THE MOLECULAR FORMS OF ALZHEIMER DISEASE IN HUMANS: A REVIEW

A Review Paper Submitted to the Department of Physiology, School of Medicine, College of Health Sciences, and Addis Ababa University, in Partial Fulfillment of the Requirements for the Degree of Master of Science in Medical Physiology.

By

Oumer Jibril

August, 2016

Addis Ababa, Ethiopia
1. Introduction

Alzheimer’s disease (AD) was originally described by Alois Alzheimer in 1906 and was renamed several years later by Emil Kraepelin and he defined Alzheimer as a disease in human brain disease that affects a significant fraction of the population by causing problems with short-term memory, thinking, spatial orientation and behavior, memory loss and other intellectual abilities. Alzheimer’s disease (AD) is the most common neurodegenerative disorder manifested by progressive loss of memory followed by irreversible dementia (Mucke, 2009). AD neurodegeneration is characterized by the loss of neurons preceded by cell membrane and cytoskeleton damage due to a complex of molecular pathways that finally lead to formation of amyloid-β (Aβ) plaques and neurofibrillary tangles in the brain tissue (Mucke, 2009).

AD is characterized by memory loss, behavior changes, and impaired cognitive ability. At the very early stage, the only symptom is mild forgetfulness. Patients cannot recall the names of recent activities or events. Most people with mild forgetfulness likely do not develop AD. It is easily confused with normal aging forgetfulness, which is not progressive (Sclan & Kanowski, 2001). As the disease goes on, progressive worsening of memory is more noticeable. AD patients have trouble learning, speaking or thinking, and cannot carry out daily activities, such as combing their hair, brushing their teeth, or recognizing familiar people around them. At later stages of AD, patients can become aggressive or wander away from home (Selkoe & Schenk 2003).

Alzheimer’s disease (AD) is characterized by the progressive loss of cholinergic neurons leading to dementia. Deciphering the molecular basis underlying this multifactorial neurodegenerative disorder remains a significant challenge. Increased oxidative stress and misfolded protein formations are the basis of AD (Godoy et al., 2014). Alzheimer’s disease (AD) is a common neurodegenerative disease characterized clinically by progressive deterioration of memory, and pathologically by histopathological changes including extracellular deposits of amyloid-beta (A-beta) peptides forming senile plaques (SP) and the intracellular neurofibrillary tangles (NFT) of hyperphosphorylated tau in the brain (Dong et al., 2012).

According to Goedert and Spillantini (2006) have been reported that the Alzheimer disease (AD) is a progressive neurological disorder characterized by a degenerative breakdown of neurons in the cortex and hippocampus of the brain. AD is the most common late onset neurodegenerative disease and, although the majority of cases are apparently sporadic in occurrence, mutations in three genes have been found to be directly implicated with neuronal breakdown. These mutations are found in genes encoding the Amyloid Precursor Protein (APP), and the two catalytic γ-secretase subunits Presenilin-1 (PSEN-1) and Presenilin-2 (PSEN-2), and lead to the earliest and most severe cases of AD (Jayadev et al., 2010). The degeneration of neurons in AD is catalyzed by harmful presence of two separate protein entities: the first is an aggregation of a hyper phosphorylated form of the microtubule stabilizing protein Tau (Goedert et al., 1995), while the second is the accumulation of the protein Amyloid-Beta (Aβ) (Wright et al., 1991). In some
advanced forms of AD both of these forms of protein aggregates have been found (Merzetti & Staveley, 2013).

Alzheimer’s disease is the most common neurodegenerative disease, characterized clinically by the cognitive impairment and changes in behavior and personality. AD is associated with two hallmark lesions: senile plaques, assembled from β-amyloid (Aβ) peptides, & neurofibrillary tangles (NFT), consisting mainly of tau protein (Zekanowski et al., 2004)

Alzheimer’s disease (AD) is an insidiously progressive severe presenile and senile dementia, involving a number of cellular and biochemical mechanisms. AD affects millions of humans as the most common cause of cognitive decline worldwide, in addition to being a main medical challenge for aging population. From the clinical point of view, AD is mostly characterized by age-dependent inexorably progressing cognitive decline, affecting memory primarily associated with behavioral and mood disorders, which increasingly appear as the disease advances (Wisniewski et al., 1996). From the neuropathological point of view, AD is mostly characterized by selective neuronal loss (Duyckaerts et al., 2009), marked synaptic alterations (Baloyannis et al., 1996), morphological mitochondrial abnormalities (Baloyannis et al., 2004), tau pathology (Iqbal et al., 2010) resulting in neurofibrillary tangles (NFT) composed of hyperphosphorylated tau (Mattson, 2004), inflammatory responses and by extracellular extensive deposits of polymers of Aβ peptide, in the form of neuritic plaques, which are a main hallmark of AD. These are dispersed in the neocortex, the hippocampus, and many subcortical structures, which play an important role in cognition. In addition, AD is characterized ultrastructurally by organelle pathology involving mostly the microtubules, the mitochondria, and Golgi apparatus (Baloyannis, 1996).

In this paper, we briefly summarize the role AD plays in amyloid cascade hypothesis and invasion and its potential value in treatment and prognosis.
2. Literature Review
2.1. Molecular Mechanisms Leading to AD

Evidence suggests that the abnormal production and accumulation of misfolded, toxic proteins initiate and/or maintain AD. In AD, β-amyloid (Aβ) peptides, the microtubule-associated protein tau and the presynaptic protein α-synuclein accumulate. The most common and distinctive "hallmark" lesions present within the AD-brain are the senile plaques and neurofibrillary tangles (NFTs) (Castellani et al., 2010). Aβ self-aggregates into assemblies as oligomers, protofibrils and fibrils, forming the core of senile plaques. Tau protein and α-synuclein self-aggregate to oligomers and large inclusions forming NFTs and Lewy bodies, respectively. All patients with AD have plaques and tangles in the brain and most patients also have Lewy bodies (Mucke, 2009). Although the pathogenesis of AD is not known and maybe caused by multiple parallel mechanisms, here we summarize some hypotheses about the distinct molecular and cellular mechanisms that can cause AD or AD-like symptoms (Castellani et al., 2010).

There are two main hypotheses for the pathogenesis of AD: the amyloid-cascade and the hyperphosphorylated tau hypothesis. (1) According to the original amyloid hypothesis (Hardy & Allsop, 1991) fibrillar Aβ peptides of the amyloid plaques are responsible for neurodegeneration in AD. The hypothesis has been radically changed during the past 10 years and soluble Aβ oligomers and protofibrils were found to be the most toxic aggregation species of Aβ peptides (Walsh et al., 2000). The newest amyloid-hypothesis emphasizes the pivotal role of intracellular Aβ species in the pathogenesis of AD (La et al., 2007). Positron emission tomography (PET) ligands (e.g., 11C-labelled PIB) directly demonstrate the presence of Aβ aggregates in the brain of AD patients (Svedberg et al., 2009). (2) The tau-hypothesis focuses on the microtubular protein tau (Iqbal & Grundke, 2005). Brain injuries, hypoxia, hypoglycemia may cause signalization disturbances activating GSK3β kinase and then tau-protein will be hyperphosphorylated, resulting in collapse of the microtubular system and cell death. Very recently the modernized amyloid hypotheses for AD pathogenesis (Hardy, 2009) have a wide acceptance.

Some new findings support the amyloid hypothesis leaving place also for taupathology: (a) "mitochondrial cascade hypothesis": mitochondrial energy production plays an important role in the etiopathology of AD (Devi & Anandatheerthavarada, 2010). One of the hallmarks of AD is the very early deterioration of mitochondria in neurons. (b) A decrease of Aβ peptides and a simultaneous increase of total tau in the CSF of AD patients indicate that the brain clearance of Aβ has decreased in AD patients. It can cause Aβ accumulation in the brain tissue and the induction of AD (Ward, 2007). (c) Aβ oligomers are synaptotoxic. A very early hallmark of the disease is the slow deterioration of synapses and impaired neuronal communication. The Zn ions may contribute to the toxicity of Aβ peptides in the synapses (Zn is co-released with glutamate) (Bush et al., 1994). (d) Mutant ApolipoproteinE (ApoE epsilon 4) protein is present in 60% of the Caucasian population of sporadic AD patients. Because ApoE plays an important role in Aβ clearance, it is a clue for the importance of Aβ clearance in AD (Mucke, 2009). (e) Biochemical and immunochemical analysis of neurofibrillary tangles (NFTs, the second hallmark of AD) resulted in important information on the proteins of the paired helical filaments. It is widely accepted that the microtubule associated protein tau is the major protein of NFT.
However, according Smith (1998) has been reviewed that a wide range of other proteins and carbohydrates were found in NFTs: (1) Cytoskeletal elements, including tau, neurofilaments, high molecular weight microtubule-associated protein MAP2, vimentin, and tropomyosin. (2) Protease-related elements, including ubiquitin, α1-antichymotrypsin, α1-antitrypsin, cathepsins B and D, trypsin, and elastase. (3) Proteoglycans, including heparin, chondroitin, and keratin sulfate proteoglycans. (4) Inflammatory molecules, including acute phase proteins, cytokines and complement molecules. (5) Amyloidogenic-related molecules, including APP, presenilin, and APOE. (6) Serum-related molecules, including P-component. (7) Adducts of oxidative stress, including advanced glycation and lipid peroxidation.

2.1.1. Amyloid Cascade Hypothesis

The history of this hypothesis began with the isolation and identification of a protein-like material that was deposited in the AD patients” meningeal vessels (Glenner & Wong, 1984). It was later demonstrated that this material was identical to that obtained from blood vessels of Down syndrome patients, a disorder that is not only characterized by cognitive impairment but is also associated with a trisomy of chromosome 21 (Glenner & Wong, 1984). Subsequently, other studies confirmed that this was the same peptide found in senile plaques of AD patients (Masters et al., 1985). Finally, the identification of both the protein precursor from which Aβ is originated (the APP) and the first mutation that was associated with AD development (located in the APP gene, precisely), inevitably led to suggest that this peptide has a central role in the disease origin (Goate et al., 1991).

The amyloid hypothesis was proposed formally for the first time by Hardy and Allsop in 1991, and it still continues to be one of the etiologic hypotheses best scientifically supported nowadays. This hypothesis states that production and excessive accumulation of Aβ, both intracellular and extracellular, as well as under different physical and aggregation states, are some of the beginning events that drive the progressive neuronal damage which fully characterizes the disease (Eckman, 2007). The Aβ is a peptide of 39 to 42 amino acids and is usually produced in all neurons through sequential proteolytic processing of a membrane-attached type-1 protein, called amyloid precursor protein (APP), by means of two enzymatic complexes: the β and γ-secretases (Postina, 2008). The APP can be processed through two enzymatic pathways, the non-amyloidogenic pathway and the amyloidogenic pathway (Figure 1). Within the nonamyloidonegic pathway, the first step of the proteolysis is mainly performed by enzymes holding α-secretase activity.

These enzymes cut the APP within the ectodomain, which correspond to the Aβ fragment. This process produces bigger soluble fragments, thus avoiding the formation of smaller fragments like the Aβ (Postina, 2008). The α-secretase’s action releases the extracellular N-terminal domain of the APP, so-called soluble sαAPP, which possesses different neurotrophic and neuroprotective properties. In addition, the C-terminal fragment of APP that remains anchored to the membrane (C83 ) is once again proteolysis by the γ-secretase producing the fragments p3 (Aβ 17–40/42), which have low-potency cellular toxic properties. Simultaneously, the intracellular domain of the APP, which has demonstrated some neuroprotective properties, is released inside the cell (R. Postina, 2008) (Figure 1). In the so-called amyloidogenic pathway, the APP is first proteolysis by the β-secretase (also known as aspartyl protease BACE1), which generates a soluble fragment
from the N-terminal domain called sAPPβ as well as the CTFβ fragment that remains attached to the membrane. The latter is next proteolysis by the γ-secretase complex then produces the Aβ. The γ-secretase is composed of four proteins complex: Nicastrin, PEN-2, Aph-1, PS1, and PS2, from which both presenilins represent the catalytic site of the enzymatic complex. It is important to highlight that all mutations associated with familial type of AD (APP, PS1, and PS2), in one way or another, increase Aβ production or modify its production rate (Paul et al., 2014).

Figure 1: Schematic Diagram Showing the Two Proteolytic Pathways of Amyloid Precursor Protein: Amyloidogenic and Nonamyloidogenic (Paul et al., 2014).

Amyloid beta (Aβ) is a peptide of 39–43 amino acids found in large amounts and forming deposits in the brain tissue of patients with Alzheimer’s disease (AD). For this reason, it has been implicated in the pathophysiology of damage observed in this type of dementia (Paul et al., 2014). However, the role of Aβ in the pathophysiology of AD is not yet precisely understood. Aβ has been experimentally shown to have a wide range of toxic mechanisms in vivo and in vitro, such as excitotoxicity, mitochondrial alterations, synaptic dysfunction, altered calcium homeostasis, oxidative stress, and so forth. In contrast, Aβ has also shown some interesting neuroprotective and physiological properties under certain experimental conditions, suggesting that both physiological and pathological roles of Aβ may depend on several factors (Swerdlow & Khan, 2004)
Figure 2: Amyloid Cascade Hypothesis (Paul et al., 2014).

2.1.2. The Mitochondrial Cascade Hypothesis

According to Swerdlow & Khan (2004) have been reported that the mitochondrial cascade hypothesis asserts inheritance determines mitochondrial baseline function and durability, mitochondrial durability influences how mitochondria change with age and AD histopathology and symptoms ensue when mitochondrial change reaches a threshold. The mitochondrial cascade hypothesis attempts a unified explanation for the clinical, biochemical, and histologic features of AD (Swerdlow & Khan, 2004). The mitochondrial cascade hypothesis takes several conceptual liberties. It assumes similar physiologic mechanisms underlie AD and brain aging. It postulates because AD mitochondrial dysfunction is systemic, it cannot simply represent a consequence of neurodegeneration.

The mitochondrial cascade hypothesis argues non-Mendelian genetic factors contribute to non-autosomal dominant AD. Finally, it posits AD brain mitochondrial dysfunction drives amyloidosis, tau phosphorylation, and cell cycle re entry (Paul et al., 2014). Certainly, mitochondria are indirectly featured in past theories of aging and directly in current aging theory. The rate of living hypothesis arose in the 1920’s from observations that animals with low metabolic rates tend to outlive those with high metabolic rates (Pearl, 1928). Harman refined this when he proposed the free radical theory of aging in 1956 (Harman, 1956). The free radical theory of aging specifically postulated over time cells accumulate structural damage from oxidative byproducts. By the 1970’s mitochondria were recognized sites of free radical production and for many the free radical theory of aging morphed into the mitochondrial theory of aging (Miquel et al., 1980). The late 1980’s envisaged a role for somatic mitochondrial DNA (mtDNA) damage in aging (Wallace, 1992). Corroborating this possibility are recent studies showing mtDNA mutation acquisition accelerates aging in laboratory animals (Trifunovic et al., 2004).

Mitochondrial dysfunction is observed in multiple AD tissues (Swerdlow & Kish, 2002). At least brain, platelet, and fibroblast mitochondria are involved. Defects of three mitochondrial enzymes
are reported. This includes reduced activities of pyruvate dehydrogenase complex, alpha ketoglutarate dehydrogenase complex, and cytochrome oxidase (Gibson et al., 1998). Spectral analysis of cytochrome oxidase indicates AD brains contain normal amounts of cytochrome oxidase, but the enzyme itself is structurally altered (Parker & Parks., 1995). Various mechanisms, such as oxidative stress and proteasome dysfunction, have been postulated to facilitate mitochondrial dysfunction in neurodegenerative diseases such as AD (Ding et al., 2006). Also, cytoplasmic hybrid (cybrid) studies indicate mtDNA at least in part accounts for reduced cytochrome oxidase activity in AD, and through this perhaps oxidative stress (Swerdlow, 2007).

Cybrid studies involve transfer of exogenous mtDNA to cultured cells depleted of endogenous mtDNA (Khan et al., 2006). “ρ” DNA is an alternative term for mtDNA. These mtDNA-depleted cells are therefore called ρ0 cells. They do not produce mtDNA-encoded proteins and lack cytochrome oxidase activity. Mitochondrial DNA transferred to ρ0 cells replicates and accomplishes mtDNA replacement. This enables expression of mtDNA-encoded ETC subunits and restoration of cytochrome oxidase activity. When cybrid lines containing mtDNA from AD subject platelets are compared to cybrid lines containing mtDNA from age-matched controls without AD, cytochrome oxidase activity is lower in the cybrid lines containing AD subject mtDNA (Swerdlow et al., 1997). Because nuclear genetic and cell culture conditions are equivalent between all cybrid cell lines, differences between mtDNA from the donor subjects likely account for the observed differences in cytochrome oxidase activity.

The exact nature of the implied AD cybrid-control cybrid mtDNA difference is unclear. This uncertainty is partly due to mtDNA-related complexities. Mitochondrial genetics and nuclear genetics differ in several key ways. One difference is that cells contain multiple copies of mtDNA. The mtDNA molecules within a cell can be identical, a state referred to as homoplasmy. However, the nucleotide sequences of mtDNA molecules within a cell can also vary. This is called heteroplasmy. If mtDNA sequence variation is present within a cell, it is necessary to consider whether it represents a homoplasmic or heteroplasmic variation. If the variation is heteroplasmic, it is necessary to further consider whether it represents a majority (high abundance heteroplasmy) or minority (low abundance heteroplasmy) of that cell’s mtDNA copies. Distinct homoplasmic or high abundance heteroplasmic mutations of mtDNA cytochrome oxidase (CO) likely account for at most a very small percentage of AD (Hamblet et al., 2006), and therefore should not account for AD-control cybrid differences.

Mitochondrial DNA polymorphisms could potentially account for AD-control cybrid differences, as these are a common source of mtDNA inter-individual variability. Although AD-mtDNA polymorphism associations are reported (Van et al., 2004), the effect of specific polymorphisms or linked polymorphisms (haplogroups) on cybrid cytochrome oxidase activity is unstudied. Low abundance heteroplasmacy might also cause AD-control cybrid cytochrome oxidase differences. A recent study did in fact suggest unique low abundance mtDNA heteroplasmies occur in AD (Coskun et al., 2004).

Other data indicate low abundance mtDNA heteroplasmies manifest with increasing age, and these heteroplasmies are associated with reduced cytochrome oxidase activity (Lin et al., 2002). However, the presence and role of low abundance mtDNA heteroplasmies in AD cybrids has not
been critically evaluated. Whether somatic or inherited mtDNA features account for AD-control cybrid cytochrome oxidase differences also requires consideration. Data pertinent to this question permit speculation. First, complex I activity is normal in AD cybrids (Ghost et al., 1999). Complex I contain seven mtDNA encoded subunits. Absence of complex I dysfunction suggests somatic mutation does not account for reduced cytochrome oxidase activity in AD cybrids, since acquired mtDNA somatic mutation should also reduce complex I activity. Second, reduction of cytochrome oxidase activity in multiple non-degenerating tissues is more consistent with mtDNA inheritance than somatic mutation. Third, plotting one AD cybrid study’s cytochrome oxidase data by mtDNA donor age actually reveals the youngest AD subjects show the biggest activity reductions (Figure 3).

These data suggest mtDNA signatures might affect intrinsic cognitive decline trajectories. One interpretation of this is the lower the cytochrome oxidase activity, the sooner the subject reaches the dementia threshold. Epidemiologic data showing maternal AD status correlates better with offspring AD status than paternal AD status is consistent with this possibility (Edland et al., 1996). Any comprehensive theory of AD pathogenesis must explain the different pathologies observed in Alzheimer’s disease. AD cybrids with reduced cytochrome oxidase activity overproduce Aβ42 and Aβ40 (Khan et al., 2000). Under in vitro conditions sodium azide, a cytochrome oxidase inhibitor alters APP processing towards amyloidogenic pathways (Gabuzda et al., 1994). Administering sodium azide to mice also causes tau phosphorylation (Szabados et al., 2004). Fibroblasts from FAD subjects phosphorylate tau following exposure to CCCP, a mitochondrial uncoupler (Blass et al., 1990). Mitochondrial ETC dysfunction increases free radical production (Swerdlow, 2002). Enhanced reliance on anaerobic metabolism is associated with cell cycling (Swerdlow & Khan, 2004).

Interestingly, according to Devi et al. (2006) have been reported that the Aβ inhibits ETC activity in general and cytochrome oxidase activity specifically. Therefore, a reciprocal relationship exists between mitochondrial function and amyloidosis. Several independent observations reinforce this concept. Neuronal-like NT2 human teratocarcinoma cells exposed to Aβ show high rates of demise. NT2 ρ0 cells, though, are impervious to Aβ (Cardoso et al., 2001). The main difference between native NT2 cells and NT2 ρ0 cells is the absence of a functional ETC in the ρ0 cells. This suggests under cell culture conditions the mitochondrial ETC mediates Aβ toxicity. Also, APP, Aβ, and the entire γ-secretase complex are found either within mitochondria or mitochondrial membranes (Manczak et al., 2006).

The role APP, Aβ, and γ-secretase play in mitochondrial function is currently unknown. Recent work showing oligomeric β-sheet proteins permeabilize membranes (Glabe & Kayed, 2006) raises the possibility these proteins allow cells to disable mitochondria when certain conditions are met. If mitochondrial dysfunction is one of these conditions, one physiologic role of APP or Aβ may be to “shut down” abnormal mitochondria. The mitochondrial cascade hypothesis takes
aging phenomena into account, and applies to individuals long before they develop AD.

**Mitochondrial Cascade Hypothesis**

![Diagram](image)

Fig 3. Mitochondrial Cascade Hypothesis (Swerdlow & Khan 2004).

### 2.1.3. The Neuroinflammation Hypothesis of AD

Inflammation is a complex protective response to defend against injury, stress or infection. The inflammatory mechanisms employed by neurons (neuroinflammation) in the defense of cellular and molecular harm associated with AD includes the recruitment of microglia, astrocytes, macrophages, and lymphocytes. These, in turn, release cytokines, chemokines, eicosanoids, and ROS/RNS. The release of these intermediaries leads to the activation of additional microglia and recruits lymphocytes and monocytes across the blood-brain barrier (Simard et al., 2006). Furthermore, the production of ROS/RNS enhances downstream activation of transcription factors controlling the expression of cytokines, chemokines, paracrine molecule metabolizing-enzymes and other ROS/RNS-generating enzymes (Gabbita et al., 2000). In the neuroinflammation hypothesis of AD, then, this pattern of activity works in a non-linear cyclic proliferative manner that can actually initiate or hasten AD-associated neurodegeneration in the aging brain. Thus, cellular damage from oxidative stress or other aging related injury commences a pro-inflammatory spiral that propagates forward to accomplish additional cellular damage.

Research to identify the presence of classic immune molecules at the site of AD plaques or tangles has yielded many results including, but not limited to, complement components (McGeer et al., 1996), cytokines such as interleukin 1β and interleukin 6 (IL-6), transforming growth factor-β (TGF-β), tumour necrosis factor-α, macrophage colony stimulating factor and the receptor for advanced glycation end products (RAGE) (Lue et al., 2001). Additionally, pro-inflammatory lipid paracrine signaling metabolizing component cyclooxygenase 2 and its product, eicosanoid prostaglandin E2 have been reported to be elevated in AD patients. Arachidonate 5-lipoxygenase (5LOX), another eicosanoid product, has also been reported to be elevated in the brains of AD patients (Ikonomovi et al., 2008). Promoters of the Aβ cascade hypothesis argue that Aβ alone is sufficient to activate microglia and stimulate the production of TNF-α, IL-1β, IL-6, IL-18 and PGE2 (Akiyama et al., 2000).

Most of the evidence regarding neuroinflammation therapy revolves around the use of nonsteroidal anti-inflammatory drugs (NSAIDs) or peroxisome proliferator-activated receptor isoform gamma (PPAR-γ). One method NSAIDs utilize to suppress inflammation is to inhibit the...
COX enzyme, subsequently reducing the availability PGE2. However, there are also COX independent mechanisms of action that appear to also be employed in reducing AD risk (Weggen et al., 2001). When ligand-bound, PPAR-γ is a nuclear hormone receptor that can inhibit the transcription rates of proinflammatory cytokines IL-1β, IL-6, and TNF-α. In addition, under inflammatory conditions, NSAIDs can act at PPAR-γ activators, which then inhibit BACE1 transcription to decrease Aβ levels (Ikonomović et al., 2008).

Trials of anti-inflammatory drugs in patients with mild-to-moderate AD were mostly neutral with only 3 of 16 studies showing beneficial effects of trends and four others showing potential detrimental effects. Results were ambiguous for three studies of NSAID trials in patients with Mild Cognitive Impairment (MCI). Again, timing – getting the jump on the inflammatory cascade – may be crucial in the treatment. Unfortunately, COX-2 inhibitors such as NSAIDs have also been found to elevate blood pressure and thrombotic response to the rupture of an atherosclerotic plaque (Fitzgerald, 2004). Due to that and other perceived cardiovascular risks, the Food and Drug Administration (FDA) suspended a large, randomized, controlled, prevention study of NSAIDs in AD. Other (non-NSAID) anti-inflammatory approaches are also in Phase II clinical trials, including a RAGE inhibitor, IVIg (which could act to boost immune reactions), and an adenovirus-aided delivery of a nerve growth factor gene that is surgically administered (Gravitz, 2011).

![Fig 4. The potential protective mechanisms of NSAIDs in AD. Cartoon adopted from Weggen et al., 2007).](image)

2.2. Biology of Tau

Tau is a microtubule-associated protein encoded by the MAPT gene on chromosome 17. Alternative splicing of 16 axons yields six isoforms of human tau that differ in number of amino acids, in number of N-terminal inserts (0–2N) and in number of microtubule binding domains (3R or 4R). In normal adult brain, the ratio of 3R:4R tau is ~1 and in AD this ratio is shifted toward excess 4R tau (Huang & Mucke, 2012). Many of the tau mutations leading to dementia alter the splicing of the tau protein also increasing the presence of 4R tau (Spires et al., 2009).

According to Gendron & Petrucelli (2009) have been reported that the number of microtubule binding domains in tau determines the affinity for microtubules (3R < 4R), thereby mediating the primary function of tau in stabilizing axonal microtubules and enabling their polymerization and
assembly. As microtubules serve as the highways for trafficking of molecules within the axon, this places tau as a central player in neuronal transport and therefore function (Ballatore et al., 2007). Also contributing to the microtubule stabilizing capacity of tau are post-translational modifications, particularly phosphorylation, which when elevated decreases affinity of tau for microtubules and causes it to detach (Spires et al., 2009).

According to Ittner et al (2011) have been reported that the tau has over 80 phosphorylation sites, some of which are considered physiological while others are „de-novo” phosphorylated in disease states. Two families of protein kinases contribute to tau phosphorylation, those that are proline directed and tend to phosphorylate serine and threonine motifs outside the microtubule binding domain and those that are KXGS-motif and non-proline directed and tend to phosphorylate within the repeat domain. Proline directed kinases that phosphorylate tau include GSK3β, MAPK (mitogen activated protein kinase), JNK (c-Jun N terminal kinase) and cyclindependent kinase 5 (Cdk5) and have been the target of some of the preliminary tau-targeted therapies. Kinases targeting KXGS and other non-proline motives include microtubule affinity regulating kinase (MARK), P70S6K, BRSK, PKA and CaMKII (Zempel et al., 2010), with MARK recently being implicated as a critical mediator of both Aβ and tau toxicity (Yu et al., 2012). In physiological conditions the activities of the kinases are dynamically counterbalanced by the primary tau directed protein phosphatases PP2A and PP1 (Gendron & Petrucelli, 2009).

2.2.1. Pathological Forms of Tau
According to Gendron & Petrucelli (2009) have been reported that in disease states such as AD, it is thought that the balance of kinase and phosphatase activity is shifted, creating a hyperphosphorylated species of tau. This increases the fraction of tau that is no longer attached to microtubules, allowing for monomeric hyperphosphorylated tau to bind one another to produce oligomers (Alonso et al., 1996). These oligomers are missorted from the axonal to somatodendritic compartment (Eckermann et al., 2007), where they undergo further hyperphosphorylation and conformational change and take on a beta sheet structure that is considered insoluble. Fusion of these oligomeric species contributes to the formation of paired helical filaments (PHF), the primary constituent of NFT (Takashima, 2008). As is the case with other proteins involved in neurodegenerative diseases, the question of which variety of tau is most toxic and whether that toxicity represents a gain or loss of function continues to be debated (Brunden et al., 2008). As tau progresses from normal to NFT it passes through a loosely defined „soluble” state in which the protein may be hyperphosphorylated, mislocalized, conformationally changed and/or oligomeric but not yet fibrillar. It is these forms of soluble tau protein that vie against NFT in the debate of pathogenic entity in neurodegenerative disease.

The toxicity of tau can be argued in several ways 1) aggregated fibrillar tau (NFT) is toxic and causative of cell death and cognitive decline in AD, 2) soluble species of hyperphosphorylated, misfolded tau that accumulate in abnormal cellular compartments are toxic and NFT act as a sink for these toxic species, implicating NFT as protective, or 3) both soluble forms of pathological tau and NFT are toxic to cells in different ways and on different time scales. We favor the third model of toxicity as will be discussed. Historically, NFT were considered indicators of cell death, particularly given that their progression and number correlate well with severity of cognitive decline in AD, while Aβ plaque deposition does not (Braak et al., 1995).
neurons of human AD brain have been shown to have abnormal quantity or distribution of molecules necessary for proper function such as synaptic proteins, and calcium binding proteins (Bezprozvanny & Mattson, 2008) and NFT have been suggested to interfere with basic cell function as they serve as a physical disruption or space occupying lesion (Ballatore et al., 2007). In a mouse model of tauopathy, expression of an aggregation prone tau molecule causes morphological and functional deficits while expression of a similar but anti-aggregation tau molecule has no such negative consequences. NFT toxicity is also supported by cell culture models in which tau aggregation leads to activation of caspase cascades and cell death (Mocanu et al., 2008).

2.2.2. Mechanisms of Tau Toxicity

Tau toxicity has largely been attributed to disruption of neuronal transport, particularly as the main function of tau is stipulated to be maintaining microtubule stability and assembly in CNS axons. Neurons, due to their morphological structure with extended processes and high-energy demands, rely heavily upon regulated transport of organelles and vital materials for cell function (Hollenbeck & Saxton, 2009). Impairment of these transport processes has been proposed as an early pathological phenomenon and underlying cause of neurodegenerative diseases, including AD (Wang & Schwarz, 2009). Both loss and gain of function mechanisms for tau interference with transport have been proposed, but interestingly, recent studies in tau knock out models have shown that the loss of tau, even in its entirety, is not lethal and actually demonstrates very mild phenotypes (Yuan et al., 2008).

According to Muresan (2009) has been reported that the tau over-expression in cell culture without the formation of NFT has been shown to inhibit fast axonal transport, with mitochondria emerging as the cargo most susceptible to deficits in localization, implicating soluble tau in these deficits. Patches of “soluble” tau bound to microtubules have been proposed to serve as a roadblock, preferentially causing anterograde-moving kinesins to detach from the microtubule (Dixit et al., 2008). A second theory suggests that soluble tau can interfere with transport by directly competing with cargo or hampering signaling cascades (Tackenberg & Brandt, 2009). Ittner et al (2011) demonstrated that pathologically hyperphosphorylated tau directly interacts with JIP1, a protein involved in linking cargoes to the kinesin motor, thereby preventing proper association of cargo and motor.

Other studies have suggested that tau may bind influential molecules such as GSK3β, or mitochondria themselves to alter transport and localization of organelles in a more indirect manner (Amadoro et al., 2010). Yet other studies have suggested that tau itself is a kinesin cargo and when cytosolic concentration of tau is pathologically increased, it may out compete other cargoes. Aggregates of misfolded tau (NFT and neuropil threads) have been argued to interfere with transport by serving as a space-occupying lesion, thereby inhibiting kinesin binding and movement (Muresan, 2009).

2.2.3. Are Tau Pathology and Cell Cycle Re-entry Related Events?

According to Johnson and Hartigan (1999) have been reported that the tangles consist of aggregated microtubule associated protein (MAP) tau, tangle tau is excessively phosphorylated
at serine and threonine residues, and tau phosphorylation presumably facilitates aggregation. Although MAP tau gene mutations drive neurodegeneration, the resultant phenotype is characteristic of frontotemporal dementia rather than AD and altered Aβ production is not characteristic of primary tauopathies (Cairns et al., 2007). Polymorphic variation of the tau gene defines two extended haplotypes, H1 and H2; H1 associates with progressive supranuclear palsy, corticobasal degeneration, Parkinson’s disease, but probably not AD (Mukherjee et al., 2007).

Cell growth and cycling are regulated by trophic stimulation and energy status. Trophins bind insulin, insulin-like growth factor 1 (IGF1), and other related receptors belonging to the tyrosine kinase receptor family. In neurons, ligand binding induces insulin receptor substrate 2 (IRS2) tyrosine phosphorylation, pha

Cell growth sometimes occurs in conjunction with division and sometimes it does not. Growing to divide and growing not to divide (hypertrophy), though, are fundamentally different processes. Cytoskeletal maintenance is not advantageous in dividing cells and high energy demand precludes energy storage. Growth without division requires a cytoskeleton and energy storage is possible. It is not surprising a single Akt-regulated protein, glycogen synthase kinase 3β (GSK3β), coordinates cytoskeletal maintenance with energy storage. GSK3β phosphorylates tau and inhibits glycogen synthesis (Bhat & Budd, 2002). Tau phosphorylation prevents cytoskeleton formation or promotes cytoskeleton disassembly, while glycogen synthase inhibition blocks glucose storage. GSK3β activation is therefore conducive to cell division. Substantial evidence reveals AD neuron cell cycle re-entry is more common than it is in non-AD brain (Mosch et al., 2007). This reentry proceeds through several parts of the cycle but fails at the G2-M interface (Zhu et al., 2004).

According to McShea et al (2007) have been reported that the mitochondrial cascade hypothesis proposes tau phosphorylation/tangle formation and cell cycle re-entry are related events, and experimental support for this prediction was recently published. We speculate preserved trophic stimulation in the face of low energy mimics a cycling profile and causes AD neurons to act as dividing cells. If correct, Akt must function differently under conditions of trophic stimulation/high energy than it does under conditions of trophic stimulation/low energy. Trophic stimulation/high energy would presumably cause Akt to inhibit GSK3β, thus facilitating growth without division. Trophic stimulation/low energy would have to activate the Akt proto-oncogene protein in such a way that GSK3β inhibition does not occur. We are currently exploring this possibility. Finally, it is necessary to postulate another factor, perhaps oxidative stress, causes phosphorylated tau to form tangle aggregations (Swerdlow & Khan, 2004).
2.3. Oxidative Stress

2.3.1. Reactive Oxygen Species and Oxidative Stress

Oxidative stress represents an imbalance between the production of reactive oxygen species (ROS) / reactive nitrogen species (RNS) and the biological system's ability to readily detoxify the reactive intermediates, or to repair the resulting damage (antioxidant systems) (Lin, 2012). Oxidants, such as ROS and RNS are a part of normal physiological process and produced at low levels in all aerobic organisms as a consequence of normal respiration. ROS include superoxide radical anion (O2·−), hydrogen peroxide (H2O2), and hydroxyl radical (OH·). Mitochondria are the major place to produce ROS through the respiratory chain. The most important targets of ROS damage are nucleic acids (DNA/RNA damage), carbohydrates, lipids (lipid peroxidation) and proteins (protein oxidation) (Chakroborty & Stutzmann, 2011). According to Khachaturian (1994) have been showed that the reactive nitrogen species (RNS) are a family of antimicrobial molecules derived from nitric oxide (NO) and superoxide (O2·−) produced via the enzymatic activity of inducible nitric oxide synthase 2 (NOS2) and NADPH oxidase respectively. Reactive nitrogen species act together with reactive oxygen species (ROS) to damage cells, causing nitrosative stress (Sabatini et al., 2001). Therefore, these two species are often collectively referred to as ROS/RNS. RNS are also continuously produced in plants as by-products of aerobic metabolism or in response to stress (Zempel & Mandelkow., 2011).

To defend against ROS- and RNS-mediated injury, cells develop several antioxidant system responses that prevent the formation, detoxification or scavenging of oxidant species. Antioxidants include both enzymes (superoxide dismutase (SOD), catalase, glutathione peroxidase and several sulfur-containing enzymes like (thioredoxin and glutaredoxin) and low molecular weight compound (glutathione and NADPH) (Chakroborty & Stutzmann., 2011). Glutathione peroxidase and catalase detoxify hydrogen peroxide (H2O2), which generates hydroxyl radicals (OH·) in the presence of transition metals (Fe2+). In addition to other antioxidants are vitamins (e.g. α-tocopherol, ascorbic acid and β-carotene), synthetic (e.g. butylated hydroxytoluene), natural (e.g. plant-derived polyphenols) and inorganic (e.g. selenium). Some act as chain-breaking molecules because they prevent the propagation of or stop radical chain reactions (i.e. α-tocopherol) (Hermes et al., 2010). In the case that the production of oxidant species exceeds the endogenous antioxidant defending system, an oxidative imbalance occurs. This results in cellular oxidative stress and subsequently leads to molecular oxidative damage, which can result in altered cellular functions and eventually cell death (Crimins et al., 2011).

2.4. What causes Alzheimer’s disease?

Many theories as to the cause of AD have consequently been proposed. It is not the intention to discuss every theory but to concentrate on those most likely to be involved. Hence, the theories are discussed in eight categories: (1) acceleration of aging, (2) degeneration of anatomical pathways, including the cholinergic and cortico-cortical pathways, (3) environmental factors such as exposure to aluminium, head injury, and malnutrition, (4) genetic factors including mutations of amyloid precursor protein (APP) and presenilin (PSEN) genes, and allelic variation in apolipoprotein E (Apo E), (5) a metabolic disorder resulting from mitochondrial dysfunction, (6) vascular factors such as a compromised blood brain barrier, (7) immune system dysfunction, and (8) infectious agents.
2.4.1. Theories Based on Aging

According to Reisberg (1983) has been reported that the AD may be an accelerated form of natural aging is based on the observation that the many pathological changes in AD are similar to those present in normal aging apart from their severity. Hence, in cognitively normal brain, there is an age-related reduction in brain volume and weight, enlargement of ventricles, and loss of synapses and dendrites in selected areas (Imhof et al., 2007). Accompanying these changes are the characteristic pathological features of AD, including SP and NFT (Anderton, 2002). Hence, in 60 normal elderly cases (Mann et al., 1987), 32/60 had no SP, although 30/60 were younger than 65 years, 13/60 had SP in the hippocampus, mainly in sector CA1 and the subiculum, and 12/60 had SP in temporal cortex comprising mainly the „primitive/mature“ and „burnt-out“ types of plaque. It was concluded that it was not possible to distinguish early-stage AD from normal aging at post-mortem (Mann et al., 1987).

Similarly, SP have been observed in 60% of normal elderly cases, albeit at lower density than in AD (Bergeron et al., 1987). Moreover, Arrigada et al. (1992) reported SP in most normal individuals greater than 55 years of age and concluded that there could be a „continuum of pathological change from elderly non-demented brains, early stage AD, to advanced AD (Armstrong, 2012). According to Bell & Ball (1990) have been reported that the density of neuritic plaques (NP), which incorporate dystrophic neurites (DN), and SP with a distinct „core“ („classic“ plaques), may not be significantly different in AD and aging. A greater vascular involvement in the formation of SP has been observed in aging than in AD (Bell & Ball, 1990). Hence, SP have been observed in the frontal and temporal cortex in 15/20 patients with critical stenosis, most often in the depths of the gyri (Sparks et al., 1993).

Alterations in cerebral perfusion may therefore play a role in SP formation and SP may not always be causally related to dementia A further study of non-demented patients with critical coronary artery disease suggested that some patients had similar densities of SP to AD, numbers of SP being directly proportional to the duration of arterial disease (Sparks et al., 1993). In addition, there may a close link between ischaemic brain episodes and sporadic cases of AD (SAD) suggesting that neovascular factors could aggravate the progression of the disease (Pluta et al., 2012). The most important molecular constituent of the SP is β-amyloid (Aβ) (Glenner & Wong, 1984), an approximately 4kDa peptide arising by constitutive cleavage of a trans-membrane amyloid precursor protein (APP) (Greenberg, 1995). Three subtypes of Aβ deposit are commonly observed in AD, viz., diffuse, primitive, and classic deposits (Armstrong, 1998). Studies of Aβ deposition have also demonstrated a clear overlap between AD and normal aging.

Hence, Aβ deposits were present in non-demented individuals greater than 60 years but were rare before this age (Mann & Jones, 1990). After 60 years of age, Aβ deposits were present in a variety of diseases as a result of aging, especially in the temporal cortex, thus blurring the distinction between AD and related disorders (Mann & Jones, 1990). In 14 non-demented elderly cases, Aβ deposits were present in the temporal lobe in 8/14, but only in cortical gyri, the CA sectors of the hippocampus and dentate gyrus being spared (Armstrong, 1998). Moreover, there was a considerable variation in the density of deposits in control cases and a significant overlap with AD.
According to Armstrong RA (1998) has been reported that the pattern of clustering of Aβ deposits was also similar in control and AD cases, i.e., in cortical gyri, deposits were aggregated into clusters regularly distributed parallel to the pia mater, suggesting a similar pathogenesis. In a further study of centenarians (Delaere et al., 1993), Aβ deposits were recorded in the parahippocampal gyrus (PHG) of patients, whether demented or not, but the hippocampus was unaffected, suggesting a little relationship between lesion density and severity of mental deficits.

2.4.1.1. Oxidative Stress and Aging

Aging is defined as “gradual irreversible changes in structure and function of an organism that occur as a result of the passage of time.” These changes are commonly harmful, decreasing normal functioning and adaptability, and simultaneously increasing the probability of death. Regarding cellular aging, or senescence, the emphasis is usually placed on the decreased ability to proliferate as a result of either exceeded proliferative limit (explicative senescence) (Lee et al., 2004) or cellular stress (stress-induced senescence) (Levy et al., 1995). Free radicals were first associated with aging when Denham Harman presented the “the free radical theory of aging” in 1956. According to the theory, biologic aging (senescence) occurs because of the accumulation of oxidatively damaged macromolecules. Today, although other factors may also be involved in the aging process (e.g., evolution, somatic mutations, errors in protein synthesis, accumulation of waste products, neuroendocrine and immunologic disturbances), the role of free radicals to aging is considered to be an important contributor in various biologic species ranging from yeast to humans (Lopez & Shelanski, 2004).

2.4.1.2. Oxidative Stress and AD

Ample evidence implicates oxidative stress as an early event that is widespread in the AD brain, and which plays an important role in the pathogenesis of AD (Kawarabayashi et al., 1991). Increased ROS/RNS and the dysfunctional antioxidant system (Kawai et al., 1990) might lead to further increase of ROS, thereby causing oxidatively damaging biomolecules, including proteins, lipids, carbohydrates, DNA and RNA (McCaddon & Kelly, 1994). ROS are responsible for progressive age related neuronal damage involving the accumulation of aberrant proteins, defective mitochondria and lipofuscin loaded lysosomes (McCoy et al., 1993). These changes may, finally, culminate in neuronal apoptosis and release of Aβ from dying cells (McGeer et al., 1991). Moreover, oxidative stress upregulating APP processing leads to the increase in intracellular content of Aβ (McKenzie et al., 1994).

Aβ is aggregated in the presence of free radicals and acts as a prooxidant by generating more free radicals, thus inducing cell death by a ROS-mediated mechanism (Mirra et al., 1991). In AD, levels of oxidative stress and protein oxidation increase predominantly in cognition-associated Aβ-rich regions, such as the cortex and hippocampus (Moro et al., 2012). Aβ has been shown to exert neurotoxicity by increasing neuronal sensitivity to oxidative stress (Morrison & Hof, 2007). Furthermore, there is extensive evidence that redox-active transition metals are involved in AD pathogenesis (Nandy, 1983). AD brains have increased concentrations of metals that catalyze the production of free radicals, including iron and aluminum (Nochlin et al., 1993). Other metals such as copper and zinc may also be involved. Copper is reduced in the hippocampus of AD brains and it is essential for the activity of many enzymes such as cytochrome-c oxidase.
(Ohgami et al., 1991). Both copper and zinc bind APP; and it is believed that this can modulate the functional properties of the molecule (Oyama et al., 1995).

In addition, the source of oxidant species in central nervous system (CNS) includes altered mitochondrial function, the Aβ peptides and the presence of unbound transition metals (Oyama et al., 1995). These factors are related to each other. In early stages of the disease, Aβ could enter the mitochondria where it would increase the generation of ROS and induce oxidative stress. Interestingly, Aβ and APP found in mitochondrial membranes can block transport of protein and disrupt the electron transport chain with final, irreversible cell damage. Evidence indicates that accumulating effects of long and gradual oxidative damage precedes the appearance of clinical and pathological AD symptoms, including Aβ deposition, neurofibrillary tangle formation, metabolic dysfunction, and cognitive decline (Panja et al., 2006). The markers of oxidative stress such as protein, DNA, RNA oxidation or lipid peroxidation have been identified in the AD brain, which supports the „oxidative stress hypothesis” (Perez et al., 2004). Consistent with the role of oxidative stress in AD pathogenesis, some studies reported positive effects of antioxidant intake that lowered the risk for AD (Perl, 1985).

Genetic mutations of APP or PS1 increase Aβ formation. Oxidative stress can increase APP levels or modulate the activity, elevating levels of β-secretase (BACE) and γ-secretase, influence Aβ formation. Aβ is pro-oxidant factor and can induce more oxidative stress, creates positive feedback on APP levels and on its proteolytic pathway (Panja et al., 2006). The elevated levels of Aβ oligomers favor the phosphorylation of tau protein. With time, Aβ oligomers are deposited in the extracellular space forming senile plaques (SPs), whereas inside neurons, the hyperphosphorylated tau form neurofibrillary tangles (NFTs). Both lesions trigger further oxidative stress reactions and sustained inflammatory responses, which ultimately will result in irreversible cell damage, slow degeneration and eventual cell death. These cell-biologic events will clinically manifest with progressive cognitive decline, early signs of dementia and, finally, full clinical AD (Reddy et al., 2010).

2.4.2. Aβ, Synaptic Alterations, and Mitochondrial Damage in Alzheimer’s disease

According to Reddy & Beal (2008) have been showed that the synapses are the sites of high-energy demand. Synaptic damage is considered the earliest cellular event in AD pathogenesis, and synaptic loss is the best correlate of cognitive impairment in AD (Hamos et al., 1989). Damaged synapses due to insufficient mitochondrial ATP lead to synaptic degeneration (Reddy & Beal, 2008). Synapses and neurites are mostly damaged in the vicinity of Aβ plaques (Dong et al., 2007). In healthy subjects, synaptic terminals transmit signals between cells in order to process information. During aging, the number of synapses and their transmissions of signals dramatically decrease (Scheff et al., 1985). The decrease in synapses has been documented in different brain regions of aged persons, supporting the hypothesis that synaptic changes are ubiquitous features of normal brain aging (Reddy et al., 2010).

2.4.2.1. Synaptic loss and Alzheimer’s disease

According to Bertoni et al (1990) have been showed that by using electron microscopy and AD postmortem brains revealed a loss of synapses in the hippocampus of AD patients compared the
number of synapses in control subjects. This loss correlates with cognitive decline in AD patients. Bretoni et al (1990) studied the number of synapses per neurons in cerebellar and hippocampal brain tissues from adult and aged control subjects and from AD-affected and unaffected brain tissues in patients with AD. The synapse-to-neuron ratio varied according to the brain regions from which the samples were taken and the individual’s health. No significant differences in the synapse-to-neuron ratio were found in samples taken from the cerebellum of adult and aged persons without AD and of aged AD patients.

However, in the hippocampal samples, the synapse-to-neuron ratio decreased more than 50% in the adult and aged persons without AD, compared to the ratio in the aged AD patients. In several studies investigating the extent that synaptic loss correlates with cognitive decline in AD patients (DeKosky et al., 1996) researchers found a 25–30% decrease in synapses in the cortex and a 15–35% decrease in synapses per cortical neuron, suggesting that synaptic loss in AD patients may correlate more with cognitive decline than with the number of Aβ plaques, neurofibrillary tangles, neuronal loss, or the extent of cortical gliosis (Reddy et al., 2010).

2.4.2.2. Loss of Synaptic Proteins and Alzheimer’s disease

Using immunoblotting and immunohistochemical analyses to determine synaptic proteins, several studies revealed decreased levels of presynaptic (synaptophysin) and postsynaptic proteins in AD patients compared to age-matched control subjects (Sze et al., 1997), suggesting that presynaptic and postsynaptic proteins are critically involved in AD progression. Further, immunoblotting analysis of postmortem brain tissues from the cerebral cortex of AD transgenic mice also revealed decreased levels synaptic in AD transgenic mice (Almeida et al., 2005), suggesting that the loss of synaptic proteins are confined to brain regions known to be affected in AD(Reddy et al., 2010).

2.4.2.3. Cell to Cell Transfer of Pathogenic Proteins

One of the first studies to suggest that the degeneration in AD could spread across normal synaptic projections, and involve the transfer of substances between neurons, was by Saper et al (1987). More recent research confirms these ideas and suggests that pathogenic proteins, including tau, α-synuclein, the disease form of prion protein (PrPsc), and Aβ may be secreted from cells, enter other cells, and seed small intracellular aggregates within these cells (Steiner et al., 2011). Hence, tau and Aβ could exit cells via exocytosis or secretion and enter a new cell by endocytosis or by interactions with membrane lipids. Transfer may also occur via tunnelling nanotubes (TNT) which connect various neurons (Steiner et al., 2011). For example, if tau spreads from cell to cell in the cortex, the resulting NFT may exhibit a spatial pattern which reflects this spread. Previous studies have suggested non-random distributions of NCI in the cerebral cortex of various disorders, the inclusions often exhibiting a distinct clustering pattern consistent with their spread via the cortico-cortical pathways (Armstrong et al., 2001).
2.4. 3. Theories based on environmental factors

Many environmental factors have been linked to AD, but most studies relate to three such variables, viz., exposure to aluminium (Al), effect of head trauma, and the influence of diet and malnutrition.

2.4.3.1. Aluminium
Much of the evidence that Al is a cause of AD is circumstantial and controversial (Armstrong RA et al., 1996). Epidemiological studies (Graves et al., 1990) have found little correlation between environmental Al and AD. In addition, out of 13 studies, in which gross brain tissue has been analyzed for Al, 9/13 found enhanced levels in AD brain, while 4/13 found no significant differences compared with control brains (Basun et al., 1991). The significance of enhanced Al levels in brain is also unclear since damaged brains may accumulate Al (Armstrong et al., 2001). The presence of Al may also be linked to the formation of SP and NFT.

2.4.3.2. Head Injury

Head trauma results in a primary injury which frequently spreads via inflammatory cytokines to initially unaffected regions, thus amplifying the original injury due to the activation of microglia and central nervous system immune cells (Armstrong et al., 2001). Several observations suggest a link between head injury and AD. In survivors of head injury, APP is observed in neuronal perikarya and in DN surrounding Aβ deposits, as in AD (Gentleman et al., 1993). The formation of Aβ from APP occurs within the synaptic terminal fold of axons, the presence of glia not being necessary for this conversion. Hence, the production of APP may be a component of the brain’s response to neuronal injury (Gentleman et al., 1993). Subsequently, it was shown that specific neurons in the medial temporal lobe secreted large quantities of APP and that there were more APP-immunoreactive neurons in these areas in head injury patients (McKenzie et al., 1994).

Hence, an increased expression of APP in head trauma cases may be an acute phase response to neuronal injury (Roberts et al., 1994), the overexpression of APP leading to the deposition of Aβ. Several acute phase proteins are localised within Aβ deposits in AD including amyloid-P, complement factors, and α-antichymotrypsin (Kalaria & Perry, 1993). Furthermore, Regland and Gottfries (1992) proposed that APP maintains cell function by supporting neuronal growth and survival. The possible neurotrophic action of APP is supported by the observation that it shares structural features with the precursor for the epidermal growth factor (Armstrong et al., 2001). NFT may also be a part of the neurons response to injury (Wisniewski et al., 1994). These studies suggest that the formation of pathological proteins as a result of brain injury is one method by which AD pathology develops and is then propagated within the brain by cell to cell transfer.

2.4.3.3. Diet and malnutrition

Abalan (1984) was one of the first authors to propose that AD could be caused by malnutrition. This hypothesis is based on clinical observation of AD patients who often exhibit emaciation and cachexia, urinary tract infections, terminal bronchopneumonia, and low triceps skin fold. Low serum albumin, iron, folate, tryptophan, vitamin B12, and low cerebral metabolism of glucose
and oxygen may also be present. These symptoms suggest a protein calorie malnutrition syndrome in AD which could result in the development of NFT due to chronic nutritional deficiencies of calcium and magnesium. A problem with this type of hypothesis, however, is in determining cause and effect, as malnutrition could be a consequence of the disease resulting from the mental state of the patient (Abalan, 1984). A more direct demonstration of a link between diet and AD has been reported by Sparks et al (1994) in which deposition of Aβ was induced in rabbits fed with high levels of dietary cholesterol.

In addition, McCaddon and Kelly (1994) found in a human family carrying a mutation of the APP gene (APP<sub>717</sub> Val-Glycine), that individuals with AD had a greater vitamin B12 deficiency, compared with unaffected members. It was concluded that this link was unlikely to be secondary and to be a consequence of impaired dietary intake. A B12 deficiency could then result in a reduction of monoamine transmitters and in cholinergic activity.

![Fig 4. The original Amyloid Cascade Hypothesis (ACH). The ACH proposes that the deposition of β-amyloid (Aβ) peptides is the initial pathological event in AD leading to the formation of senile plaques (SP) and neurofibrillary tangles (NFT), and then to cell death and dementia, both SP and NFT acquiring several additional proteins during their formation, such as apolipoprotein E (Apo E), glial fibrillary acidic protein (GFAP), ubiquitin (Ub), and complement. Other abbreviations: Amyloid precursor protein mutations (APPm), Presenilin genes 1 and 2 mutations (PSEN1/2m) (Abalan, 1984).](image)

### 2.4.4. Theories Based on Genetics

In the 1990s, strong evidence emerged of the connection between familial AD (FAD) and specific genetic factors. Hence, small numbers of cases were linked to APP mutations (Chartier et al., 1991) and a larger subgroup to PSEN1/2 mutations (Sherrington et al., 1993), while others genes are currently unidentified (Sorbi et al., 2001). In addition, allelic variation in the Apo E locus on chromosome 19 was identified as a significant risk factor, especially in late-onset AD.
2.4.5. Theories Based on Blood Brain Barrier Dysfunction

The involvement of the cerebral blood vessels in the pathogenesis of AD has been controversial (Attems et al., 2004). Some studies have found spatial correlations between Aβ deposits and blood vessels suggesting that degeneration of blood vessels or diffusion of substances from vessels could be involved in the formation of Aβ deposits (Miyakawa et al., 1992). By contrast, other studies conclude that the spatial correlations observed between Aβ deposits and blood vessels are fortuitous and arise because of the presence of high densities of capillary profiles and Aβ (Kawai et al., 1990). In the cerebral cortex of cases of SAD, however, of the three Aβ deposit subtypes, only the classic Aβ deposits exhibited a consistent spatial relationship with blood vessels (Armstrong, 2011).

Classic deposits were clustered specifically around larger blood vessel profiles, such as the vertically penetrating arterioles, the number of classic deposits declining exponentially with distance from the vessels (Armstrong, 2011). A number of factors could explain the correlation between Aβ deposits and blood vessels in AD (Fig. 5). First, Aβ could develop in association with the basement membranes or smooth muscle of blood vessel walls (Perlmutter & Chui, 1990). Tian et al (2006) found that blood vessels underwent degenerative changes in AD accompanied by Aβ deposition and loss of smooth muscle cells. Second, Aβ could be released by axon terminals or reactive glial cells juxtaposed to vessel walls. Attems et al (2004), for example, found that Aβ42 was deposited within the glia limitans rather than the capillary walls. Third, diffusion could occur from degenerating arterioles or from clusters of capillaries surrounding the larger blood vessels. Blood vessels with collapsed or degenerated endothelia are evident in more than 90% of AD cases (Kalaria & Hedera, 1995) and occur concurrently with Aβ deposition.

Fig 5. Relationships between the cerebral microvasculature and the development of cored (classical) senile plaques (SP). β-amyloid (Aβ) could develop in association with the basement membranes or smooth muscle (S) of blood vessel walls or Aβ could be released by axon terminals or reactive glial cells juxtaposed to vessel wall. Diffusion of proteins such as amyloid-P could also occur from degenerating arterioles or from clusters of capillaries surrounding the
larger blood vessels and influence cored SP development. Deposition of Aβ around the larger blood vessels could also be a result of impaired drainage (Allsop et al., 1989).

According to Mann (1985) has been reported that the integrity of the brain microvasculature may be related to neuronal degeneration and especially to age-related cell losses in the LC. In AD transgenic mice, deposition of Aβ in blood vessels is associated with endothelial cell activation and apoptosis (Schultheiss et al., 2006), which could encourage diffusion. Endothelial cell injury could also depend on duration of dementia (Tian et al., 2006). If diffusion is involved in the pathogenesis of the classic Aβ deposits, then various plasma proteins may be implicated. Amyloid fibrils have been observed projecting directly from blood vessels towards classic-type deposits (Vuia, 1978) suggesting that Aβ itself could diffuse from blood vessels. However, most Aβ deposited in the cortex is likely to be of neuronal origin (Allsop et al., 1989).

2.4.6. Theories Based on Infectious Agents

Indirect evidence that infection could be a cause of AD has been reported by Wisniewski et al (1994) who suggested that invasion by a virus could cause activation of microglia and pericytes and ultimately, amyloid deposition. In addition, Libikova et al (1975) suggested that the virus herpes simplex (HSV), to which antibodies may be observed in the cerebral spinal fluid (CSF) in AD, could induce abnormal protein formation and result in PHF and NFT. This type of theory has been given further credibility by observations of marked structural and biochemical alterations in regions associated with olfaction, most notably the olfactory bulb and EC (Doty et al., 1987). The olfactory system is a possible point of entry into the brain for an infectious agent or protein (Mann et al., 1987). Hence, experimentally introduced viruses into body cavities and organs often result in high titres of the virus in olfactory epithelium, olfactory bulb, and other brain regions. Nevertheless, it is unlikely that HSV is the responsible virus, as it does not typically enter the brain via the olfactory system (Salazar et al., 1983).

2.5. Current Alzheimer’s Therapeutics

Alzheimer’s disease is a complex disease with a prolonged trajectory of etiopathogenic changes in brain bioenergetics decades prior to the clinical onset of the disease. Therefore, targeting only the pathological aspect, particularly the β-amyloid pathway, is insufficient to achieve full efficacy to prevent, delay, or even reverse the disease progression. Recently, Eli Lilly announced cessation of its phase III clinical trial of semagacestat, a γ-secretase inhibitor, as the drug candidate failed to achieve efficacy in slowing disease progression and was associated with worsening of clinical measurement of cognitive function (Extance, 2010). Similarly, other Aβ targeting candidates, tarenflurbil, tramiprosate and flurizan, also failed in Phase II or phase III clinical trials (Raybon & Albright, 2010), although these candidates have been demonstrated to lower or reduce Aβ production in preclinical and early phase clinical studies (Raybon & Albright, 2010). Further, the clinical potential of the γ-secretase inhibitors are often complicated by its off-target interferences on the Notch signaling pathway.

Another category of anti-Aβ therapeutics includes Aβ42 vaccines, monoclonal Aβ antibodies and polyclonal antibodies. These candidates act via an immunotherapeutic mechanism to promote the
clearance of amyloid. Yet, the active immunization approach, such as the amyloid vaccine AN-1792 (Elan/Wyeth), was discontinued due to increased risk of severe meningoencephalitis in the patients despite the trend towards positive efficacy it achieved (Rafii & Aisen, 2009). On the other hand, bapineuzumab (Elan/Wyeth), a passive immunization monoclonal antibody of Aβ, failed to achieve expected efficacy in a Phase II clinical trial, although it did exhibit some benefits in AD patients who did not carry the APOE4 genetic risk factor (Rafii & Aisen, 2009).

Other Aβ antibodies are currently under various stages of clinical trials and the efficacy is yet to be determined. Similarly, candidates that target Tau pathology as disease modifying therapeutics for AD are still in the early stage of development despite the potential benefits exhibited in preclinical animal studies. Aside from the anti-Aβ/tau strategy, antioxidants have been proposed as potential therapeutics for AD. Analyses on specimens obtained from AD patients and various preclinical animal models clearly documented elevated oxidative damage on cellular components (Yao et al., 2009).

The therapeutic potential of antioxidants is further supported by vast epidemiological analyses that demonstrated a positive correlation between the usage of antioxidant, particularly vitamin E and C, and cognitive function. Further early administration of antioxidants such as curcumin or vitamin E, has been demonstrated to suppress amyloidogenesis in several preclinical AD rodent models (Lim et al., 2001). However, multiple randomized clinical trials of high dosage of vitamin E usage failed to achieve significant efficacy (Brewer, 2010), indicating the therapeutic limit of using exogenous antioxidants to scavenge oxidative insults rather than suppress the generation of oxidative insults.
3. Conclusion

Increasing evidence suggests that Aβ, mitochondrial dysfunction, and synaptic damage are critically involved in AD progression and development. The latest research into AD revealed that Aβ and mitochondrial abnormalities are key factors that cause synaptic damage in AD neurons. Aβ is reported to accumulate in subcellular compartments and to impair the normal function of neurons. Further, recent in vitro and in vivo studies of Aβ using biochemical methods and electron microscopy revealed that the accumulation of Aβ at nerve terminals damages synaptic activities, including the release of neurotransmitters and synaptic vesicles. Further, recent discoveries of mitochondria in AD suggest that structural changes in mitochondria, including increased mitochondrial fragmentation and decreased mitochondrial fusion, are critical factors associated with mitochondrial dysfunction and synaptic damage in AD. Despite tremendous progress that has been made in AD research, we still do not have drugs or other agents to prevent or slow down disease progression. Further, we still do not know the precise toxic effects that are caused by Aβ and mitochondrial abnormalities at synapses, particularly in neurons, that are involved in cognitive decline. Further research is needed to develop drugs capable of crossing the blood-brain barrier and targeting mitochondria, and to develop the agents to boost mitochondrial function and decrease Aβ toxicity and improve synaptic branching and cognitive functions in elderly people and patients with AD.
4. Reference


Armstrong RA. (2011). Spatial patterns of β-amyloid (Aβ) deposits in familial and sporadic


# Table of contents

<table>
<thead>
<tr>
<th>Contents</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table of Contents</td>
<td>i</td>
</tr>
<tr>
<td>List of Figures</td>
<td>iii</td>
</tr>
<tr>
<td>Acronyms</td>
<td>iv</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>vi</td>
</tr>
<tr>
<td>Abstract</td>
<td>vii</td>
</tr>
<tr>
<td>1. Introduction</td>
<td>1</td>
</tr>
<tr>
<td>2. Literature Review</td>
<td>3</td>
</tr>
<tr>
<td>2.1. Molecular mechanisms leading to AD</td>
<td>3</td>
</tr>
<tr>
<td>2.1.1. Amyloid Cascade Hypothesis</td>
<td>4</td>
</tr>
<tr>
<td>2.1.2. The Mitochondrial Cascade Hypothesis</td>
<td>6</td>
</tr>
<tr>
<td>2.1.3. The Neuroinflammation Hypothesis of AD</td>
<td>9</td>
</tr>
<tr>
<td>2.2. Biology of Tau</td>
<td>10</td>
</tr>
<tr>
<td>2.2.1. Pathological Forms of Tau</td>
<td>11</td>
</tr>
<tr>
<td>2.2.2. Mechanisms of Tau Toxicity</td>
<td>12</td>
</tr>
<tr>
<td>2.2.3. Are Tau Pathology and Cell Cycle Re-entry Related Events?</td>
<td>12</td>
</tr>
<tr>
<td>2.3. Oxidative Stress</td>
<td>14</td>
</tr>
<tr>
<td>2.3.1. Reactive Oxygen Species and Oxidative Stress</td>
<td>14</td>
</tr>
<tr>
<td>2.4. What causes Alzheimer’s disease</td>
<td>14</td>
</tr>
<tr>
<td>2.4.1. Theories Based on Aging</td>
<td>15</td>
</tr>
<tr>
<td>2.4.1.1. Oxidative Stress and Aging</td>
<td>16</td>
</tr>
<tr>
<td>2.4.1.2. Oxidative Stress and AD</td>
<td>16</td>
</tr>
<tr>
<td>2.4.2. Aβ Synaptic Alterations, and Mitochondrial Damage in Alzheimer’s disease</td>
<td>17</td>
</tr>
<tr>
<td>2.4.2.1. Synaptic loss and Alzheimer’s disease</td>
<td>17</td>
</tr>
<tr>
<td>2.4.2.2. Loss of Synaptic Proteins and Alzheimer’s disease</td>
<td>18</td>
</tr>
<tr>
<td>2.4.2.3. Cell to Cell Transfer of Pathogenic Proteins</td>
<td>18</td>
</tr>
</tbody>
</table>
2.4.3. Theories Based on Environmental Factors...........................................................19
  2.4.3.1. Aluminium.......................................................................................................19
  2.4.3.2. Head Injury...............................................................................................19
  2.4.3.3. Diet and Malnutrition.............................................................................19
  2.4.4. Theories Based on Genetics...........................................................................20
  2.4.5. Theories Based on Blood Brain Barrier Dysfunction.................................21
  2.4.6. Theories Based on Infectious Agents............................................................22
  2.5. Current Alzheimer’s Therapeutics......................................................................22
3. Conclusion...............................................................................................................24
4. Reference................................................................................................................25
List of Figures

<table>
<thead>
<tr>
<th>Contents</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1: Schematic Diagram Shows the Two Proteolytic Pathways of Amyloid Precursor Protein: Amyloidogenic and Nonamyloidogenic</td>
<td>5</td>
</tr>
<tr>
<td>Figure 2: Shows the Amyloid Cascade Hypothesis</td>
<td>6</td>
</tr>
<tr>
<td>Figure 3: Shows the Mitochondrial Cascade Hypothesis</td>
<td>9</td>
</tr>
<tr>
<td>Figure 4: Shows the Potential Protective Mechanisms of NSAIDs in AD</td>
<td>10</td>
</tr>
</tbody>
</table>
Acronyms

ACH............................................Amyloid Cascade Hypothesis
AD................................................Alzheimer’s disease
Aβ..................................................Amyloid-Beta
Al................................................Aluminium
ApoE.............................................Apolipoprotein Epsilon 4)
APP..................................................Amyloid Precursor Protein
APPm...............................................Amyloid Precursor Protein Mutations
Cdk5.............................................Cyclindependent Kinase 5
CNS..................................................Central Nerves System
CO..................................................Cytochrome Oxidase
COX-2.............................................Cyclooxygenase 2
CSF..................................................Cerebral Spinal Fluid
DN..................................................Dystrophic Neurites
FDA..................................................Food and Drug Administration
GFAP..............................................Glial Fibrillary Acidic Protein
GSK3β.............................................Glycogen Synthase Kinase 3β
H2O2...............................................Hydrogen Peroxide
HSV..................................................Virus Herpes Simplex
IL-1β.............................................Interleukin 1β
IGF1..................................................Insulin-Like Growth Factor 1
IRS2..................................................Insulin Receptor Substrate 2
5LOX...............................................5-Lipoxygenase
MAP...............................................Microtubule Associated Protein
MAPK...............................................Mitogen Activated Protein Kinase
MARK...........................................Microtubule Affinity Regulating Kinase
MCI...........................................Mild Cognitive Impairment
M-CSF...........................................Macrophage Colony Stimulating Factor
mTOR...........................................Mammalian Target of Rapamycin
NSAIDs........................................Nonsteroidal Anti-Inflammatory Drugs
NFT............................................Neurofibrillary Tangles
NOS2...........................................Nitric Oxide Synthase 2
(O2−)...........................................Superoxide Radical Anion
(OH•)...........................................Hydroxyl Radical
PGE2...........................................Product, Eicosanoid Prostaglandin E2
PHG...........................................Parahippocampal Gyrus
PHF...........................................Paired Helical Filaments
PI3K ...........................................Phosphoinositide 3 Kinase
PPAR-γ......................................Peroxisome Proliferator-Activated Receptor Isoform
Gamma
PSEN-1/2......................................Presenilin-1/2
RAGE..........................................Receptor for Advanced Glycation End Products
ROS...........................................Reactive Oxygen Species
RNS...........................................Reactive Nitrogen Species
SOD...........................................Superoxide Dismutase
SP..............................................Senile Plaques
TGF-β.........................................Transforming Growth Factor-B
TNF-α..........................................Tumour Necrosis Factor-Alpa
TNT.............................................Tunnelling Nanotubes
Acknowledgements

Above all, I would like to thank the Almighty Allah who blessed my life in all movements, also I want to thank everyone that have helped and supported me during this time. I thank him for the wonderful change, peace, joy and love brought to my life in my stay as well as working this project. I would also like to thank my enthusiastic and supportive advisor Dr.Getahun Shibru for his wonderful and kindest encouragement, constructive comments and invaluable assistance throughout the whole academic year as well as this project paper. Moreover, I wish to extend my most sincere gratitude to my friends for their excellent guidance by giving me supportive ideas throughout my work. Lastly but not least I am highly indebted to all physiology staff members, and my beloved friends and family outside academia as well as my sponsorship Arsi University for their unreserved support and remarkable contribution in this project and my academic success.
Abstract

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder characterized clinically by memory and cognitive dysfunction and also protein misfolding-based rapid cognitive impairment in the aging brain. It can inhibit the protein expression of specific genes by activating a sequence-specific RNA degradation process. This is a powerful tool with which to study gene function, investigate the mechanism of the disease, and validate drug targets. In this review, we summarize the systems biology data on AD and pay particular attention to the proteomic changes in AD. Applying a systems biology model of the synapse, we attempt to integrate protein changes and provide an explanation of why seemingly diverse molecular changes result in memory impairment. Finally, we give a systems biology model of AD explaining how AD can develop in an individual manner in each particular subject but always results in a rapidly developing dementia and memory impairment. Unfortunately, there is no effective therapeutic method for AD treatment or ways to halt disease progression. Many mechanisms are involved in the disease, including genes mutation and protein dysfunction.

Keywords: Alzheimer’s disease, Neurodegerative disease, Senile plaques, Assembled from β-amyloid (Aβ) peptides, & neurofibrillary tangles (NFT), Tau protein