen et al., 2004). Further, there are some other potential clinical candidates such as inositol and cyclohexanol isomers, polypeptide colostrinin (Coman & Nemeş, 2017; Lannfelt et al., 2008) and polyphenolic compounds (Wang et al., 2008). Additionally, Tramiprosate, which is a glycosaminoglycan, has the ability to bind on monomeric Aβ peptides preventing both oligomerization and aggregation of Aβ. A second drug developed for its anti-oligomerization properties, the ELND005 (Scyllo-Inositol) effectively exhibited decreased insoluble Aβ oligomers and reversed cognitive decline in transgenic mice. Further, the Colostrinin (CLN) or proline rich polypeptide complex has strong immunoregulatory properties and affects the ability of learning, memory and cognitive functioning. More specific, the action of colostrinin is via the inhibition of Aβ aggregation and dissolve the pre-formed fibrils (Coman & Nemeş, 2017).

2.3.4 Increase of Aβ clearance

The enhance of Aβ clearance is another therapeutic strategy of AD. Aβ is degraded by various enzymes, including neprilysin which has also been considered for drug development (El-Amouri et al., 2008). Natura endopeptidase neprilysin mediate the degradation and clearance of Aβ peptides (Shirotani et al., 2001). Moreover, some other enzymes which act in the same way is; angiotensin-converting enzyme (ACE) (Hu et al., 2001), insulin dependent enzyme (IDE) (Farris et al., 2003), matrix metalloproteinase-9 (MMP-9) (Backstrom et al., 1996), and plasmin (Melchor et al., 2003). Further, RAGE (Receptor for advanced glycation end product) is an enzyme responsible for the Aβ accumulation in neurons (Deane et al., 2003). Thus, apart from the previous mentioned enzymes a potential target is an inhibitor of RAGE signaling which could block its damaging effects (Cai et al., 2016).

2.3.5 anti-Aβ immunotherapy

A great attention has been placed to design anti-Aβ immunotherapeutic agents as a reasonable treatment of AD. These therapeutic approach of AD is classified into two categories: active immunization (the use of synthetic Aβ42 or fragments of Aβ42, Aβ vaccination) or passive immunization (the use of monoclonal antibodies against Aβ). Immunization techniques are

successful to prevent the generation of both senile plaques and degradation/clearance of Aβ from neurons (Coman & Nemeş, 2017). According to studies Aβ vaccination could result to reduction of NFTs formation which is linked to tauopathy (Dai et al., 2015). However, Aβ antibodies act through various mechanisms such as mediating the phagocytic response by microglial cells (Weiner and Frenkel, 2006), resolve Aβ through interaction with them, producing specific conditions for the clearance of Aβ peptide (DeMattos et al., 2001), bound to Aβ, and block their toxicity (Lannfelt et al., 2014).

Aβ acts as a proinflammatory agent, mediating the activation of many inflammatory components. In the early stages of AD progression, those components may have a protective role (anti-neuroinflammatory) to clear the Aβ and release nerve growth factors. Nevertheless, in the case of over-accumulation either Aβ or other toxic products, proinflammatory agents are activated leading to the damage of neurons (Hamelin et al., 2016). Lymphatic system and microglia are the two major biological clearance systems responsible for the removal of undesirable toxins from the brain (Tarasoff-Conway et al., 2015). The difficulty in these case is the understanding of problems linked with the brain's immune system and it is unclear whether these immune cells are either underactive or overactive.

AN-1792 was the first reported active immunotherapy approach for AD which consists of synthetic full-length Aβ peptide. However, due to serious signs of CNS inflammation in 2002, was discontinued in its phase II trial (Orgogozo et al., 2003). 6% of the participants of these trial developed aseptic meningoencephalitis, brain iron deposits in choroid plexus, and cerebral microbleeds as side effects (Joseph-Mathurin et al., 2013). These events of brain immune system failure, for the clearance of toxic proteins, is a major contributor of dementia and cognitive dysfunctionalities (Jian et al., 2018).

The amyloid based immunotherapy, is a therapeutic strategy which includes the vaccination of the individuals with Aβ oligomers. Thus, triggers the immune response which causes the inhibition of Aβ oligomers aggregation and its clearance. The use of an anti-inflammatory approach is based on the neuroinflammation during the pathology of AD (Rogers et al., 1996). According to evidence non-steroidal anti-inflammatory drugs (NSAIDs) may be a way of prevention or a strategy to delay the onset of the disorder (Moore & O' Banion, 2002). Further, anti-inflammatory drugs can effect also the cleavage of APP through the action of γ-secretase

(Weggen et al., 2001). Future research have to focus on Aβ vaccination which mediate the activation of glial cells in order to avoid the growth of neuritic plaques.

Tailor-made vaccines seem to be a great improvement for the personalized treatment of each AD patient. Until now have been designed several monoclonal antibodies which are considered in the passive immunotherapy strategy that slows down, in a dose-dependent way, the cognitive decline in patients. Some of them have made it to proceed to the final stage, but then failed. In 2019, five drug trials were done using monoclonal antibody (mAbs) which targets Aβ; crenezumab, gantenerumab from Roche (Ostrowitzki et al., 2017), solanezumab, aducanumab, from Eli Lilly, bapineuzumab from Pfizer (Doody et al., 2014; Lasser et al., 2016; Moreth et al., 2013; Sevigny et al., 2016) and one trial with a combination of gantenerumab and solanezumab. In all clinical studies of vaccines, the participants were only AD patients with brain Aβ accumulation and were in the earliest stages of AD (absent or subtle memory loss). According to the results was found that only 0.1% of those antibodies circulating in the bloodstream can pass the BBB. Thus, remained in the bloodstream without reaching the target in sufficient quantities (Zhang et al., 2005).

Crenezumab is a humanized monoclonal antibody (IgG4) with high affinity for Aβ monomers, oligomers and fibrils (Vos et al., 2013). These drug failed in its phase III trials, on January 30, 2019. Additionally, Gantenerumab, also a human recombinant monoclonal IgG1 Ab which binds to amino-terminal and central regions of large, soluble Aβ (Logovinsky et al., 2016; Piton et al., 2018). It was reported that these drug had higher affinities for Aβ oligomers and fibrils than Aβ monomers (Bohrmann et al., 2012). During the study researchers evaluated monthly subcutaneous injections of these drug in mild AD patients. Also, Gantenerumab was reported to activate the microglia-mediated phagocytic clearance of neurotic plaques. However, it was terminated in phase III trial (Ostrowitzki et al., 2017). Last, Bapineuzumab, the first monoclonal antibody, was used for passive immunotherapy to target Aβ. Further trials of this drug were also discontinued after the first two trials were successfully completed and reported no treatment effect on cognitive or functional outcomes (Salloway et al., 2014).

The pre-mentioned antibody solanezumab, is a humanized IgG1 monoclonal antibody that has as a target the central region of Aβ. In its phase III trials, enrolled patients with mild to moderate Alzheimer's Disease (Honig et al., 2018). To those participants was transferred intravenous solanezumab infusions. According the results, solanezumab caused worsened

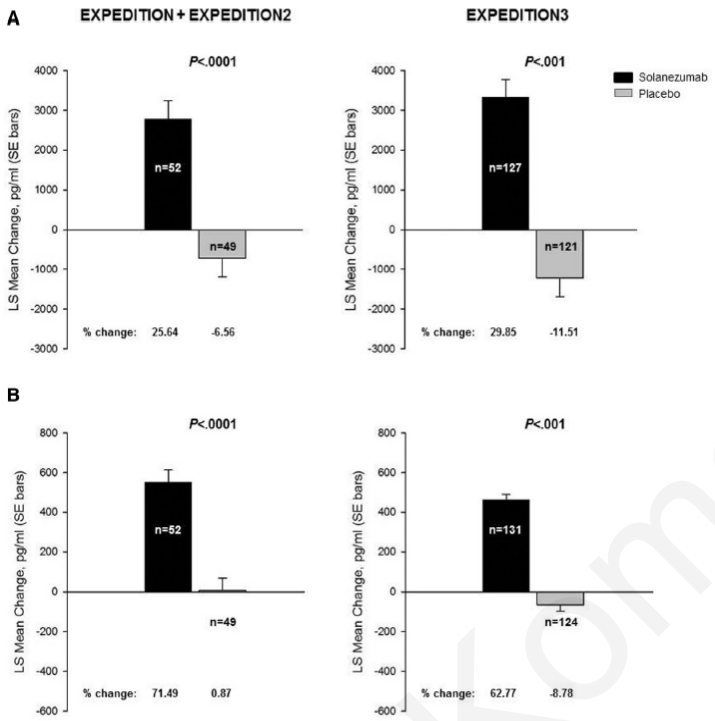

Figure 17 Changes of Aβ40 (A) and Aβ42 (B) total concentrations from three different EXPEDITION studies.

cognition and many side effects. Also, according to Florbetapir PET analysis revealed that was lack of reduction in brain amyloid aggregates (Doody et al., 2014). Willis et al., also reported the CSF concentration of Aβ in patients with mild AD. They considered into their research three studies with AD patients. EXPEDITION3 was a double-blind, placebo-controlled, phase III study with the participation of 2129 AD patients with mild dementia, with a florbetapir PET scan or CSF result which reports the presence of amyloid pathology at screening. Florbetapir PET scan is a method designed specifically for AD diagnosis which contains the radionuclide fluorine-18. These radionuclide binds to Aβ, thus can identify the presence of Aβ deposits in the brains of AD patients. EXPEDITION and EXPEDITION2 studies were designed in the same way, double-blind, placebo-controlled, phase III studies with 2052 mild to moderate AD patients with dementia. However, in those two studies did not have any objective biomarker-based evidence of amyloid deposits. According to their observations, solanezumab resulted in a significant increase in the total levels of CSF Aβ isoforms (Figure 17, Figure 18). Additionally, they identified low penetration of the drug

into the CNS. Last, they demonstrated that even higher exposure to solanezumab increased CSF total Aβ levels.

Aducanumab (Biogen Idec), a monoclonal Ab, has as a target the aggregated Aβ species and binds to parenchymal over vascular amyloid. In 2017 and 2018, a clinical trial was started

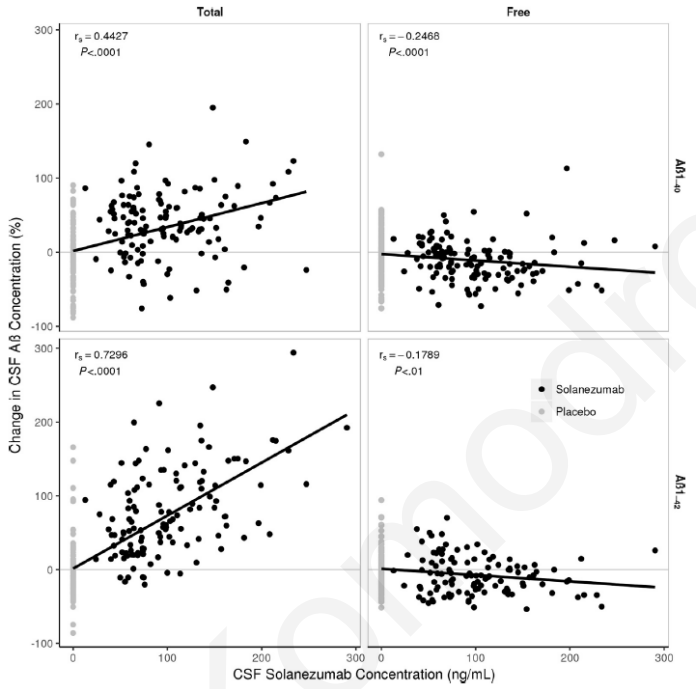

Figure 18 Linear regression analyses of CSF solanezumab concentrations compared to the total and free Aβ40 and Aβ42. The top left panel determines the Aβ40 total levels; The top right panel illustrated the free Aβ;The bottom left panel are the Aβ42 total levels and the bottom right panel represents the free Aβ42.

with the use of these recombinant, human Anti-Aβ IgG1 monoclonal antibody. In these study the participants were patients of prodromal or mild AD in order to evaluate the safety, pharmacokinetics and tolerability of these drug in aged patients (50-90 year old) with a positive amyloid PET scan. According to evidence, amyloid deposition was eliminated in all treatment groups, in a dose- and time-dependent manner (Figure 2019, 20). They also reported a slowing cognitive decline.

Despite its significant reduction on Aβ deposition, Biogen and Eisai on March 21, 2019 announced the termination of the clinical trial at phase III. The clinical trial ended due to a futility analysis which supported that these trials will not achieve the deceleration of AD progress which

was the primary endpoint. Together, we conclude that all these failures of these trials of antibody approaches highlights the theory that amyloid plaques are not an actual mechanistic cause of AD.

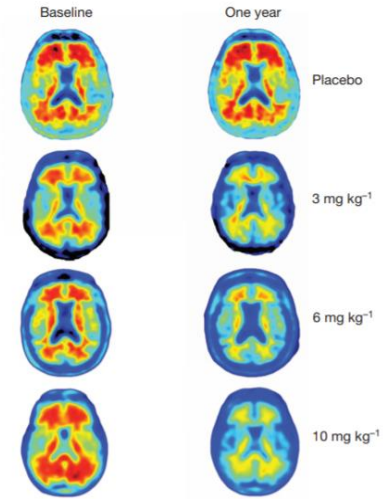

Figure 19 Pet images illustrating the reduction of Amyloid plaques after treatment with aducanumab. Those images are from the baseline until the 54 week of therapy. Axial slice represents anatomical regions in the posterior part of the brain with is related to AD pathology.

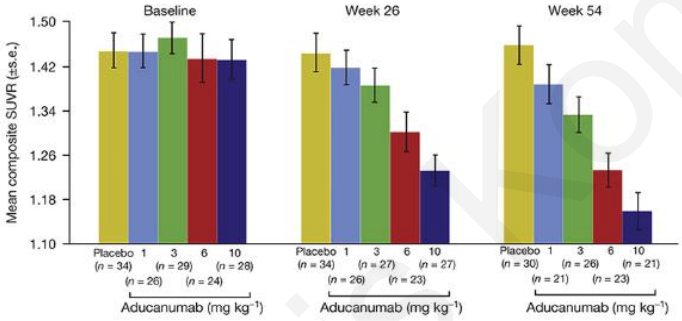

Figure 19 SUVR values at the baseline and treatment at week 26 and 54.

2.4 The Cholinergic Hypothesis and Aβ

Cholinergic hypothesis is the oldest statement of AD causation. These hypothesis proposes that AD is caused by a reduction of ACh, a neurotransmitter which plays a key role in learning and memory (**Figure 21**). When a nerve impulse reaches the pre-synaptic terminal it stimulates the release of the neurotransmitter ACh into the cholinergic synapse. Then ACh diffuses and cross the synapse to the presynaptic nerve terminal and binds to receptors embedded in the membrane of the postsynaptic nerve terminal. The binding of ACh to receptors on the post-synaptic neuron re-initiates nerve impulse. Finally, acetylcholinesterase anchored to the membrane of postsynaptic

nerve terminal hydrolyses acetocholine to acetate and choline resulting in the termination of the nerve impulse at the synapse (Hampel et al., 2017).

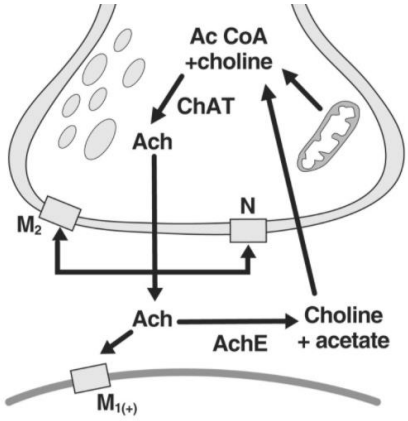

Figure 20 Synthesis, release and reuptake of acetylcholine.

Adapted from: (Hampel et al., 2017)

Cholinergic synapses are altered by Aβ oligomers neurotoxicity leading to synaptic loss which is correlated with cognitive impairment. During AD, ChAT transcription in cholinergic neurons is reduced which leads to decreased ChAT activity and progression of neurodegeneration (Ferreira-Vieira et al., 2016). This event of cholinergic alterations during AD involves with the impaired attention and memory loss of the patient. It is also known, that AChE interacts with Aβ mediating the formation of amyloid fibrils.

According to studies, the cortical cholinergic deficit begins at the early stage of AD (Mormino et al., 2008). Also, in vitro studies have shown that M1 muscarinic receptor activation leads to changes in the cleavage process of the β-APP that favor decreased production of Aβ (Davis et al., 2010). This effect has been confirmed in vivo, in both animals and humans. However, reduction in the activation of M1 receptors results to high expression of βAPP and increased amyloidogenic cleavage or high aggregation of Aβ (Davis et al., 2010). Further, have been demonstrated that M1 receptor becomes uncoupled from its G-protein in AD. However, the coupling of M1 receptors to G-proteins has not been quantitatively demonstrated in preclinical AD.

Potter et la., sought to determine if the cortical cholinergic dysfunction begins at preclinical stages of AD. Their results are the first report of a preclinical alteration of post-synaptic function in AD (Potter et al., 2011). Thus, in their research the participants were separated into five groups. The young control (YG) group, the group of participants with no plaques (NPL), the group of sparse

plaques (SPL), the group of participants with many plaques and the group of AD patients. The groups were categorized according to their percentages of apoE-ε4 allele. The MPL and AD groups had the higher percentages. First, they tested the cortical ChAT activity in all groups where they observed a progressive reduction in ChAT in non-demented elderly with plaques. The ChAT activity loss was proportionate to the plaque density (Figure 22).

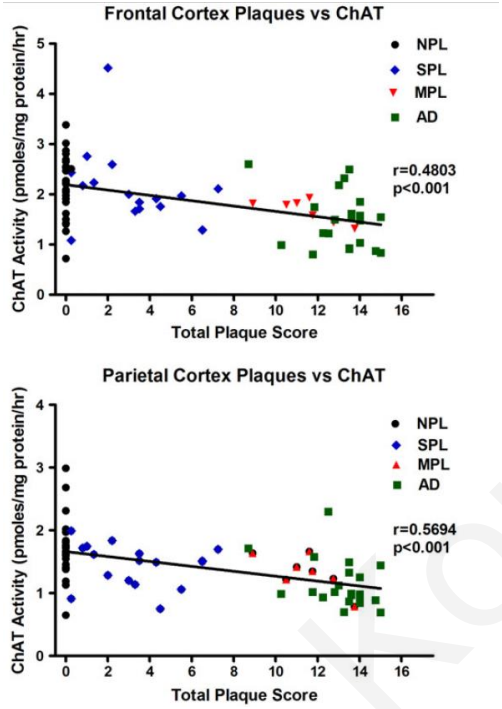

Figure 21 Correlation of ChAT activity and plaque density.

Adapted from: Potter et al., 2011

They have also identified that the coupling of M1 receptors to G-proteins is altered in non-demented elderly with plaques, than that in non-demented elderly without plaques. Thus, they concluded that cortical cholinergic dysfunctions and degeneration begins at the early stages of AD in parallel with the aggregation of cortical amyloid plaques but before the onset of dementia. To sum up, they reported that non-demented elderly group with plaques have tangles, plaques, cholinergic deficit and high prevalence of the apoE-e4-allele. According to other studies, even a small increase in the number of M1 muscarinic receptors, measures of M1 receptor signal transduction-related markers or function are reduced or impaired in AD (Tsang et al., 2007). However, it is still unclear the cause of receptor uncoupling in the presence of plaques. A hypotheses, is that could be a result from high concentrations of soluble Aβ. Reports in animals have illustrated that Aβ exposure can result to M1 receptor uncoupling (Kelly et al., 2005). To sum up, the early identification of cortical cholinergic deficit in AD illustrates that could have a causative or accelerating effect on AD pathogenesis. Thus it could be an important therapeutic target for AD prevention.

3. Discussion

Here was demonstrated the pathophysiology and the progression of AD and an overview into the potential and current therapeutic targets. Further, was reported some aspects of APP biology, processing, trafficking of APP. The crucial role of APP and its importance in AD relies in its role as the precursor protein of Aβ peptide, a central player of amyloid hypothesis. Beyond this APP function, APP has several other biological activities involving in neuronal function and development.

Over the years pharmaceutical companies focused on the amyloid hypothesis which has been the mainstream explanation for AD pathogenesis. Thus, many drugs were developed and proceeded to clinical trials on amyloid reduction but all of them failed. Figure 23 demonstrate all of the drugs were used in clinical trials for the anti-amyloid therapy since 2016. These table highlight the mechanism of action for each drug and the reason of failure according to their reports on different patient groups. Therefore, the amyloid hypothesis may not be strong enough and researchers emphasized on other hypothesis of AD pathogenesis. Thus, the leading point was the number of trials decreased in 2019.

Year	Drug	Mechanism of action	Participants	Main reasons for failure	Remarks
2016	Solanezumab	Monoclonal antibody	Mild AD	Lack of efficacy	
	Solanezumab	Monoclonal antibody	Prodromal AD	Strategic	
	Verubecestat	BACE inhibitor	Mild to moderate AD	Lack of efficacy	
2018	Verubecestat	BACE inhibitor	Prodromal AD	Lack of efficacy	Worsens cognition
	Atabecestat	BACE inhibitor	Preclinical AD	Toxicity	Worsens cognition
	Lanabecestat	BACE inhibitor	Early AD	Lack of efficacy	Worsens cognition
	Lanabecestat	BACE inhibitor	Mild AD	Lack of efficacy	Worsens cognition
2019	Aducanumab	Monoclonal antibody	Early AD	Lack of efficacy	
	CNP520	BACE inhibitor	Preclinical AD	Lack of efficacy	Worsens cognition

Figure 22 A sum up, of the failed clinical trials on anti-amyloid therapy since 2019.

However, an in-depth and comprehensive understanding of the mechanistic contribution not only for Aβ but also other factors of AD is crucial for designing novel pharmacotherapies. In 2019, nine phase III trials for eight drugs developed targeting the amyloid protein. The two trials enrolled patients with preclinical Alzheimer's Disease; however only one clinical trial required positive amyloid PET scanning for its participants, and the other required a genetic mutation or strong genetic background. Additionally, four trials enrolled individuals with prodromal AD with positive biomarkers, also one trial the enrolled prodromal and mild AD patients. Last two trials

enrolled patients with mild to moderate stages AD with dementia. Also, the criteria for these trials were positive results in amyloid PET or cerebrospinal fluid (CSF) biomarker thus was an evidence of early AD. Until now, no drug trials have enrolled patients with advanced AD, which highlights that anti-amyloid therapy is not efficient in late stage AD patients. However, an innovative therapeutic approach is the development of disease-modifying agents for AD. All the drugs developed so far focused on the Aβ deposition because it is an event that could occur at the very beginning of AD clinical symptoms. Thus, researchers, do not prefer later stages of AD because it may be too late, the brain has already been damaged by Aβ protein and most of the processes have been initiated and are irreversible. Overall, as mentioned before many BACE inhibitor trials have demonstrated that even patients who receive treatment have worse cognitive impairment. Moreover, some evidence support that AD patients with Aβ plaques in their brains even if they were virtually cleared by an anti-Aβ immunotherapy they did not show any cognitive improvement benefit. However, needs further investigation to find out if it is beneficial the decreasing of amyloid load in the brain. Some further targets for drug development is to take into account the concept that Aβ oligomers might alter the neuronal function by causing synaptic impairment through the inducing mitochondrial dysregulation and affecting microglia. For example, a small molecule antagonist of the receptor for glycation products, as a potential strategy of slowing cognition function in mild Alzheimer's disease. The other suggestion after the anti-amyloid trials is the need for further basic research focused on the molecular structure, metabolism, immune responses, and amyloid toxicity.

So, we hope for novel treatments to target the root of the disease process and stop the progressive accumulation of Aβ. The failure of clinical trails focusing to Aβ peptides may be due to failure of compound, of experimental procedures, failure to include efficient biomarkers, incomplete reporting of the data during the trial, population heterogeneity, inappropriate dosage. Also can be attributed to the incomplete understanding of the pharmacokinetics and bioavailability of drugs developed until now.

For many years AD theories and research focused on amyloid plaques and tangles an increasing body of evidence points to the cholinergic system which plays an important role in memory and attention. The loss of cholinergic neurons identified in AD patients is an important factor contributing to AD memory deficit. Currently there are only four drugs that have been

approved by the US Food and Drug for treating AD patients. This drugs includes cholinesterase inhibitors (AChEIs) – tacrine, donepezil, galantamine, and rivastigmine – and memantine, an NMDA receptor antagonist. None of them are disease-modifying drugs they are used to relief the patient for AD symptoms. The idea to replenish lost neurons or avoid neuronal death can be considered as potential therapeutics to modify AD. According to this idea, new drugs are being developed to achieve the elimination of Aβ production or increase trophic factors. Therapies like this have the potential to prevent cholinergic neuronal death and the loss of cortical and hippocampal neurons.

The need for drug development nowadays is greater due to the seriousness of AD and the continual increase number of patients. Despite the complexity of AD existing insights are important for launching disease-modifying treatments for AD. Advances in cellular biology and genetics are required to enhance our understanding of both pathogenesis and the various mechanisms that must be targeted for the efficient development of treatment. A great contribution will be the determination not only the structure but also the function and location of toxic Aβ species and tau protein. Also, it is an urgent need the identify several biomarkers of Aβ and tau protein. Thus, it will be easier for us to find their presence and their production, accumulation, or their actions.

Bibliography

1. Ando K, Iijima K, Elliott J, Kirino Y and Suzuki T (2001) Phosphorylation-dependent Regulation of the Interaction of Amyloid Precursor Protein with Fe65 Affects the Production of β-Amyloid. Journal of Biological Chemistry. 276 (43), 40353-40361.
2. Arendt, T., Holzer, M., Großmann, A., Zedlick, D. and 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), pp.5-18.
3. Arun K, G. and Brindisi, M., 2012. Developing β-secretase inhibitors for treatment of Alzheimer's disease. Journal of Neurochemistry.
4. Avramopoulos D (2009) Genetics of Alzheimer's disease: recent advances. Genome Medicine. 1 (3), 34.
5. Ballatore C, Lee V and Trojanowski J (2007) Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. Nature Reviews Neuroscience. 8 (9), 663-672.
6. Balmanno, K. and Cook, S., 1999. Sustained MAP kinase activation is required for the expression of cyclin D1, p21Cip1 and a subset of AP-1 proteins in CCL39 cells. Oncogene, 18(20), pp.3085-3097.
7. Blessed, G., Tomlinson, B. and Roth, M., 1968. The Association Between Quantitative Measures of Dementia and of Senile Change in the Cerebral Grey Matter of Elderly Subjects. British Journal of Psychiatry, 114(512), pp.797-811.
8. Bohrmann B, Baumann K, Benz J, Gerber F, Huber W, Knoflach F, Messer J, Oroszlan K, Rauchenberger R, Richter W, Rothe C, Urban M, Bardroff M, Winter M, Nordstedt C and Loetscher H (2012) Gantenerumab: A Novel Human Anti-Aβ Antibody Demonstrates Sustained Cerebral Amyloid-β Binding and Elicits Cell-Mediated Removal of Human Amyloid-β. Journal of Alzheimer's Disease. 28 (1), 49-69.
9. Braak E, Griffing K, Arai K, Bohl J, Bratzke H and Braak H (1999) Neuropathology of Alzheimer’s disease: what is new since A. Alzheimer?. European Archives of Psychiatry and Clinical Neurosciences. 249 (S3), S14-S22.
10. Brunden K, Trojanowski J and Lee V (2009) Advances in tau-focused drug discovery for Alzheimer's disease and related tauopathies. Nature Reviews Drug Discovery. 8 (10), 783-793.

11. Buée L, Troquier L, Burnouf S, Belarbi K, Van der Jeugd A, Ahmed T, Fernandez-Gomez F, Caillierez R, Grosjean M, Begard S, Barbot B, Demeyer D, Obriot H, Brion I, Buée-Scherrer V, Maurage C, Balschun D, D'Hooge R, Hamdane M, Blum D and Sergeant N (2010) From tau phosphorylation to tau aggregation: what about neuronal death?. Biochemical Society Transactions. 38 (4), 967-972.
12. Busciglio, J., Lorenzo, A., Yeh, J. and Yankner, B., 1995. β-Amyloid fibrils induce tau phosphorylation and loss of microtubule binding. Neuron, 14(4), pp.879-888.
13. Busser, J., Geldmacher, D. and Herrup, K., 1998. Ectopic Cell Cycle Proteins Predict the Sites of Neuronal Cell Death in Alzheimer's Disease Brain. The Journal of Neuroscience, 18(8), pp.2801-2807.
14. Cai X, Golde T and Younkin S (1993) Release of excess amyloid beta protein from a mutant amyloid beta protein precursor. Science. 259 (5094), 514-516.
15. Castro A, Pinheiro P, Pinheiro M and Tamanini I (2010) Applied hybrid model in the neuropsychological diagnosis of the Alzheimer's disease: a decision making study case. International Journal of Social and Humanistic Computing. 1 (3), 331.
16. Chang, K., Kim, H., Ha, T., Ha, J., Shin, K., Jeong, Y., Lee, J., Park, C., Kim, S., Baik, T. and Suh, Y., 2006. Phosphorylation of Amyloid Precursor Protein (APP) at Thr668 Regulates the Nuclear Translocation of the APP Intracellular Domain and Induces Neurodegeneration. Molecular and Cellular Biology, 26(11), pp.4327-4338.
17. Chaput, D., Kirouac, L., Bell-Temin, H., Stevens, S. and Padmanabhan, J., 2012. SILAC-based proteomic analysis to investigate the impact of amyloid precursor protein expression in neuronal-like B103 cells. ELECTROPHORESIS, 33(24), pp.3728-3737.
18. Citron M, Oltersdorf T, Haass C, McConlogue L, Hung A, Seubert P, Vigo-Pelfrey C, Lieberburg I and Selkoe D (1992) Mutation of the β-amyloid precursor protein in familial Alzheimer's disease increases β-protein production. Nature. 360 (6405), 672-674.
19. Cornutiu G (2015) The Epidemiological Scale of Alzheimer's Disease. Journal of Clinical Medicine Research. 7 (9), 657-666.
20. Davies P (1976) Selective loss of central cholinergic neurons in Alzheimer's Disease. The Lancet. 308 (8000), 1403.
21. De Strooper, B. and Karran, E., 2016. The Cellular Phase of Alzheimer's Disease. Cell, 164(4), pp.603-615.

22. Dodart, J., Mathis, C., Saura, J., Paul, S. and Ungerer, A., 2000. Neuroanatomical abnormalities in behaviorally characterized APP(V717F) transgenic mice. Neurobiology of Disease, 7(2), pp.71-85.
23. Doody R, Thomas R, Farlow M, Iwatsubo T, Vellas B, Joffe S, Kieburtz K, Raman R, Sun X, Aisen P, Siemers E, Liu-Seifert H and Mohs R (2014) Phase 3 Trials of Solanezumab for Mild-to-Moderate Alzheimer's Disease. New England Journal of Medicine. 370 (4), 311-321.
24. Ebneth A, Godemann R, Stamer K, Illenberger S, Trinczek B, Mandelkow E and Mandelkow E (1998) Overexpression of Tau Protein Inhibits Kinesin-dependent Trafficking of Vesicles, Mitochondria, and Endoplasmic Reticulum: Implications for Alzheimer's Disease. Journal of Cell Biology. 143 (3), 777-794
25. El-Amouri S, Zhu H, Yu J, Marr R, Verma I and Kindy M (2008) Neprilysin: An Enzyme Candidate to Slow the Progression of Alzheimer's Disease. The American Journal of Pathology. 172 (5), 1342-1354.
26. Farzan, M., Schnitzler, C., Vasilieva, N., Leung, D. and Choe, H., 2000. BACE2, a beta - secretase homolog, cleaves at the beta site and within the amyloid-beta region of the amyloid-beta precursor protein. Proceedings of the National Academy of Sciences, 97(17), pp.9712-9717.
27. Ferrer, I., Blanco, R., Carmona, M., Ribera, R., Goutan, E., Puig, B., Rey, M., Cardozo, A., Viñals, F. and Ribalta, T., 2006. Phosphorylated Map Kinase (ERK1, ERK2) Expression is Associated with Early Tau Deposition in Neurones and Glial Cells, but not with Increased Nuclear DNA Vulnerability and Cell Death, in Alzheimer Disease, Pick's Disease, Progressive Supranuclear Palsy an. Brain Pathology, 11(2), pp.144-158.
28. Fotiou D, Kaltsatou A, Tsiptsios D and Nakou M (2015) Evaluation of the cholinergic hypothesis in Alzheimer’s disease with neuropsychological methods. Aging Clinical and Experimental Research. 27 (5), 727-733.
29. Games, D., Adams, D., Allessandrini, R., Barbour, R., Berthelette, P., Blackwell, C., Carr, T., Clemens, J., Donaldson, T. and Gillespie, F., 1995. Alzheimer-type neuropathology in transgenic mice overexpressing V717F beta-amyloid precursor protein. Nature, 373(6514), pp.523-7.

30. Gärtner, U., Holzer, M. and Arendt, T., 1999. Elevated expression of p21ras is an early event in Alzheimer's disease and precedes neurofibrillary degeneration. Neuroscience, 91(1), pp.1-5.
31. Gärtner, U., Holzer, M., Heumann, R. and Arendt, T., 1995. Induction of p21ras in Alzheimer pathology. NeuroReport, 6(10), pp.1441-1444.
32. Glenner, G. and Wong, C., 1984. Alzheimer's disease and Down's syndrome: Sharing of a unique cerebrovascular amyloid fibril protein. Biochemical and Biophysical Research Communications, 122(3), pp.1131-1135.
33. Goedert M, Klug A and Crowther R (2006) Tau protein, the paired helical filament and Alzheimer's disease. Journal of Alzheimer's Disease. 9 (s3), 195-207.
34. Golde, T., 2014. Open questions for Alzheimer's disease immunotherapy. Alzheimer Res, 6(1), p.3.
35. Gotz, J., 2001. Formation of Neurofibrillary Tangles in P301L Tau Transgenic Mice Induced by Abeta 42 Fibrils. Science, 293(5534), pp.1491-1495.
36. Guela, C., Wu, C., Saroff, D., Lorenzo, A., Yuan, M. and Yankner, B., 1998. Aging renders the brain vulnerable to amyloid β-protein neurotoxicity. Nature Medicine, 4(7), pp.827-831.
37. H. Ferreira-Vieira T, M. Guimaraes I, R. Silva F and M. Ribeiro F (2016) Alzheimer's disease: Targeting the Cholinergic System. Current Neuropharmacology. 14 (1), 101-115.
38. Habtemariam (2019) Natural Products in Alzheimer's Disease Therapy: Would Old Therapeutic Approaches Fix the Broken Promise of Modern Medicines?. Molecules. 24 (8), 1519.
39. Hamelin L, Lagarde J, Dorothée G, Leroy C, Labit M, Comley R, de Souza L, Corne H, Dauphinot L, Bertoux M, Dubois B, Gervais P, Colliot O, Potier M, Bottlaender M and Sarazin M (2016) Early and protective microglial activation in Alzheimer's disease: a prospective study using18F-DPA-714 PET imaging. Brain. 139 (4), 1252-1264.
40. Hampel, H., Mesulam, M., Cuello, A., Khachaturian, A., Farlow, M., Snyder, P., Giacobini, E. and Khachaturian, Z., 2017. WITHDRAWN: Revisiting the cholinergic hypothesis in Alzheimer's disease: Emerging evidence from translational and clinical research. Alzheimer's & Dementia,.

41. Hardy, J., 1997. Amyloid, the presenilins and Alzheimer's disease. Trends in Neurosciences, 20(4), pp.154-159.
42. Hardy J (2006) Alzheimer's disease: The amyloid cascade hypothesis: An update and reappraisal. Journal of Alzheimer's Disease. 9 (s3), 151-153.
43. Hardy J and Allsop D (1991) Amyloid deposition as the central event in the aetiology of Alzheimer's disease. Trends in Pharmacological Sciences. 12, 383-388.
44. Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., Yang, F. and Cole, G., 1996. Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. Science, 274(5284), pp.99-102.
45. Hyman, B., Reiter, J., Moss, M., Rosene, D. and Pandya, D., 1994. Extracellular signal-regulated kinase (MAP kinase) immunoreactivity in the rhesus monkey brain. Neuroscience Letters, 166(1), pp.113-116.
46. Irizzary, M., Soriano, F., McNamara, M., Page, K., Schenk, D., Games, D. and Hyman, B., 1997. Abeta deposition is associated with neurophil changes, but not with overt neuronal loss in the human amyloid precursor protein V717F (PDAPP) transgenic mouse. Neuroscience, 17(18), pp.7053-7059.
47. Jouanne, M., Rault, S. and Voisin-Chiret, A., 2017. Tau protein aggregation in Alzheimer's disease: An attractive target for the development of novel therapeutic agents. European Journal of Medicinal Chemistry, 139, pp.153-167.1
48. Judge, M., Hornbeck, L., Potter, H. and Padmanabhan, J., 2011. Mitosis-specific phosphorylation of amyloid precursor protein at Threonine 668 leads to its altered processing and association with centrosomes. Molecular Neurodegeneration, 6(1), p.80.
49. Kedikian, G., Heredia, F., Salvador, V., Raimunda, D., Isoardi, N., Heredia, L. and Lorenzo, A., 2010. Secreted amyloid precursor protein and holo-APP bind amyloid β through distinct domains eliciting different toxic responses on hippocampal neurons. Journal of Neuroscience Research, p.NA-NA.
50. Kelly JF, Storie K, Skamra C, Bienias J, Beck T, Bennett DA (2005) Relationship between Alzheimer's disease clinical stage and Gq/11 in subcellular fractions of frontal cortex. J Neural Transm 112:1049–1056

51. Kirouac, L., Rajic, A., Cribbs, D. and Padmanabhan, J., 2017. Activation of Ras-ERK Signaling and GSK-3 by Amyloid Precursor Protein and Amyloid Beta Facilitates Neurodegeneration in Alzheimer's Disease. eneuro, 4(2), pp.ENEURO.0149-16.2017.
52. Klein, W., 2001. Targeting small Aβ oligomers: the solution to an Alzheimer's disease conundrum?. Trends in Neurosciences, 24(4), pp.219-224.
53. Kobayashi, S., Ishiguro, K., Omori, A., Takamatsu, M., Arioka, M., Imahori, K. and Uchida, T., 1993. A cdc2-related kinase PSSALRE/cdk5 is homologous with the 30 kDa subunit of tau protein kinase II, a proline-directed protein kinase associated with microtubule. FEBS Letters, 335(2), pp.171-175.
54. Kolarova M, García-Sierra F, Bartos A, Ricny J and Ripova D (2012) Structure and Pathology of Tau Protein in Alzheimer Disease. International Journal of Alzheimer's Disease. 2012, 1-13.
55. Lane R, Potkin S and Enz A (2005) Targeting acetylcholinesterase and butyrylcholinesterase in dementia. The International Journal of Neuropsychopharmacology. 9 (01), 101.
56. Le, W., Xie, W., Kong, R. and Appel, S., 2002. β-Amyloid-Induced Neurotoxicity of a Hybrid Septal Cell Line Associated with Increased Tau Phosphorylation and Expression of β-Amyloid Precursor Protein. Journal of Neurochemistry, 69(3), pp.978-985.
57. Lee, K., Moussa, C., Lee, Y., Sung, Y., Howell, B., Turner, R., Pak, D. and Hoe, H., 2010. Beta amyloid-independent role of amyloid precursor protein in generation and maintenance of dendritic spines. Elsevier, 169(11), pp.344-356.
58. Lee, M., Kao, S., Lemere, C., Xia, W., Tseng, H., Zhou, Y., Neve, R., Ahlijanian, M. and Tsai, L., 2003. APP processing is regulated by cytoplasmic phosphorylation. Journal of Cell Biology, 163(1), pp.83-95.
59. Leissring, M., Murphy, M., Mead, T., Akbari, Y., Sugarman, M., Jannatipour, M., Anliker, B., Muller, U., Saftig, P., Strooper, B., Wolfe, M., Golde, T. and LaFerla, F., 2002. A physiologic signaling role for the γ-secretase-derived intracellular fragment of APP. Proc. Natl. Acad. Sci. U. S. A., 99(7), pp.4697-702.
60. Lemere C, Blusztajn J, Yamaguchi H, Wisniewski T, Saido T and Selkoe D (1996) Sequence of Deposition of Heterogeneous Amyloid β-Peptides and APO E in Down

Syndrome: Implications for Initial Events in Amyloid Plaque Formation. Neurobiology of Disease. 3 (1), 16-32.

61. Lichtenthaler, S., Haass, C. and Steiner, H., 2011. Regulated intramembrane proteolysis - lessons from amyloid precursor protein processing. Journal of Neurochemistry, 117(5), pp.779-796.
62. Ling, Y., Morgan, K. and Kalsheker, N., 2003. Amyloid precursor protein (APP) and the biology of proteolytic processing: relevance to Alzheimer's disease. The International Journal of Biochemistry & Cell Biology, 35(11), pp.1505-1535.
63. Liu P, Xie Y, Meng X and Kang J (2019) Author Correction: History and progress of hypotheses and clinical trials for Alzheimer's disease. Signal Transduction and Targeted Therapy. 4 (1).
64. Lorenzo, A., Yuan, M., Zhang, Z., Paganetti, P., Sturchler-Pierrat, C., Staufenbiel, M., Mautino, J., Vigo, F., Sommer, B. and Yankner, B., 2000. Amyloid β interacts with the amyloid precursor protein: a potential toxic mechanism in Alzheimer's disease. Nature Neuroscience, 3(5), pp.460-464.
65. Mantile, F. and Prisco, A., 2020. Vaccination against β-Amyloid as a Strategy for the Prevention of Alzheimer's Disease. Biology, 9(12), p.425.
66. Masters, C., Bateman, R., Blennow, K., Rowe, C., Sperling, R. and Cummings, J., 2015. Alzheimer's disease. Nature Reviews Disease Primers, 1(1).
67. Medeiros R, Baglietto-Vargas D and LaFerla F (2010) The Role of Tau in Alzheimer's Disease and Related Disorders. CNS Neuroscience & Therapeutics. 17 (5), 514-524.
68. Merlini G and Bellotti V (2003) Molecular Mechanisms of Amyloidosis. New England Journal of Medicine. 349 (19), 1872-1873.
69. Moore, A. M., & O'Banion, M. K. (2002). Neuroinflammation and anti-inflammatory therapy for Alzheimer's disease. Advanced Drug Delivery Reviews, 54, 1627–1656.
70. Mormino, E., Kluth, J., Madison, C., Rabinovici, G., Baker, S., Miller, B., Koeppe, R., Mathis, C., Weiner, M. and Jagust, W., 2008. Episodic memory loss is related to hippocampal-mediated -amyloid deposition in elderly subjects. Brain, 132(5), pp.1310-1323.
71. Muller, U. and Zheng, H., 2011. Physiological Functions of APP Family Proteins. Cold Spring Harbor Perspectives in Medicine, 2(2), pp.a006288-a006288.

72. Muller U. C. and Zheng H. (2012) Physiological functions of APPfamily proteins. Cold Spring Harb. Perspect. Med. 2, a006288.
73. Munter, L., Botev, A., Richter, L., Hildebrand, P., Althoff, V., Weise, C., Kaden, D. and Multhaup, G., 2010. Aberrant Amyloid Precursor Protein (APP) Processing in Hereditary Forms of Alzheimer Disease Caused by APP Familial Alzheimer Disease Mutations Can Be Rescued by Mutations in the APP GxxxG Motif. Journal of Biological Chemistry, 285(28), pp.21636-21643.
74. Muresan, Z. and Muresan, V., 2005. Coordinated transport of phosphorylated amyloid-β precursor protein and c-Jun NH2-terminal kinase–interacting protein-1. Journal of Cell Biology, 171(4), pp.615-625.
75. Nishitomi, K., Sakaguchi, G., Horikoshi, Y., Sakaguchi, I., Minami, S., Nakajima, Y., Li, H., Takeyaman, C., Kihara, T., Ota, A., Wong, P., Aisen, P., Kato, A., Kinoshita, N. and Matsuoka, Y., 2006. BACE1 inhibition reduces endogenous Abeta and alters APP processing in wild-type mice. Journal of Neurochemistry, 99(6), pp.1555-63.
76. Nizzari, M., Thellung, S., Corsaro, A., Villa, V., Pagano, A., Porcile, C., Russo, C. and Florio, T., 2012. Neurodegeneration in Alzheimer Disease: Role of Amyloid Precursor Protein and Presenilin 1 Intracellular Signaling. Journal of Toxicology, 2012, pp.1-13.
77. Nizzari, M., Venezia, V., Repetto, E., Caorsi, V., Magrassi, R., Gagliani, M., Carlo, P., Florio, T., Schettini, G., Tacchetti, C., Russo, T., Diaspro, A. and Russo, C., 2007. Amyloid Precursor Protein and Presenilin1 Interact with the Adaptor GRB2 and Modulate ERK 1,2 Signaling. Journal of Biological Chemistry, 282(18), pp.13833-13844.
78. Nizynski B, Dzwolak W and Nieznanski K (2017) Amyloidogenesis of Tau protein. Protein Science. 26 (11), 2126-2150.
79. O'Brien, R. and Wong, P., 2011. Amyloid Precursor Protein Processing and Alzheimer's Disease. Annual Review of Neuroscience, 34(1), pp.185-204.
80. Ohno, M., Sametsky, E., Younkin, L., Oakley, H., Yunkin, S., Citron, M., Vassar, R. and Disterhoft, J., 2004. BACE1 deficiency rescues memory deficits and cholinergic dysfunction in a mouse model of Alzheimer's disease. Neuron, 41(1), pp.27-33.
81. Ostrowitzki S, Lasser R, Dorflinger E, Scheltens P, Barkhof F, Nikolcheva T, Ashford E, Retout S, Hofmann C, Delmar P, Klein G, Andjelkovic M, Dubois B, Boada M, Blennow

K, Santarelli L and Fontoura P (2017) A phase III randomized trial of gantenerumab in prodromal Alzheimer's disease. Alzheimer's Research & Therapy. 9 (1).

82. Perry E, Perry R, Blessed G and Tomlinson B (1977) Necropsy evidence of central cholinergic deficits in senile dementia. The Lancet. 309 (8004), 189.
83. Potter, P., Rauschkolb, P., Pandya, Y., Sue, L., Sabbagh, M., Walker, D. and Beach, T., 2011. Pre- and post-synaptic cortical cholinergic deficits are proportional to amyloid plaque presence and density at preclinical stages of Alzheimer's disease. Acta Neuropathol, 122(1), pp.49-60.
84. Raina, A., Monteiro, M., Mcshea, A. and Smith, M., 2001. The role of cell cycle-mediated events in Alzheimer's disease. International Journal of Experimental Pathology, 80(2), pp.71-76.
85. Raina P, Santaguida P, Ismaila A, Patterson C, Cowan D, Levine M, Booker L and Oremus M (2008) Effectiveness of Cholinesterase Inhibitors and Memantine for Treating Dementia: Evidence Review for a Clinical Practice Guideline. Annals of Internal Medicine. 148 (5), 379.
86. Reynolds, C., Betts, J., Blackstock, W., Nebreda, A. and Anderton, B., 2002. Phosphorylation Sites on Tau Identified by Nanoelectrospray Mass Spectrometry. Journal of Neurochemistry, 74(4), pp.1587-1595.
87. Robakis N (2016) Mechanisms of neuronal death in familial Alzheimer disease and therapeutic implications. Neurobiology of Aging. 39, S14.
88. Rogers, J., Webster, S., Lue, L. F., Brachova, L., Civin, W. H., Emmerling, M., Shivers, B., Walker, D., & McGeer, P. (1996). Inflammation and Alzheimer's disease pathogenesis. Neurobiology of Aging, 17, 681–686.
89. Rohan de Silva H, Jen A, Wickenden C, Jen L, Wilkinson S and Patel A (1997) Cell-specific expression of β-amyloid precursor protein isoform mRNAs and proteins in neurons and astrocytes. Molecular Brain Research. 47 (1-2), 147-156.
90. Rojas-Fernandez, C. H., Chen, M., & Fernandez, H. L. (2002). Implications of amyloid precursor protein and subsequent -amyloid production to the pharmacotherapy of Alzheimer's disease. harmacotherapy, 22, 1547–1563.
91. Salloway S, Sperling R, Fox N, Blennow K, Klunk W, Raskind M, Sabbagh M, Honig L, Porsteinsson A, Ferris S, Reichert M, Ketter N, Nejadnik B, Guenzler V, Miloslavsky M,

Wang D, Lu Y, Lull J, Tudor I, Liu E, Grundman M, Yuen E, Black R and Brashear H (2014) Two Phase 3 Trials of Bapineuzumab in Mild-to-Moderate Alzheimer's Disease. New England Journal of Medicine. 370 (4), 322-333.

92. Selkoe D (2001) Alzheimer's Disease: Genes, Proteins, and Therapy. Physiological Reviews. 81 (2), 741-766.
93. Selkoe, D. J., & Schenk, D. (2003). Alzheimer's disease: Molecular understanding predicts amyloid-based therapeutics. Annual Review of Pharmacology and Toxicology, 43, 545–584.
94. Shaked, G., Kummer, M., Lu, D., Galvan, V., Bredesen, D., Koo, E., Shaked, G., Kummer, M., Lu, D., Galvan, V., Bredesen, D. and Koo, E., 2006. Aβ induces cell death by direct interaction with its cognate extracellular domain on APP (APP 597–624). The FASEB Journal, 20(8), pp.1254-1256.
95. Shen, J. and Kelleher, R., 2006. The presenilin hypothesis of Alzheimer's disease: Evidence for a loss-of-function pathogenic mechanism. Proceedings of the National Academy of Sciences, 104(2), pp.403-409.
96. Skaper, S., 2012. Alzheimer's Disease and Amyloid: Culprit or Coincidence?. International Review of Neurobiology, pp.277-316.
97. Sola Vigo, F., Kedikian, G., Heredia, L., Heredia, F., Añel, A., Rosa, A. and Lorenzo, A., 2009. Amyloid-β precursor protein mediates neuronal toxicity of amyloid β through Go protein activation. Neurobiology of Aging, 30(9), pp.1379-1392.
98. Sondag, C. and Combs, C., 2006. Amyloid precursor protein cross-linking stimulates beta amyloid production and pro-inflammatory cytokine release in monocytic lineage cells. Journal of Neurochemistry, 97(2), pp.449-461.
99. Stelzmann R, Norman Schnitzlein H and Reed Murtagh F (1995) An english translation of alzheimer's 1907 paper, "Uber eine eigenartige erkankung der hirnrinde". Clinical Anatomy. 8 (6), 429-431.
100. Suzuki N, Cheung T, Cai X, Odaka A, Otvos L, Eckman C, Golde T and Younkin S (1994) An increased percentage of long amyloid beta protein secreted by familial amyloid beta protein precursor (beta APP717) mutants. Science. 264 (5163), 1336-1340.

101. Suzuki, T., Oishi, M., Marshak, D., Czernik, A., Nairn, A. and Greengard, P., 1994. Cell cycle-dependent regulation of the phosphorylation and metabolism of the Alzheimer amyloid precursor protein. The EMBO Journal, 13(5), pp.1114-1122.

102. Takashima, A., Honda, T., Yasutake, K., Michel, G., Murayama, O., Murayama, M., Ishiguro, K. and Yamaguchi, H., 1998. Activation of tau protein kinase I/glycogen synthase kinase-3β by amyloid β peptide (25–35) enhances phosphorylation of tau in hippocampal neurons. Neuroscience Research, 31(4), pp.317-323.

103. Tanaka, T., Zhong, J., Iqbal, K., Trenkner, E. and Grundke-Iqbal, I., 1998. The regulation of phosphorylation of τ in SY5Y neuroblastoma cells: the role of protein phosphatases. FEBS Letters, 426(2), pp.248-254.

104. Thinakaran G and Koo E (2008) Amyloid Precursor Protein Trafficking, Processing, and Function. Journal of Biological Chemistry. 283 (44), 29615-29619.

105. Tokuda T, Fukushima T, Ikeda S, Sekijima Y, Shoji S, Yanagisawa N and Tamaoka A (1997) Plasma Levels of amyloid ? proteins A?1-40 and A?1-42(43) are elevated in Down's syndrome. Annals of Neurology. 41 (2), 271-273.

106. Trojanowski, J., Mawal-Dewan, M., Schmidt, M., Martin, J. and Lee, V., 1993. Localization of the mitogen activated protein kinase ERK2 in Alzheimer's disease neurofibrillary tangles and senile plaque neurites. Brain Research, 618(2), pp.333-337.

107. Tsang SW, Pomakian J, Marshall GA, Vinters HV, Cummings JL, Chen CP, Wong PT, Lai MK (2007) Disrupted muscarinic M1 receptor signaling correlates with loss of protein kinase C activity and glutamatergic deficit in Alzheimer's disease. Neurobiol Aging 28:1381–1387

108. Tsubuki, S., Takai, Y. and Saido, T., 2003. Dutch, Flemish, Italian, and Arctic mutations of APP and resistance of Aβ to physiologically relevant proteolytic degradation. The Lancet, 361(9373), pp.1957-1958.

109. Utton, M., Vandecandelaere, A., Wagner, U., Reynolds, C., Gibb, G., Miller, C., Bayley, P. and Anderton, B., 1997. Phosphorylation of tau by glycogen synthase kinase 3β affects the ability of tau to promote microtubule self-assembly. Biochemical Journal, 323(3), pp.741-747.

110. Vassar R (1999) Beta-Secretase Cleavage of Alzheimer's Amyloid Precursor Protein by the Transmembrane Aspartic Protease BACE. Science. 286 (5440), 735-741.

111. Vassar, R., Kovacs, D., Yan, R. and Wong, P., 2009. The beta-secretase enzyme BACE in health and Alzheimer's disease: regulation, cell biology, function, and therapeutic potential. Neuroscience, 29(41), pp.12787-94.

112. Villarreal, S., Zhao, F., Hyde, L., Holder, D., Forest, T., Sondey, M., Chen, X., Sur, C., Parker, E. and Kennedy, M., 2017. Chronic Verubecestat Treatment Suppresses Amyloid Accumulation in Advanced Aged Tg2576-AβPPswe Mice Without Inducing Microhemorrhage. Journal of Alzheimer's Disease, 59(4), pp.1393-1413.

113. Vos S, Xiong C, Visser P, Jasielec M, Hassenstab J, Grant E, Cairns N, Morris J, Holtzman D and Fagan A (2013) Preclinical Alzheimer's disease and its outcome: a longitudinal cohort study. The Lancet Neurology. 12 (10), 957-965.

114. Walsh, D. and Selkoe, D., 2004. Oligomers on the Brain: the Emerging Role of Soluble Protein Aggregates in Neurodegeneration. Protein & Peptide Letters, 11(3), pp.213-228.

115. Weggen, S., Erickson, J. L., Das, P., Sagi, S. A., Wang, R., Pietrzik, C. U., Findlay, K. A., Smith, T. E., Murphy, M. P., Butler, T., Kang, D. E., Marquez-Sterling, N., Golde, T. E., & Koo, E. H. (2001). A subset of NSAIDs lower amyloidogenic A beta 42 independently of cyclooxygenase activity. Nature, 414, 212–216.

116. Weidemann A, König G, Bunke D, Fischer P, Salbaum J, Masters C and Beyreuther K (1989) Identification, biogenesis, and localization of precursors of Alzheimer's disease A4 amyloid protein. Cell. 57 (1), 115-126.

117. Wilquet, V. and Strooper, B., 2004. Amyloid-beta precursor protein processing in neurodegeneration. Current Opinion in Neurobiology, 14(5), pp.582-588.

118. Yoshikai S, Sasaki H, Doh-ura K, Furuya H and Sakaki Y (1991) Genomic organization of the human-amyloid beta-protein precursor gene. Gene. 102 (2), 291-292.

119. Zheng, H. and Koo, E., 2006. Molecular Neurodegeneration, 1(1), p.5.

120. Zhou, F., Gong, K., Song, B., Ma, T., van Laar, T., Gong, Y. and Zhang, L., 2012. The APP intracellular domain (AICD) inhibits Wnt signalling and promotes neurite outgrowth. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, 1823(8), pp.1233-1241.