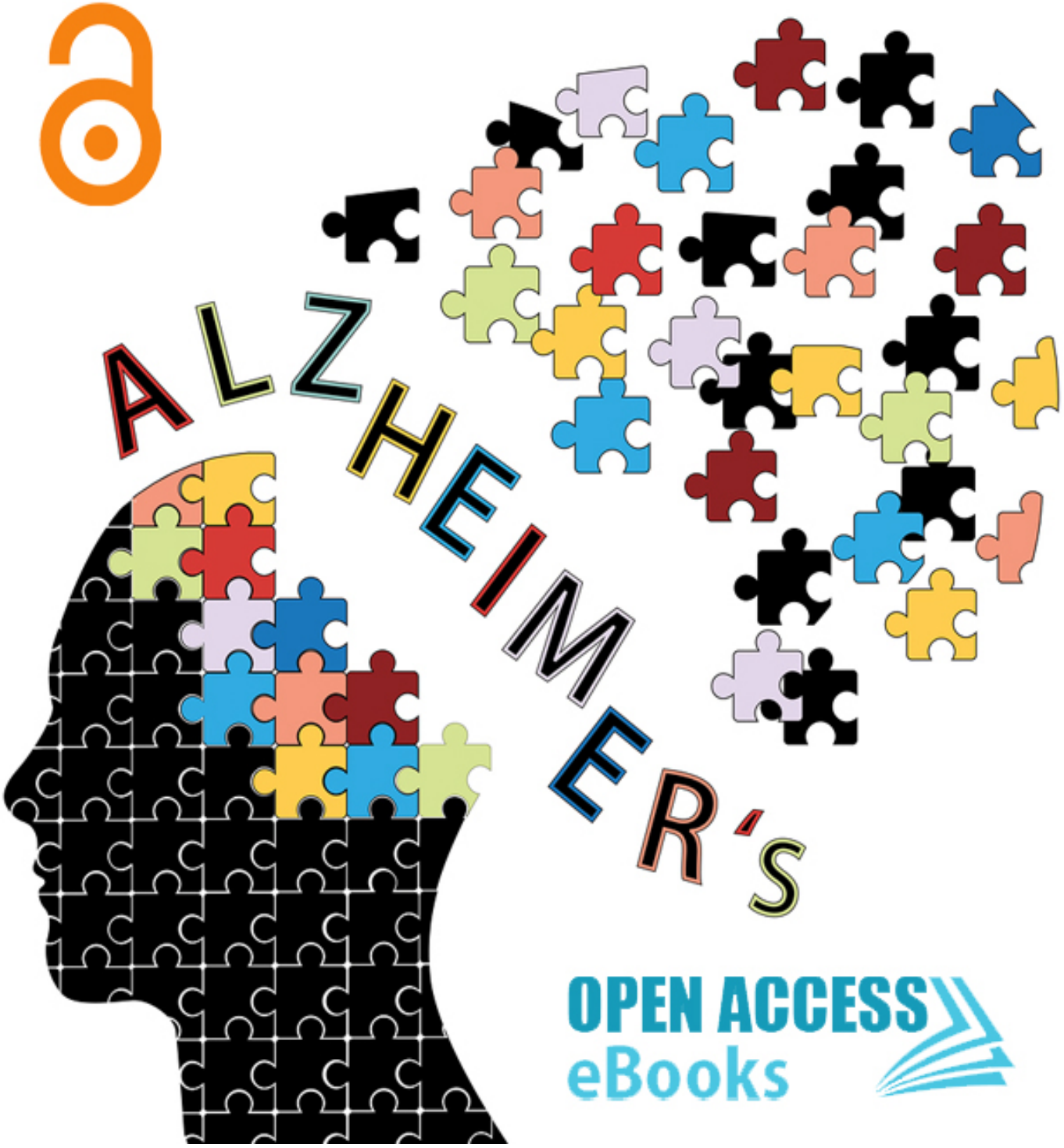
Alzheimer's Disease & Treatment



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Alzheimer's Disease & Treatment

Chapter 1

Therapeutic Strategies for the Treatment of Alzheimer's Disease-Recent Advances and Future Perspective

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Abstract

Alzheimer's disease (AD) a progressive and fatal neurodegenerative disorder is considered as scourge of 21st century, as it acts as a biggest health challenge and socioeconomic burden for the developed countries. Neuropathological hallmarks of AD involves extracellular deposition of senile plaques composed of fibrillar β amyloid peptide (Aβ) in the synaptic junction and presence of neurofibrillary tangles (NFT) in the axon leading to impairment of neurotransmission causing neuronal death. AD is multifactorial disorder, with complex biochemical pathways leading to its pathogenesis; hence single targeted drugs will not be efficient for the treatment of AD. Current symptomatic treatment for AD includes cholinesterase inhibitors and N-methyl-D aspartate receptor antagonist, which are poorly effective for mild to moderate AD and also possess severe side effects. Recent research on pathogenesis of AD unfolded several therapeutic strategies for the treatment of AD which includes (1) drugs interfering with Aβ deposition such as β and γ secretase inhibitors, attenuators of Aβ aggregation, deaggregators of β amyloid plaque, metal chelators, active and passive vaccination against Aβ peptide (2) drugs modulating Tau protein phosphorylation and deposition like glycogen synthase kinase 3β inhibitors (GSK-3β), vaccination against τ-protein (3) Anti-inflammatory and antioxidant drugs which attenuates the oxidative/nitrosative stress induced by Aβ peptide (4) modulators of cholesterol and vascular risk factor. The present review focuses on summarizing the recent findings and advances in the therapeutic strategies for the treatment of AD.

Keywords: β-amyloid peptide; Oxidative stress; Neuroinflammation; Tau protein; Immunotherapy; Traditional medicine

Citation: *Suganthy N, Hanan F Aly, Lars-Gunnar Gunnarsson, Ganiyu Oboh, Karthikeyan Muthusamy, Belkhelfa M (2018) Alzheimer's Disease & Treatment, Vol. 1, pp. 1-125.*

Abbreviations: Aβ: β amyloid; ACE I: Angiotensin converting enzyme I; AChE: Acetylcholinesterase; ACh: Acetylcholine; AD: Alzheimer's disease; ApoE: Apolipoprotein E; APP: Amyloid precursor protein; BACE1: β Secretase; BDNF: Brain-derived neurotrophic factor; BuChE: Butyrylcholineesterase; CDK5: Serine/threonine cyclin dependent kinase; ChAT: Choline acetyl transferase; CNS: Central nervous system; CSF: Cerebrospinal fluid; CREB: cAMP response element-binding protein , ECGC: Epigallocatechin gallate; GSK-3β : Glycogen synthase kinase 3β; IDE: Insulin degrading enzyme; IL-1β: Interleukin 1 beta; IL-6: Interleukin-6; IF-γ: Interferon gamma; LRP: Low-density lipoprotein; MAPT: Microtubule-associated protein Tau; MAPK: mitogen activated protein kinase; NFT: Neurofibrallary tangles (NFT); NGF: Neuronal growth factor; NSAIDs: Nonsteroidal anti-inflammatory drugs; PKC: protein kinase C; ROS: Reactive oxygen species; TNF-α: Tumor necrosis factor alpha.

1. Introduction

Alzheimer's disease is an irreversible degenerative brain disorder comprising 60-80% of dementia cases affecting particularly the elderly persons above the age of 60. AD is one of the fifth leading causatives of death creating huge socioeconomic burden to the developed countries [1]. Clinically, AD is characterized by progressive loss of cognitive function mostly memory and learning affecting the daily activities of life with death occurring within 9 years of incidence [2]. Epidemiological studies reported that worldwide approximately 36 million (3 per 1000 individuals) people are suffering from AD and this expected to quadruple to 115 million by 2050, if no effective therapy intervenes [3,4]. Alzheimer's disease is classified as familial and sporadic AD, both of which share similar clinical and pathological features such as deposition of abnormal protein aggregates, defects in axonal transport, synaptic loss ultimately leading to neuronal death. Familial type AD also termed as early onset AD constitute 5% of the total AD cases affecting people aged between 30-60 years. Mutation in gene encoding for amyloid precursor protein (APP; chromosome 21), Presenillin 1 and 2 encoding for γ secretase enzyme (PSEN 1, 2; Chromosome 1 and 14) and patient suffering from Down's syndrome (21st trisomy) have high risk of familial type AD. About 95% of the total AD cases are sporadic type (late onset), which is incident in elderly population above the age of 65 [5]. Several reversible and irreversible factors such as aging, nutrition, life style, environmental factors, increased expression of ApoE4 gene and chronic metabolic disorders provokes the incidence of late onset AD. Among the etiological factors, aging is the major risk factor as incidence of AD increases exponentially with age [6].

Neuropathological hall marks of AD include both positive and negative lesions leading to neuronal death. Classical positive lesion includes extracellular deposition of amyloid plaques composed of Aβ (1-42) peptide in the synaptic junction and intracellular accumulation of neurofibrallary tangles (NFT) containing hyperphosphorylated tau protein in the axonal region. Deposition of amyloid plaques and NFT is accompanied by activation of astrocytes and microglial cells causing impairment in the synaptic junction leading to neuronal death, the core negative features of AD. Unique lesions like amyloid plaques are widely found in the cortical mantle, while the NFT are present in limbic and associated cortices. Degeneration

of neurons occurs early in the entorhinal/peirhinal cortex followed by hippocampus region, the associated cortex, finally in the primary cortex causing neuronal loss leading to cognitive impairment. The disease primarily affects cognitive function like learning and memory initially, while in the advanced stages patient suffer from loss of motor functions, which are essential for the basic activities of daily life [7]. As etiological factors leading to AD are not well defined, multiple hypotheses were proposed as causatives for the pathophysiology of AD, among, which amyloidogenic pathway that causes deposition of the amyloid-β peptide (Aβ) in the brain is a considered as central event. Other pathways including oxidative stress, inflammation, tau aggregation and cholinergic dysfunction are the secondary pathways triggered as a result of amyloidogenic pathway [8].

2. Processing of Amyloid Precursor Protein (APP)

Amyloid precursor protein is a ubiquitous transmembrane protein of about 639-770 amino acid long, highly expressed in the nervous system. APP exists in three isoforms, APP695 expressed in neurons, while APP751 and APP770 isoforms are highly expressed in non-neuronal cells. APP plays significant role in differentiation of neurons, neurogenesis, maintenance of synaptic plasticity, regulation of apoptosis and cell proliferation. About 90% of APP is processed by non-amyloidogenic, while 10% enters into the amyloidogenic pathway (**Figure 1**) the ratio may vary according to environmental factors, mutation and aging [9]. In the non-amyloidogenic pathway, γ-secretase cleaves APP between aminoacids 16(Lys) and 17(Leu) forming N-terminal secreted APP (sAPPα) exhibiting neurotrophic effect and 83- aminoacid containing C-terminal fragment (CTF), which plays crucial role in neurotrophic and calcium regulation. CTF will be further cleaved by γ secretase to form 3kDa product (P3) and APP intracellular domain (AICD), which is involved in nuclear signaling via transcriptional regulation, as well as axonal transport through its ability to associate with different proteins [10].

In the amyloidogenic pathway, APP is cleaved by β-secretase yielding N-terminal fragment (sAPPβ) and membrane bound C-terminal fragment (CTF-β) comprising of 99 amino-acid. CTFβ is further cleaved by γ secretase forming AICD and soluble Aβ fragment, which tends to accumulate into microscopic plaques. Level of Aβ in the extracellular space is influenced by several factors like neural activity and synaptic release, aging, and high cholesterol level. Length of Aβ peptides varies from 38 to 42 amino acids, among, which Aβ acts as major species deposited as plaques [11].

3. Pathogenesis Leading to AD

Presence of high concentration of transition metal ions like Cu^{2+}, Zn^{2+} and Fe^{2+} in the brain alleviates the aggregation of Aβ fragment formed in amyloidogenic pathway to oligomers, proto-fibrils and mature plaques. Deposition of mature plaques in the synaptic junction disrupts the cholinergic neurotransmission leading to neuronal death. In addition, plaques acti-

vate the associated astrocytes and microglial cells initiating inflammatory mechanism provoking death of neurons. Furthermore, reactive oxygen species (ROS) released during Aβ aggregation causes peroxidation of neuronal membrane lipids forming neurotoxic 4hydroxynonenal (4HNE), which alters the calcium homeostasis. Enhanced intracellular calcium activates kinases promoting hyperphosphorylation of tau protein forming neurofibriallary tangles (NFT), which gets deposited in the axonal region, affecting microtubules leading to neuronal death. Increased Ca^{2+} level also induce glutamate excitotoxicity, endoplasmic reticulum stress, mitochondrial dysfunction triggering caspase dependant apoptosis ultimately leading to neuronal death [12,13]. All these multiple cascade of events occurs primarily in the cholinergic neurons of hippocampal region of brain leading to impairment in cognitive function such as learning and memory (**Figure 2**).

4. Therapeutic Strategies for AD

Although genetical, biochemical and neuropathological evidences strongly illustrate Aβ and amyloid plaque formation as a central event in AD pathogenesis, the etiopathology of AD still remains unclear. Considerable evidences suggest AD as multifactorial disorder and metabolism of Aβ influences multiple pathways, which shift towards the pathogenic pathway under unfavorable condition leading to AD [12]. Several biochemical pathways including amyloidogenic pathway, cholinergic pathway, mitochondrial cascade, oxidative stress and neuroinflammation were implicated in the pathogenesis of AD. Hence current treatment strategies are focusing on therapeutic molecules targeting these pathways to prevent AD. Drug molecules targeting multiple pathways (**Figure 2**), which are under clinical trials, are tabulated in **Table 1**.

4.1. Cholinesterase inhibitors

According to cholinergic hypothesis decline in cognitive function in AD patients are caused due to deficit in cholinergic neurotransmission due to loss of acetylcholine level (ACh). The enzyme choline acetyltransferase (ChAT) plays a significant role in the synthesis of ACh, and the degradation of ACh is catalysed by cholinesterase. Deterioration of synaptic junction of cholinergic neurons together with decline in ChAT leads to reduction in level of ACh ultimately leading to impairment in cognitive function in AD patients [14]. In addition scientific evidences illustrated that acetylcholinesterase (AChE) interacts with Aβ peptide forming AChE-Aβ complex inducing the aggregation of Aβ peptide to form oligomers and mature plaques. This AChE-Aβ complex in the synaptic junction triggers neurotoxicity by dysregulating the calcium homestasis leading to mitochondrial dysfunction culminating to apoptosis [15,16]. Butyrylcholinesterase (Pseudocholinesterase) primarily localized in glial cells and subcortical neurons are also involved in the extra synaptic cleavage of ACh. Recent evidences s illustrated that BuChE with Aβ in senile plaques promotes its maturation from benign to ma

lignant plaques [17,18]. Based on these evidences, current therapeutic approach focuses on enhancing the cognitive function by inhibiting cholinesterase, thereby restoring the level of ACh necessary to stimulate nicotinic and muscarinic receptors within the brain [19]. Moreover, inhibition of ChE might block the aggregation of Aβ peptide attenuating the pathological casacade of AD [20]. FDA approved drugs like Galanthamine (Razadyne), Rivastigmine (Exelon) for mild to moderate AD and Donepezil (Aircept) for all stages of AD. Systemic reviews and clinical trials illustrated these drugs possess severe side effects and were effective only for mild to moderate and not for longer progressive disease [21]. Despite of FDA approved drugs, several drug molecules with potent AChE and BuChE activities were identified from natural source which are under clinical trials. One such drug Huperzine A, an alkaloid isolated from Huperzine serrata with potent AChE inhibitory activity has been commercialized as a dietary supplement for the treatment of AD in china [22]. Ladostigil (TV3326) which exhibits dual inhibition of AChE and monoamine oxidase are currently used for the treatment of AD [23].

4.2. N-methyl-D-aspartate antagonist (NMDA)

N-methyl-D-aspartate (NMDA) receptors have received must attention nowadays, despite of several post synaptic neuronal receptors as they play major role in neurodegenerative mechanism. L- Glutamate the predominant excitatory neurotransmitter of mammalian central nervous system plays important role in establishing new neural networks, maintaining synaptic plasticity, learning and memory. Glutamate binds to NMDA receptor in the post synaptic terminal transmitting neurosignals via the entry of calcium, which gets terminated by the uptake of glutamate by the excitatory aminoacid transporter in glial cells and astrocytes around the synapse. Increased glutamate level in the post synaptic cleft of AD causes hyperpolarisation of neurons leading to synaptic loss and neuronal shrinkage [24]. NMDA receptor antagonist binds to the receptor blocking the action of glutamate thereby preventing neuronal excitotoxicity and damage. Currently memantine the FDA approved glutaminergic NMDA receptor antagonist has been used for the treatment of moderate to severe AD. Memantine exhibits mild to moderate affinity to NMDA receptor in uncompetitive mode of inhibition. Experimental studies illustrated that memantine also showed protective effect against Aβ induced toxicity by attenuating neuronal apoptosis and increased synaptic density in the hippocampus. Randomized clinical trial showed improvement in cognition, behavior and ADL in people suffering from moderate to severe AD [25]. Administration of memantine caused certain adverse effects such dizziness, headache and confusion [26]. Clinical trials were attempted for combination therapy of donepezil and memantine, which was effective for severe AD and not for mild to moderate AD [27,28].

4.3. Therapeutic strategy modulating Aβ formation and amyloid deposition

4.3.1. Modulation of secretase enzymes

APP is processed by two competing proteases, α and β secretase to form sAPPα/sAPPβ followed by γ secretase activity to form P3/Aβ fragment. Formation of Aβ peptide in AD is regulated by β secretase (BACE1/memapsin 2) followed by γ-secretase cleavage, hence inhibiting β secretase and γ secretase or by promoting α-secretase cleavage can reduce Aβ load [29].

4.3.1.1. β secretase inhibitors/modulators

β secretase (BACE1) also termed as Asp2, and memapsin 2 is transmembrane aspartic protease comprising of 501 amino acid closely related to the pepsin family of aspartic proteases. BACE1 is the key enzyme involved in the formation of Aβ (1-42) peptide fragment in the amyloidogenic pathway. Scientific evidences illustrated that inhibition of BACE1 effectively reduced 90% Aβ production in cerebrospinal fluid (CSF) in human. BACE1 in addition to APP processing are involved in other biological function such as processing neuregulin -1 involved in maintaining synaptic plasticity, neurogenesis, so inhibition of BACE1 might cause severe secondary effects [30,31]. Second major limitation is poor oral bioavailability and obstacles like blood brain barrier permeability and efflux system P-glycoprotein, which acts as hurdle for the entry of BACE1 inhibitors in to brain. Current researches are focusing on selective BACE 1 modulators, which blocks APP processing without interfering its other signaling pathways [32]. Structure based design has led to identification of several peptide and nonpeptidomimetic compounds with BACE 1 inhibitory/modulating activity, which are still under preclinical and clinical trials. KMI-429 when administered in to the hippocampal region of APP transgenic mice significantly reduced Aβ production [33]. BACE1 inhibitor CTS-21166 reported to reduce the level of human plasma Aβ is under phase I clinical trials [34]. MK8931 (Verubecestat - Merck laboratories) entered in to Phase II clinical trials in 2012 effectively reduced the Aβ levels in human CSF., however it was discontinued due to lack of cognitive and clinical efficacy in mild to moderate AD patients (Kennedy et al., 2016). Similarly, AZD3839 from AstraZeneca and LY2811376 from Eli Lill, which entered in to phase IIa/b clinical trials in 2012, ended in 2013 due to anamolous finding in hepatic biochemical parameters of participants [35,36]. Ultimately the next move will be to develop inhibitors with better pharmaceutical properties and well defined clinical trials to rescue cognitive decline in patients with AD.

4.3.1.2. γ- secretase inhibitors/ modulators

γ Secretase is a nucleoprotein complex comprising four different proteins, among which Presenilin PS-1 and PS-2 are involved in the cleavage of α/β secretase cleaved fragments. As γ-secretase acts as therapeutic target in AD, a plethora of γ-secretase inhibitors (GSIs) have been developed that effectively inhibit γ-secretase cleavage of APP in humans [37]. Moreover γ-secretase in addition to APP are also involved in processing Notch receptor 1 necessary for

cell growth and development, so inhibition of γ secretase causes notch-related side effects hampering its pharmacological action. Current scientific investigation focuses on search for GSI with oral bioavailability, BBB permeability and substrate specificity [38]. Semagacestat (LY-450139) the potent γ-secretase inhibitor, decreases the generation of Aβ in concentration dependant manner in CSF of the healthy volunteers. However phase III clinical trial with semagacestat at higher dose (140 mg) was discontinued due to its unfavorable effects on cognition and brain function when compared to control due to the inhibition of Notch signaling pathway [39]. Other side effects such as weight loss, increased incidence of skin cancer and high risk of infection were also observed [40,41]. BMS-708163 (Avagacestat; Bristol-Myers Squibb, New York, NY, USA) a GSI showed decrease in level of CSF Aβ 40 and 42, following daily intake of 100 mg [42]. Phase II clinical trials with Avagacestat (100 and 125 mg) was interrupted due to its lack of efficacy in cognitive enhancement and adverse effects in gastrointestinal tract and skin [43]. Administration of LY450139 di-hydrate for 6 weeks in about 70 patients during RCT decreased the plasma Aβ 1-40 level with no significant toxicity [44]. Mayer et al. [45] identified two notch sparing GSIs Begacestat BMS-708163 and ELN-475516, which lowered the Aβ level in transgenic mice Tg257 mice at dosage of 100 mg/kg bw. Clinical trials with γ - secretase inhibitors are closely scrutinized because these agents might affect other protein like Notch causing deleterious effect in gastrointestinal tract and thymus. To avoid adverse effects, investigations are underway for search of γ - secretase modulators (GSM), which modulates the PSEN1 and 2 proteins, with notch sparing activity, BBB permeability without affecting other signaling pathway. Several nonsteriodal anti-inflammatory drugs (NSAIDs) such as ibuprofen, sulindac, indomethacin and flurbioprofen, which exhibited cylooxygenase (COX) inhibitory activity also showed decline in the level of Aβ (1-42) peptide under *in vitro* and *in vivo* condition [46]. Tarenflurbil (or R-flurbiprofen), a NSAID devoid of COX inhibitory activity acts as GSM, reducing Aβ level in preclinical studies, however Phase III clinical trials showed negative effect due to its low potency and brain penetration difficulty [47] CHF 5074 another NSAID devoid of COX inhibition with GSM activity, on chronic treatment in AD transgenic mice model system reduced brain β- amyloid burden, associated microglia inflammation and attenuated spatial memory deficit [48]. Phase I clinical studies with 144 volunteers reported CHF 5074 as safe and tolerable drug, but caused mild diarrhea. Similarly, Phase II was performed in 96 patients with amnestic / nonamnestic mild cognitive impairment (MCI) and the results of the study showed enhancement in cognitive function and memory [49]. Naturally occurring NSAID NIC-15 also known as pinitol a cyclic sugar alcohol, which modulates γ - secretase activity, reduced Aβ load, improving cognitive function, without affecting Notch signaling pathway in preclinical AD model system [50]

4.3.1.3. *α*-secretase activator

α-secretase a disintegrin and metalloproteinase (ADAM) cleaves at L688 residue lo-

cated within the Aβ peptide sequence preventing Aβ formation and its cleavage product sAPP α exhibits neuroprotective potential [51]. Mounting evidences indicated that activation of non-amyloidogenic pathway by stimulating α- secretase activity is one of the promising therapeutic strategy for the treatment of AD. Some potential therapeutics and lead compounds such as benzolactam derivative, TPPB [(2S,5S)-(E,E)-8-(5-(4-(trifluoromethyl)phenyl)-2,4-pentadienoylamino) benzolactam] and green tea polyphenol (-)-epigallocatechin-3-gallate activates α-secretase shifting towards nonamyloidogenic pathway reducing the level of Aβ in brain [52]. Etazolate (EHT 0202, ExonHit Therapeutics, Paris, France) a GABA receptor modulator stimulates the neurotrophic α-secretase attenuating Aβ induced neuronal death providing symptomatic relief for AD. Phase II clinical trials of EHT0202 with 159 randomized patients with mild to moderate AD was found to be safe, generally well tolerated except the fact it cause adverse events in the central nervous system at high dose [53]. Drug showed no significant difference in cognitive function between the treated and control group, which led to the discontinuation of drug [54]. Selective monoamine oxidase inhibitor selegiline slowed down the progression of AD by enhancing the activity of α-secretase via a protein trafficking related mechanism [55]. Atorvastatin the lipid lowering drug used to treat AD induced the activation of α-secretase [56]. PRX-03140, a serotonin type receptor (5-HT4) agonist stimulated α-secretase activity improving cognition in AD patients in phase II clinical trials, which was terminated as results were not declared (http://clinicaltrials.gov/show/NCT00693004). Bryostatin, a powerful PKC modulator showed increased sAPP α level in Phase II clinical trials indicating enhanced activity of α secretase activity [57].

4.3.2 Antiamyloid therapy

4.3.2.1. Compounds with antiaggregation potential

The production of Aβ from APP and its aggregation to toxic oligomers, protofibrils and mature plaques are the crucial step in the pathogenesis of AD [29]. Based on these facts, recent investigation focuses on identifying compounds, which prevent the aggregation of Aβ and disaggregates the preformed mature plaques [58]. 3-amino-1-propaneosulfonic acid (3-APS, Alzhemed, tramiprosate) designed as antagonist to prevent the interaction of Aβ with glycosaminoglycans, which promotes Aβ aggregation showed negative results in Phase III clinical trials [59]. Assessment of clinical efficacy, safety and disease modification of tramiprostate in mild to moderate AD showed slowing down of decline in Alzheimer's disease cognitive scale with no effect in clinical dementia illustrating the clinical inefficacy of tramiprosate for the treatment of heterogeneous dementia [60]. Colostrinin a proline-rich polypeptides distributed in ovine, bovine, and human colostrum inhibited aggregation of Aβ and its neurotoxicity under *in vitro* condition, improved cognitive function in AD mice model system, however phase II clinical trials showed only mild improvement in patients with mild AD [61]. Oral administration of scyllo-inositol (ELND005) halted the aggregation of Aβ reducing its toxicity in mouse

hippocampus. Phase II clinical trials with ELND005 for 18 months did not meet the primary clinical efficiency outcomes [62]. Metal chelating compounds such as 8-hydroxiquinolines (8-HQ), clioquinol and PBT2 blocks the interaction of metals with brain Aβ peptide attenuating oxidative stress mediated damage restoring cellular metal homeostasis. However, Phase I and II clinical trials lack the clinical efficacy of drug [63]. Sulfated glycosaminoglycan mimetic tramiprosate (NC-531) attenuated Aβ induced toxicity in cell lines and reduced amyloid plaques in transgenic mice. Phase II clinical trials in about 58 patients with mild to moderate AD showed reduction in Aβ (1-42) level in CSF in concentration dependant manner. Moreover the treatment was well tolerated with no adverse effect. Two RCT in phase III for 18 month to evaluate the safety and efficacy of drug are underway [59].

4.3.2.2. Compounds promoting removal of amyloid plaques

As deposition of amyloid plaques in the synaptic junction impairs the synaptic plasticity leading to neuronal death, clearance of amyloid plaques might act as one of the promising therapeutic strategies for AD. Different strategies used for disaggregation of plaques are

- Activation of multiple proteases enzyme, which degrade amyloid plaques such as neprilysin, insulin degrading enzyme, plasmin, endothelin converting enzyme, angiotensin converting enzyme and metalloproteinases, which are effective only under *in vitro* condition and not under clinical trials due to lack of specificity [64].

The transport of Aβ between the CNS and pheripheral circulation is mediated by (i) apoplipoprotein which transport Aβ from the blood to brain, (2) low-density lipoprotein receptor-related protein (LRP-1), which increases Aβ outflow from the brain to the blood, (3) receptor for advanced glycation end products (RAGE), which facilitates the transport of Aβ across the blood-brain barrier (BBB). The ultimate aim of the treatment is to reduce the amyloid load by regulating the transport of Aβ between CNS and peripheral circulation. Azeliragon is a small molecule inhibitor of RAGE, which was discovered by TransTech Pharma as TTP488 and licensed to Pfizer as PF-04494700. Phase II clinical trials in 67 people suffering from mild to moderate AD for 10 weeks showed safe and well tolerated results in treatment groups [65]. Phase II clinical trials with oral administration of TTP488 (60 mg for 6 days followed by 20 mg/day; 15 mg for 6 days followed by 5 mg/day) in 399 patients of mild to moderate AD for 18 months showed negative results with respect to safety and efficacy, hence the usage of drug was halted [66]. In April 2015, Phase III clinical trials of azeliragon (5mg/day daily) was initiated in 800 patients with mild to moderate AD for 18 months and yet to complete in 2018.

- Immunotherapy with active (Aβ (1-42) peptide) and passive immunisation (Anti Aβ antibodies) acts as one of the novel approach for the clearance of amyloid plaque. Active immunisation with synthetic full length Aβ (1-42) peptide AN1792 with adjuvant in phase II clinical trial decreased amyloid load, but caused cerebral inflammation like meningoencephalitis, led

to discontinuation of the drug [67]. A second-generation vaccine was designed with Aβ (1-6) to reduce nonspecific immune response. CAD106 (Novartis) was the first second generation vaccine, which reached clinical phase of development [68]. ACC-001 (Janssen) has recently completed two phase II trials (NCT01284387 and NCT00479557) with an additional phase II trial still ongoing (NCT01227564). Other vaccines like tetra-palmitoylated Aβ(1-15) reconstituted in liposome (ACI-24), MER5101 and AF205 are currently used in various stages of preclinical studies [69,70]. Passive immunisation deals with intravenous administration of monoclonal or polyclonal antibodies directed against Aβ. Benefit of passive immunization when compared to active immunisation is that no proinflammatory T cell mediated immune response was observed. Experimental evidence of passive immunisation of transgenic animals illustrated reduced cerebral amyloid load with improvement in cognitive function. Bapineuzumab and solanezumab are monoclonal antibodies against Aβ (1-6) and Aβ (12-28), which entered into phase III clinical trials, however bapineuzumab declined the brain amyloid plaques in the CSF with no significant improvement in cognitive function [71,72] Administration of 400 mg of solanezumab for 80 weeks in patients with mild to moderate AD showed improvement in cognition in mild AD [39]. Eli Lilly and Company (NYSE: LLY) performed EXPEDITION phase III clinical trials of solanezumab with 2100 patients with mild to moderate AD in 2015 for 18 months, which was discontinued in November 2016 as it did not meet the primary endpoint in people with mild dementia due to AD (NCT01900665 ; INDIANAPOLIS, Nov. 23, 2016 /CNW/ -- Eli Lilly and Company (NYSE: LLY). Several other monoclonal antibodies, which recognizes Aβ oligomeric and fibrillar species such as MABT5102A (Crenezumab, Genentech, San Francisco, California, USA), PF-04360365 (Ponezumab, Pfizer), R1450 (Hoffman-LaRoche, Basel, Switzerland), GSK933776A (GlaxoSmithKline, London, UK) which are effective in clearing amyloid plaques have successfully completed Phase I clinical trials related to safety [66,73,74] . Crenezumab (MABT5102A) a humanized monoclonal antibody with IgG4 backbone was subjected to phase II clinical trial to evaluate the safety and efficacy in E280A **autosomal dominant** mutation of PSEN1 (NCT01998841), which failed to reach its primary endpoints [75]. Hoffmann-La Roche initiated Phase III clinical trials in 750 people with MCI or prodromal AD (NCT02670083) termed as CREAD, which is expected to run until 2020 [76]. Ponezumab (PF-04360365) was subjected to two phase 2 clinical trials with 198 (NCT00722046)and 36 patients (NCT00945672) of mild to moderate AD which showed no effect on primary endpoints such as change in CSF Aβ burden, hence it was discontinued. Phase II open label trials of GSK933776A was performed with different doses (1,3 or 6 mg/kg bw) in patients with mild to moderate AD in Germany and Sweden. Trial showed decrease in CSF Aβ level with increase in plasma Aβ level and no significant change in CSF tau protein level [77]. However there are no current plan of developing the drug for the treatment of AD. Gantenerumab (R1450 - Hoffman-LaRoche, Basel, Switzerland) human IgG1 antibody specific to conformational epitope on β-amyloid fibres binds to amyloid plaque recruits microglial cells inducing phagocytosis mediated amyloid plaque clearance. Phase 2 clinical trial

(NCT01224106) was carried out with different doses of gantenerumab in 360 participants, which was later expanded to Phase2/3 study in 799 people (Roche) and this study was discontinued due to lack of efficiency in primary and secondary endpoints. Phase III clinical trials (NCT02051608) with 1000 patients of mild AD was initiated by Roche, which is actively ongoing till 2018 [78]. In addition, other passive immunotherapies mostly in phase I clinical development include NI-101, SAR-228810, and BAN-2401 [70,79,80]. SAR228810 a monoclonal antibody directed primarily against soluble protofibrillar and fibrillar species of Aβ. Phase I clinical trials was carried out in patients with mild to moderate AD to assess the safety, tolerability and the pharmacokinetic properties of SAR228810, which was discontinued due to lack of reports (NCT01485302). Gammagard a small fraction of polyclonal antibodies against Aβ peptide, which possess immunomodulatory and microglial cells mediated amyloid clearance, was reported to be safe and effective in phase II clinical trials [81]. Phase III trials in gammagard was carried out in two doses 400 mg/kg and 200 mg/kg doses for 18 months in 390 patients with mild to moderate AD. Primary endpoints showed no difference between study drug and placebo. Benefits were observed in higher dose treatment in moderate AD with insufficient power in other AD patients. Baxter international terminated phase III clinical trials of gammagard, which was started in 2012 due to adverse effects and inefficient results (NCT00818662; May 2013 press release).

4.4. Tau based therapeutics

Tau proteins are highly soluble microtubule stabilizing protein present abundantly in the axonal region of the neurons essential to maintain the morphology of neurons. Upregulated expression of kinase enzyme like CDK5, GSK3β, Fyn, stress activator protein kinase JNK, p38 and mitogen activated protein kinase and downregulated expression of phosphatase leads to hyperphosphorylation of tau protein, which tends to aggregate forming NFT [82,83]. Formation of NFT affects the microtubule binding capacity leading to neuronal death. Development of drugs, which inhibit phosphorylation and aggregation of tau protein, microtubule stabilizing drugs and immunomodulation are the possible disease modifying strategy for the treatment of AD [84].

4.4.1. Drugs attenuating tau hyperphosphorylation

As imbalance between the expression of phosphatase and kinase leads to hyperphosphorylation of tau protein, drug, which modulate these expression can act as potential therapeutic drug for AD. Among the kinases, GSK3β (Glycoge synthase kinase), CDK5 (serine/threonine cyclin dependent kinases) were the key enzymes involved in the tau pathology, hence inhibition of these kinases can prevent tau hyperphosrylation. Lithium exhibited GSK3β inhibitory activity and Aβ peptide accumulation in AD transgenic mice model and in MCI patients; however toxicity in adults limits its usage [85]. Compounds like pyrazolopyrazines, pyrazolopyri-

dines, the aminothiazole AR-A014418, and sodium valproate exhibited GSK3β inhibitory activity under *in vitro* and *in vivo* condition and the clinical trial is ongoing [86]. Tideglusib, an irreversible inhibitor of GSK3β showed negative results in phase II clinical trials in mild to moderate AD. Roscovitine and flavopiridol showed potent CDK5 inhibitory activity under in vitro and in vivo model system, clinical trials are yet to be carried out [87].

Activation of phosphatase also acts one of the possible drug targets, till date only one protein phosphatase 2 (PP2A)agonist has been developed. Experimental evidences reported that sodium selenite (VEL015) effectively reduced tau phosphorylation under in vitro condition and improved cognitive function in AD mouse model system. Sodium selenite is under phase II clincical trails in Australia (ACTRN12611001200976) [88].

4.4.2. Inhibitors of tau aggregation

Several compounds inhibiting tau aggregation were identified under in vitro condition. Methylene blue and its derivatives showed promising results disrupting the aggregation of both tau protein and Aβ peptide, attenuating oxidative stress and improving mitochondrial function in AD model system [89]. Rember the first generation of methylene blue stabilized AD progression in clinical trials for 50 weeks. Positive results led to the development of second generation of methylene blue, TRx 0237 which not only attenuated tau protein aggregation, but also dissolved NFT [90] . Three phase III trials have been carried out with TRx0237. First phase III study (NCT01689233) deals with administration of single dose (200 mg/day) to placebo in 800 patients with dementia or AD mild enough to score above an MMSE of 20. Trial began in 2012, its outcome have not been presented yet. Second Phase III trial (NCT01689246) deals with comparison of 150 and 250 mg/day dose in 891 patients with mild to moderate AD with MMSE of 14 or higher. Clinical, cognitive and safety outcomes showed negative results. Third Phase 3 clinical trials (NCT01626378) was carried out in 180 patients with fronto-temporal dementia administrating 200 mg/day and the outcome was observed to be negative. Overall all the phase III clinical trials with TRX0237 showed negative results in terms of safety and cognitive outcomes [91], https://clinicaltrials.gov/ct2/show/NCT02245568.

4.4.3. Stabilizers of microtubule

Microtubule stabilization may provide similar effect as that of drugs interfering tau hyperphosphorylation and aggregation. Paclitaxel an anticancer drug have been reported for microtubule-stabilizing ability, however inability to cross BBB and adverse side effects limited its usage in AD [92,93]. Another anticancer drug TPI 287 a derivative of taxane stabilized the microtubules by binding to tubulin . TPI287 has entered into clinical trials to assess its safety in mild to moderate AD patients (ClinicalTrials.gov identifier: NCT01966666) which is under way [94,95]. MT-stabilizing agent from the taxane family, TPI-287, has entered clinical testing

in patients with AD and PSP/CBD (ClinicalTrials.gov identifier: CT02133846). Epothilone D a microtubule-stabilizing compound, which showed improvement in axonal transport, reduced tau neuropathology and hippocampal neuronal loss in AD model system, was discontinued from drug development after its failure in clinical trials [22].

4.4.4. Anti-Tau immuntherapy

Active and passive immunization to prevent the aggregation of tau protein and dissolving the preformed NFT is one of the current areas of research to prevent tau pathology. AADvac-1 (Axon neuroscience) a synthetic peptide derived from the tau sequence was coupled with adjuvant keyhole limpet hemocyanin and was subjected to preclinical studies which showed good preclinical safety profile for a period of 3 months for rats, rabbits and dogs. Phase I clinical trials of AADvac1 was carried out in 30 patients for 12 weeks in which 24 patients were assigned as AADvac 1 group and six as placebo groups (NCT01850238). No cases of meningoencephalitis or vasogenic oedema were observed, except two patients who withdrew due to adverse events such as a viral infection followed by epileptic seizure. Results showed positive IgG response in 29 patients illustrating the safety profile and excellent immunogenicity, in Phase I clinical trials [96]. To substantiate the clinical efficacy of AADvac1, phase II clinical trial (24 months) was initiated in 185 patients with mild to moderate AD on March 2016 and it is slated to run up to February 2019 (clinical trial no. NCT0257952) .

4.5. Antinflammatory drugs

Biochemical and neuropathological studies in the brain of AD patients provided clear evidence of activation of inflammatory pathway and its destructive effect. In AD, deposition of amyloid plaques in the synaptic junction activates microglial cells and reactive astrocytes promoting enhanced expression of phospholipase A2 with subsequent release of proinflammatory mediators leading to inflammation mediated neuronal death. Evidences showed that long term usage of anti-inflammatory drugs such as NSAIDs reduced the risk of AD [97]. Inflammation and AD are interlinked as certain components of this molecular machinery promote AD pathology, while other components exhibit protective effect to the neuron via amyloid plaque clearance. Despite of successful preclinical studies of glucocorticoid therapy, hydroxychloroquine, and non-steroidal anti-inflammatory drugs with AD models system, results of clinical trials are still disappointing. The challenge with anti-inflammatory drug is to find ways of fine tuning inflammation to delay, prevent, or treat AD [98].

Certain NSAIDs have been reported to modulate γ-secretase activity decreasing the production of Aβ (1-42), which has been prevented from clinical trials due to toxicity [99]. Another possible mode of action of NSAIDs is to block COX-2 expression in brain, as COX-2 is upregulated in AD brain mainly in pyramidal neurons in the cerebral cortex and the hippocampal formation [100]. NSAIDS such as ibuprofen, indomethicin, and sulindac sulphide reduce

the level of Aβ1-42 peptide up to 80% in cultured cells [101]. Ibuprofen treatment in AD mice model system showed reduction in amyloid plaque in the cortex attenuating microglial activation in the mice [102]. Neurons treated with COX-1 inhibitors, such as ibuprofen and acetyl salicylic acid, were observed to be more resistant to the effects of Aβ than neurons that were treated by COX-2 inhibitors [103]. Treatment with COX-1 and COX-2 inhibitors reduced the production of prostaglandin E2. NSAIDs may also function by activating the peroxisomal proliferators-activated receptors (PPARs) which act negatively inhibiting the transcription of proinflammatory genes. *In vitro* studies illustrated that PPARα agonist inhibited microglial activation and proinflammatory mediators like NOS, COX-2 and IL-6 [104]. RCT of COX-2 inhibitor rofecoxib and naproxen showed no change in cognitive decline in patients with mild to moderate AD. Unfortunately clinical trials of NSAID in AD patients were not fruitful, particularly in the case of COX-2 inhibitors [105]. Recent hypothesis illustrated that NSAIDS reduces the incidence of the disease and are ineffective after the incidence of disease. Glucocorticoids like prednisone inhibited Aβ induced chemokines and cytokines in the CNS of animal models; however RCT showed no difference in cognitive decline in AD patients [106]. Among the plant derived polyphenols, flavonoids play crucial role in down regulating proinflammatory mediators of innate immune system [107]. Green tea rich in flavonoids like EGCG modulated T- cell response, NF-κB signaling, TNF-α production in vascular endothelial cells and improvement in cognitive function in mice model system. Results were inconsistent with the clinical trial reports, in which no significant improvement in cognition was observed in AD patients [109]. Recent report revealed that tumour necrotic factor (TNF) one of the glial transmitters play key role in glial activation mediating inflammation and synaptic dysfunction. Administration of FDA approved TNF inhibitor Etanercept (used for rheumatoid arthritis) for 6 months in 15 patients with AD provided sustained improvement in cognitive function for patients with AD. Nevertheless, etanercept merits further study in RCTs [110].

4.6. Antioxidants in AD therapy

Brain is highly vulnerable to oxidative insults because it is rich in polyunsaturated fatty acids, high demand for oxygen and relative scarcity for antioxidant systems. Aβ peptide together with altered mitochondrial function and increased level of transition metal ions in brain are the potential source of oxidative stress in brain. In AD primarily oxidative stress is induced by the Aβ aggregation, which is considered as crucial factor in the early pathogenesis of AD, leading to neuronal cell injury and death [111]. Epidemiological investigations revealed that intake of food rich in antioxidants reduced the incidence of AD [112]. Antioxidant therapy includes direct acting antioxidants (ROS scavenging and chain breakers) and indirect antioxidants (metal chelators) which are further classified as enzymatic and nonenzymatic antioxidant system [113]. Naturally occurring antioxidants such as Glutathione (GSH), ascorbate (vitamin C), α- tocopherol (vitamin E), β-carotene, NADPH, uric acid, bilirubin, sodium selenite, di-

hydrolipoic acid, melatonin and plasma protein thiol are direct acting antioxidants which attenuated ROS mediated oxidative stress in AD model system [114]. Other antioxidant such as mitoquinone, Ginkgo biloba and natural polyphenols like green tea, blue berries, curcumin, ω3 fatty acids, folate, vitamin B_6 and vitamin B_{12} supplementation showed positive results under in vivo AD model system. However clinical trials with these antioxidants showed conflicting results for AD patients. In placebo controlled trials, daily administration of Vitamin E (2000 IU for 2 years) in about 341 patients of moderate to severe AD showed significant delay in progression of AD suggesting vitamin E as prophylactic medicine [115]. High intake of vitamin E and C supplements in combination also reduced the risk of AD. But the main disadvantage of these studies are the dose of antioxidant used are low and the studies were carried out in patient who are affected by neurodegenerative diseases in which the full potency of antioxidant cannot be observed. In addition the cardiovascular risk of vitamin E reduced the usage of vitamin E for AD. Most commonly studied antioxidant curcumin, which showed positive results under in vitro conditions, is under clinical trials with AD patients. Current research focus on developing antioxidant that target the mitochondria such as lipophilic triphenylphosphonium cation conjugated to an antioxidant moiety, such as the ubiquinol moiety of coenzyme Q (mitoquinone; MitoQ), α-tocopherol (MitoVitE) [116,117]. MitoQ is a recycling antioxidant which can revert back to its active form after ROS neutralization, similarly SOD mimetic MitoSOD and MitoTEMPOL, and peroxidase mimetic MitoPeroxidase exhibit antioxidant activity [118, 119]. Szeto-Schillertetra peptides a novel class of small-cell permeable peptide concentrates in the inner mitochondrial membrane and protects against mitochondrial oxidative damage attenuating further ROS production [120]. Antioxidant therapy might be beneficial in early phase of AD or people with risk of AD incidence. BBB permeability acts as major hurdle for the smaller antioxidant molecules, hence naturally occurring small antioxidant molecules with ability to cross the BBB offers much promise for the treatment of AD.

4.7. Cholesterol lowering drugs

Human brain contains approximately 25% of total body cholesterol which are distributed in myelin sheath, glial cells (astrocytes and microglia) and in other parts of neurons. Cholesterol plays key role in signal transduction, neurotransmitter release, synaptogenesis, membrane trafficking [121]. Increased cholesterol level enhances BACE-1 activity leading to accumulation of Aβ 1-42 peptide forming amyloid plaques. In addition reports suggested that cholesterol alters the conformation of Aβ leading to the generation of amyloid seeds. ApoE is the lipid carrier protein in the CNS released by astrocytes to supply neurons with cholesterol and it exist in three isoforms. ApoE2 and ApoE3 are involved in lipid clearance, recycling particularly after injury than ApoE4. Increased expression of ApoE4 promotes the aggregation of Aβ leading to AD [122]. Enhanced level of ApoE4 together with Aβ impairs the cholesterol transport from the astrocytes to neuron affecting the neurotransmission [123]. Evidences il-

lustrated that intake of hypocholestermic drugs reduced the risk of developing AD. Statin a hypocholestermic drug exhibited safe efficacy during long term treatment and also exhibited delayed onset of AD in mice model system [124]. Statins reduces the Aβ level by enhancing APP metabolism by α -secretase activity [125]. Clinical trials of atorvastatin with 63 individual with normal cholesterol levels and mild to moderate AD exhibited clinical benefit for a period of 6 months and 1 year. Large scale Phase III clinical trials with atorvastatin in mild to moderate AD is under progress U.S. National Institute on Aging (NIA) sponsored conducted survey on cholesterol lowering agent simvastatin to slow the progression of AD in mild to moderate AD patients [126,127].

4.7. Other treatment strategies

- Presence of activated astrocytes around the Aβ plaques led to the development of astrocyte modulating agent to reduce AD pathology. Arundic acid (ONO-2506) reduced infarct size, enhanced neurologic outcome, prevented motor abnormalities and protected the dopaminergic neurons in AD and parkinson's mice model system by modulating the activation of astrocytes. This compound is under phase II clinical trial with mild to moderate AD [128,129].

- Increased concentration of sulfur containing amino acid homocysteine was observed in AD patients, which will hamper the DNA repair in neurons making the neurons more vulnerable to Aβ induced damage. Therefore lowering of homocysteine might reduce the pathology of AD. Clinical trial on homocysteine lowering vitamin combination in AD is under progress [130]

- Neuronal growth factor play significant role in maintaining neuronal integrity, lack of endogenous NGF leads to memory deficit in AD. Hence treatment with NGF and NGF-related agents were attempted to enhance the neuroprotective effect [131]. But the major limitation with exogenous NGF administration is BBB permeability, which acts as a significant challenge for AD therapy. Recently surgical implantations of NGF expressing cells (gene therapy) or administration of agents, which potentiate production of NGF were carried out [132]. Neurotrophic enhancing agent like xaliproden (SR-57746) cerebrolysin (FPF 1070), activated the endogenous neurotrophin synthesis including BDNF and NGF, enhanced the synaptic regeneration, ameliorated memory deficit in APP transgenic mice [133,134]. RCT investigation showed cerebrolysin infusions significantly improved the activities of daily living and cognitive function, while xaliproden is under phase III clinical trials with mild to moderate AD patients [135,136]. Results of phase I study on genetically modified, autologous fibroblasts producing human NGF implanted into the forebrains of six patients showed no post surgical adverse effects and moreover improvement in cognitive function was observed [137]. Clinical investigation of this approach is expected to continue.

5. Conclusion

Multiple evidences suggest AD as complex multifactorial disorder caused by several etiological factors and multiple biochemical pathways, which prompted the search for disease modifying drugs for AD therapy. This review summarizes the various therapeutic strategies available and under research for the treatment of AD. For the past 20 years, amyloid hypothesis have been considered as pathogenic pathway and enormous number of studies have been carried out on inhibition and removal of Aβ and senile plaques. Unfortunately, the amyloidogenic approaches have failed to demonstrate defects that clearly contribute to cognitive decline in AD. Mechanism underlying the pathogenesis of AD must be assessed before the development of disease modifying compounds. Understanding the events occurring in the synapse and associated microglial cells and the relationship between tau and Aβ will be instrumental in developing successful disease modifying drugs. Novel pharmacotherapies should not be limited to amyloid cascade hypothesis; it should be multitargeted focusing on other pathways involved in the pathogenesis. Early diagnosis, nutritional diet coupled with combinatorial pharmacotherapy targeting multiple pathways and change in life style will help in successful eradication of AD. As BBB acts as a major obstacle for the treatment of AD recent research focuses on improving drug delivery systems such as nanoencapasulation and microemulsion to enhance the bioavailability and blood brain permeability.

Table 1: Disease modifying drugs under clinical trials for the Alzheimer's therapy

S. No:	Mode of action	Modulating Agents/compounds	Clinical trials	Results
	Beta secretase inhibitors	Lilly AZD 3293 (Astrazeneca)	Phase II/III	
		CTS-21166 (CoMentis)	Phase I	+
		E2609 (Eisai/Biogen Idec)	Phase II	
		HPP854 (High point)	Phase I	
		LY2886721 (Lilly)	Phase II	-
		MK-8931 (Merck)	Phase II/III	-
		PF-05297909 (Pfizer)	Phase I	
		RG7129 (Roche)	Phase I	
		TAK-070(Takeda)	Phase I	
		VTP-37948 (vitae/Boehringer Ingelheim)	Phase I	
	Gamma secretase inhibitors	Semagacestat	Phase III	-
		Tarenflurbil	Phase III	-
		LY450139 (Avagacestat)	Phase II	-
	Gamma secretase modulators	Ibuprofen, sulindac, indomethacin, and R-flurbiprofen (Tarenflurbil)		
		NIC5-15		
	Alpha secretase modulators	Etazolate	Phase II	-

	Nonsteroidal inhibitory of cyclooxygenase activity (NSAIDs)	CHF5074	Phase IIa	+
	Drugs attenuating aggregation of Aβ Peptide/Anti Aβ immunotherapy	Glycosaminoglycans 3-amino acid, 1-propanesulfonic synthetic (3APS, Alzhemed, tramiprosate)	Phase III	-
		Colostrinin	Phase II	+/-
		Scyllo-inositol compound (ELND005)	Phase II	+/-
		PBT1 (clioquinol) and PBT2 (metal chelators)	Phase IIa	+
		Active immunization (vaccination)		
		AN-1792	Phase II	-
		CAD-106	Phase II	+
		ACC-001	Phase II	
		MER5101, AF205	Preclinical trials	
		Passive immunization (Monoclonal antibodies)		
		Bapineuzumab	Phase III	-
		Solanezumab	Phase III	-
		Crenezumab (MABT5102A)	Phase II	-
		Gammagard	Small number of AD patients	-
		PF-04360365 (Ponezumab), NI-101, SAR-228810, GSK933776A, and BAN-2401	Phase I Phase II	+ -
Modulators of tau pathology				
	GSK 3β inhibitor	Lithium Tideglusib Sodium selenite (VEL015)	Phase I Phase II Phase II	+ -
	CDK5 inhibitor	Roscovitine and flavopiridol	Preclinical trials	+-
	Immunotherapy	AADvacI vaccine	Phase I	Ongoing
	Inhibitors tau aggregation	Methylene blue derivatives - RemberTM, TRx0237	Preclinical trials Phase III	+ -
	Microtubule stabilizers	Paclitaxel Epothilone D TPI 287 (taxane)	Phase I Phase III	- -
	Anticholinesterase inhibitors	Donepezil, Rivastigmine, Galanthamine Ladostigil (TV3326)	FDA approved drugs	-
	N-methyl-D-aspartate antagonist	Memantine Dimebon	Treatment of mild to moderate AD Phase III	+

	Antioxidants	α-Tocopherol	Phase III	+
	Nicotinic receptor activator	AZD1446/TC-6683	Phase II/IIa/ IIb	
	Nerve growth factor	CERE-110	Phase II/IIa	

+ = Positive results, +/- = Partially positive, - = Negative results.

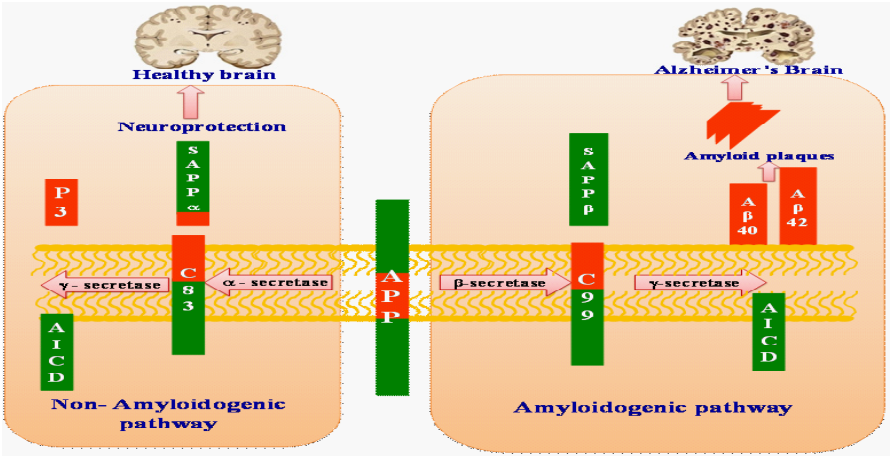

Figure 1: Sequential cleavage of Amyloid precursor protein

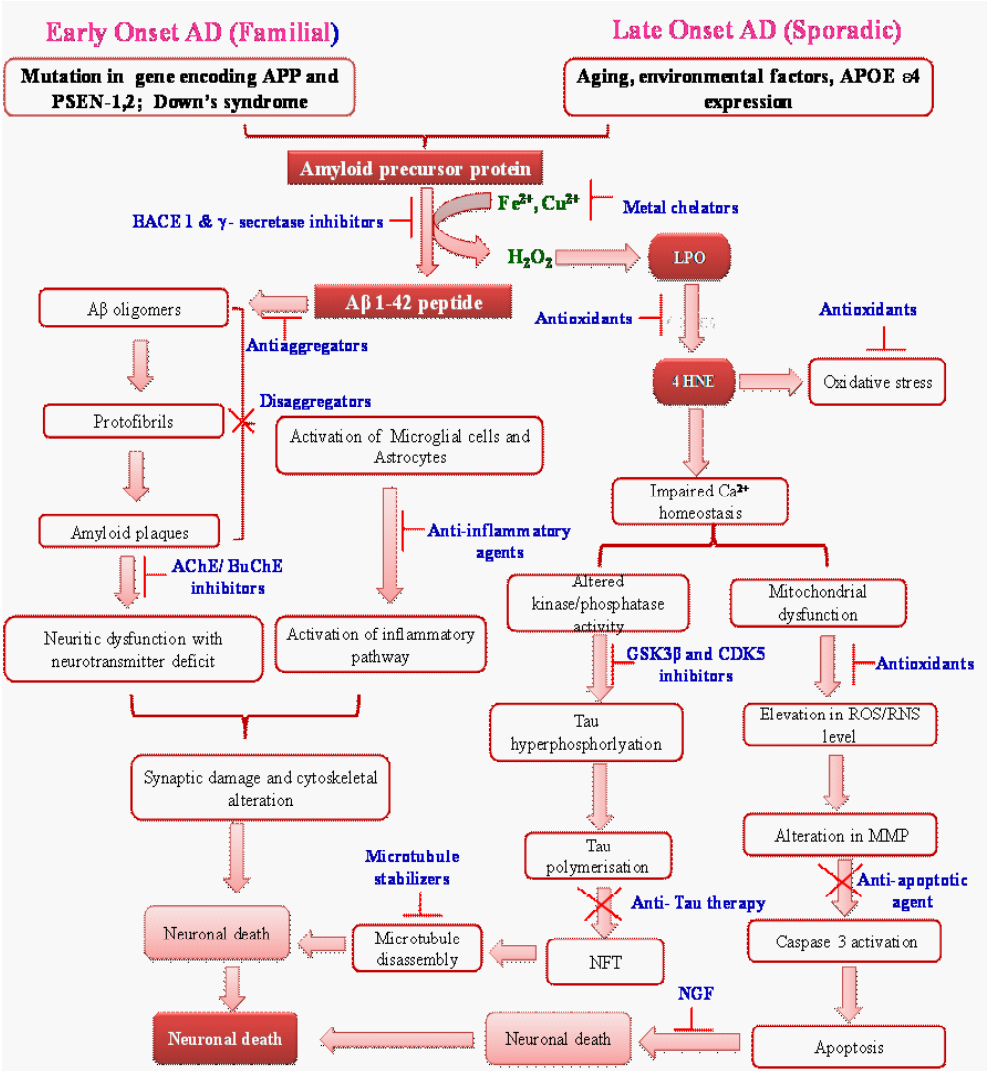

Figure 2: Pathogenesis of AD and Therapeutic strategies for AD

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Chapter 2

Natural Products for Treatment of Alzheimer's Disease

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1. Alzheimer Disease (AD)

Aging is a complex process accompanied by several changes, including shrinkage in human brain, in addition to changes in brain white matter. Loss of neurons and myelinated axons are supposed to be the cause of decrease in weight and volume of the aged brain. The common human age-related neurodegenerative disorders such as Alzheimer's disease (AD) andParkinson's diseases (PD) are characterized by the progressive loss of brain function and memory decline. It is well known that the risk to develop dementia causing diseases increases with chronological aging. Several studies indicated that the age-dependent changes at gene and protein expression level, are multifactorial as human brain aging could result from the combination of the normal decline of biological functions with environmental factors that contribute to defining disease risk of late-life brain disorders. The initiation and progression of age-related neurodegenerative disorders is a complex process not yet fully understood [1].

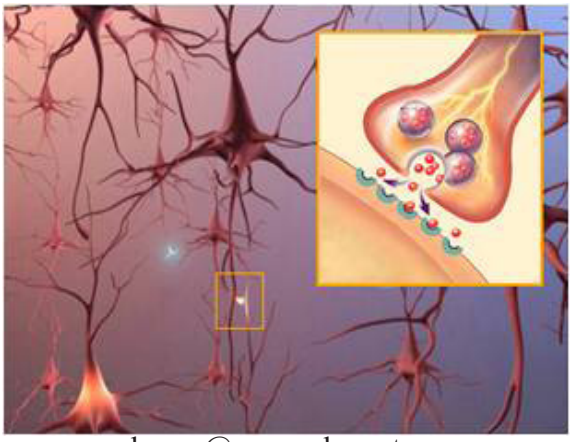
Dementia privilegeis predicted to increase at an alarming rate in the least developed and developing regions of the world despite mortality resulting from malnutrition, poverty, war, and infectious diseases. WHO suggest that by 2025, about three-quarters of the estimated 1.2 billion people aged 65 years and older will reside in developing countries [2]. Currently; dementia is diagnosed based on clinical symptoms, but significant brain damage have already occurred by the time a clinical diagnosis of dementia is made which may be too late for any effective intervention. Thus, defining a panel for biomarkers of Alzheimer's disease (AD) would be of great public health and preventive value; that precedes the clinical manifestation of dementia and could permit early detection of persons at a higher risk for developing dementia,

specifically Alzheimer's disease dementia. Nevertheless, for the purpose of large-scale screening, circulating peripheral blood-based biomarkers are more appropriate and practical as being less invasive than lumbar puncture, simple, reliable, reproducible, , and less costly than brain amyloid imaging, and can be easily accessed and non-time-consuming [1].

2. Alzheimer's and the Brain

The brain has 100 billion nerve cells (neurons). Each nerve cell connects with many others to form communication networks. Groups of nerve cells have special jobs. Some are involved in thinking, learning and remembering. Others help us see, hear and smell.

The picture below depicts nerve cells, or *neurons,* in the brain. Neurons are the chief cells destroyed by Alzheimer's disease.

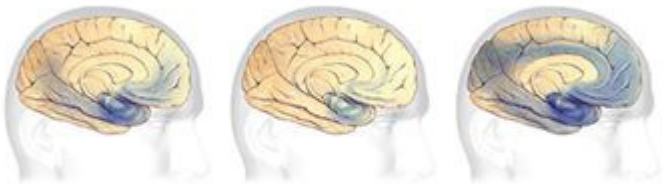


alz.org@research center

To do their work, brain cells operate like tiny factories. They receive supplies, generate energy, construct equipment and get rid of waste. Cells also process and store information and communicate with other cells. Keeping everything running requires coordination as well as large amounts of fuel and oxygen.

Scientists believe Alzheimer's disease prevents parts of a cell's factory from running well. They are not sure where the trouble starts. But just like a real factory, backups and breakdowns in one system cause problems in other areas. As damage spreads, cells lose their ability to do their jobs and, eventually die, causing irreversible changes in the brain [3].

3. The Role of Plaques and Tangles



Plaques and tangles tend to spread through the cortex as Alzheimer's progresses.Two abnormal structures called plaques and tangles are prime suspects in damaging and killing

nerve cells. **Plaques** are deposits of a protein fragment called beta-amyloid that builds up in the spaces between nerve cells.

Tangles are twisted fibers of another protein called tau that builds up inside cells. Though autopsy studies show that most people develop some plaques and tangles as they age, those with Alzheimer's tend to develop far more and in a predictable pattern, beginning in the areas important for memory before spreading to other regions.

Scientists do not know exactly what role plaques and tangles play in Alzheimer's disease. Most experts believe they somehow play a critical role in blocking communication among nerve cells and disrupting processes that cells need to survive. It's the destruction and death of nerve cells that causes memory failure, personality changes, problems carrying out daily activities and other symptoms of Alzheimer's disease [2,3].

4. Treatment of Alzheimer

FDA-approved drugs[3]

The U.S. Food and Drug Administration (FDA) have approved five medications (listed below) to treat the symptoms of Alzheimer's disease.

Drug name	Brand name	Approved For	FDA Approved
1. donepezil	Aricept	All stages	1996
2. galantamine	Razadyne	Mild to moderate	2001
3. memantine	Namenda	Moderate to severe	2003
4. rivastigmine	Exelon	All stages	2000
5. donepezil and memantine	Namzaric	Moderate to severe	2014

5. How Alzheimer's Drugs Work

In the brain, neurons connect and communicate at synapses, where tiny bursts of chemicals called neurotransmitters carry information from one cell to another. Alzheimer's disrupts this process, and eventually destroys synapses and kills neurons, damaging the brain's communication network. Current FDA-approved Alzheimer's drugs support this communication process through two different mechanisms:

1. *Cholinesterase inhibitors* work by slowing down the process that breaks down a key neurotransmitter. *Donepezil, galantamine and rivastigmine* are cholinesterase inhibitors.

2. *Memantine*, the fifth Alzheimer's drug, is an *NMDA (N-methyl-D-aspartate) receptor antagonist,* which works by regulating the activity of glutamate, an important neurotransmitter in the brain involved in learning and memory. Attachment of glutamate to cell surface "docking sites" called NMDA receptors permits calcium to enter the cell. This process is important

for cell signaling, as well as learning and memory. In Alzheimer's disease, however, excess glutamate can be released from damaged cells, leading to chronic overexposure to calcium, which can speed up cell damage. *Memantine* helps prevent this destructive chain of events by partially blocking the NMDA receptors.The effectiveness of cholinesterase inhibitors and memantine varies across the population [3].

6. Alzheimer Treatment with Natural Products

Despite modern medicine's incredible innovation and resulting accumulation of valuable knowledge, many of the world's most problematic diseases such as Alzheimer Disease (AD) still lack effective cures and treatments. Western medicine has revealed many genetic, cellular, and molecular processes that characterize AD such as protein aggregation and inflammation. As the need for novel and effective treatments increases, researchers have turned towards traditional medicine as a resource. Modern, evidence based research examining traditional and complementary remedies for AD has generated promising results within the last decade [4].

Dementia pathologies such as Alzheimer's disease (AD) are reaching epidemic proportions, yet they are not successfully managed by effective symptomatic treatments. Only five drugs have been developed to alleviate cognitive symptoms, and more effective and safe treatments are needed for both the cognitive symptoms and behavioural and psychological symptoms of dementia (BPSD). As two of these licensed drugs (cholinesterase inhibitors (ChEIs) are naturally derived (galantamine and rivastigmine), the potential for plants to yield new therapeutic agents has stimulated extensive research to discover new ChEIs together with plant extracts, phytochemicals and their derivatives with other mechanistic effects relevant to dementia treatment [5].

The current therapeutic drugs for Alzheimer's disease are predominantly derived from the alkaloid class of plant phytochemicals. These drugs, such as galantamine and rivastigmine, attenuate the decline in the cholinergic system but, as the alkaloids occupy the most dangerous end of the phytochemical spectrum (indeed they function as feeding deterrents and poisons to other organisms within the plant itself), they are often associated with unpleasant side effects. In addition, these cholinesterase inhibiting alkaloids target only one system in a disorder, which is typified by multifactorial deficits. The present paper will look at the more benign terpene (such as *Ginkgo biloba*, Ginseng, *Melissa officinalis* (lemon balm) and *Salvia lavandulaefolia* (sage)) and phenolic (such as resveratrol) phytochemicals; arguing that they offer a safer alternative and that, as well as demonstrating efficacy in cholinesterase inhibition, these phytochemicals are able to target other salient systems such as cerebral blood flow, free radical scavenging, anti-inflammation, inhibition of amyloid-β neurotoxicity, glucoregulation and interaction with other neurotransmitters (such as γ-aminobutyric acid) and signalling pathways (e.g. via kinase enzymes) [6].

Guzior et al. [7] review current development of multifunctional potential of anti-AD agents, most of which are acetylcholinesterase inhibitors that extend the pharmacological profile. Thus compounds that offer hope are symptomatic and suggest causal treatment of AD.

Advantageous properties include the amyloid-β antiaggregation activity, inhibition of β-secretase and monoamine oxidase, an antioxidant and metal chelating activity, NO releasing ability and interaction with cannabinoid, NMDA or histamine H3 receptors. These unusual molecules possess heterodimeric structures that interact with multiple targets which in turn might combine different pharmacophores, original or derived from natural products or existing therapeutics (tacrine, donepezil, galantamine, memantine). There is minimal reaction to these findings since several described compounds may be promising drug candidates. Others may be valuable inspirations as we continue to search for new effective AD therapies [7].

7. Treatment of AD with Different Natural Sources

Various natural animal and plant based products have enormous potential. We can therefore expect improved treatment and creation of drugs for certain diseases: cancer, diabetes, and heart disease, and expect that AD will be added. Natural products from invertebrates have already begun to be integrated into modern biomedicine, e.g. leeches are employed for plastic surgery or snail venom is used as an alternative to opioids for humans [8]. For example, alterations in uptake and release of glutamate, predominant excitatory neurotransmitter in the central nervous system, have been observed in various neurodegenerative diseases, including AD. Bee venom, which has been used in Traditional Korean Medicine, exerts anti-inflammatory effects, and has been assessed for its inhibition of glutamate as related to neurotoxicity. Protecting against cell death and inhibiting cellular toxicity, bee venom is a promising compound that may be helpful as a treatment against glutamatergic neurotoxicity for neurodegenerative diseases [9]. Moreover, compounds isolated from marine invertebrates have shown equally promising leads. Acetylcholinesterase (AChE) inhibition seems relevant for treating AD.

Moreover, compounds isolated from marine invertebrates have shown equally promising leads. Acetylcholinesterase (AChE) inhibition seems relevant for treating AD. Marine natural products may be effective as AChE inhibitors with curative potential [10].

Neurodegeneration is the term for the progressive loss of structure or function of neurons, including death of neurons. Many neurodegenerative diseases including Alzheimer's disease, Parkinson's disease,and Huntington's disease occur as a result of neurodegenerative processes [11]. Current interventions for Alzheimer's disease (AD) include acetylcholinesterase inhibitors (AchI), which are indicated for patients with mild to moderate symptoms. A spectrum of alternative treatments for AD has also been proposed and must be examined judiciously in preclinical, clinical, and evidence-based research (EBR) studies [12]. Therefore,

search for acetylcholinesterase inhibitors is useful for the treatment of Alzheimer's disease. Pharmacological studies with marine compounds affecting the nervous system involved three areas of neuropharmacology: the stimulation of neurogenesis, the targeting of receptors, and other miscellaneous activities on the nervous system. A new stigmastane type steroidal alkaloid 4-acetoxy-plakinamine B isolated from a Thai marine sponge Corticium sp. significantly inhibited acetylcholinesterase (IC50 = 3.75 μM). This compound is reported to be the "first marine derived acetyl cholinesteraseinhibiting steroidal alkaloid" [13]. The inflammatory component to the pathology of neurodegeneration was most notably in Alzheimer's disease but also in Parkinson's disease and motor neuron disease [14]. Hymenialdisine is an alkaloid isolated from marine sponges, such as Acanthellaaurantianca and Stylissamassa [15]. Hymenialdisine inhibits phosphorylation of the protein tau (which is hyperphosphorylated in Alzheimer's disease) with promising potential against human neurodegenerative diseases [16]. 11-Dehydrosinulariolide was obtained from formosan soft coral, S. flexibilis, promoting neuroprotective properties as a promising candidate for the treatment of Parkinson's disease [17].

8. Application of Phytochemicals and Nutraceuticals

Synthetic drugs are useful for managing AD and many other chronic illnesses; still there are side effects. Consequently, attention of researchers has inclined toward phytochemicals as promising therapeutic agents. Many are anti-inflammatory, antioxidative and possess anticholinesterase activities with minimal side effects [18]. Approaches to Traditional Chinese Medicine (TCM) remedies reveal first that traditional herbs and phytochemicals may delay AD onset and slow its progression but also allow recovery by targeting multiple pathological causes that possess antioxidative, anti-inflammatory, and antiamyloidogenicproperties [19]. Furthermore herbs regulate mitochondrial stress, apoptotic factors, free radical scavenging systems, and neurotrophic factors. Neurotrophins such as BDNF, NGF, NT3, and NT4/5 may also participate in neuronal and nonneuronal responses to AD.

Neurotrophin depletion accelerates progression of AD and therefore, replacing such neurotrophins could serve as a potential treatment for certain neurodegenerative diseases. Emphasis concerning mechanisms rests on phytochemicals that mediate signaling pathways involved in neuroprotection specifically neurotrophin-mediated activation of Trk receptors and members of the p75(NTR) superfamily. Research conducted by Venkatesan et al. [19] focused on representative phenolic derivatives, iridoid glycosides, terpenoids, alkaloids, and steroidal saponins as regulators of neurotrophin-mediated neuroprotection. There is evidence derived from encouraging advances, since these phytochemicals have attracted attention due to their in vitro neurotrophin potentiating activity, yet there is still a need for in vivo and clinical efficacy trials. Currently, proof of neuroprotective effects in certain preclinical models and in humans is unclear [19].

Here is where we are with respect to therapeutic approaches. According to Frautschy and Cole [20], AD involves a complex pathological cascade perhaps triggered initially by accumulation of beta-amyloid peptide aggregates or aberrant amyloid precursor protein processing. Moreover, there is credible information concerning factors initiating AD process significantly before onset of cognitive deficits. However, there is an unclear understanding of any events that immediately precede and precipitate cognitive decline. Without these events defined or at least clarified more adequately we are left with a major limiting factor that hinders rapid development of adequate prevention and treatment strategies. If we agree with the hypothesis of inhibiting certain pathways, clearly efforts have focused on pleiotropic activities of omega-3 fatty acids and anti-inflammatory, antioxidant, and anti-amyloid activity of curcumin in multiple models that consider many steps in the AD pathogenic cascade [20]. AD reveals that inflammation contributes to neurodegenerative disease. Knowing this it is therefore suggested that early prevention and management of inflammation might conceivably delay onset or reduce symptoms of AD, but what is a likely target? With aging, normal physiological changes in the brain include depletion of long chain omega-3 fatty acids. Analyses have shown that brains of AD patients possess lower levels of docosahexaenoic acid (DHA). In agreement, Thomas et al. [20] report that DHA supplementation reduces markers of inflammation. Research devoted to epidemiological, dietary intervention, and supplementation support roles of long chain omega-3 fatty acids in preventing or delaying cognitive decline in AD during its early stages. These results support further investigation of long chain omega-3 supplementation in early stage AD, and maintains the importance of overall quality and composition of diet to protect against AD and dementia [21].

Complementary and alternative medicine reveals nutraceuticals whose properties are both anti-inflammatory and anti-cancer. For example, mangosteen, the fruit from a tropical evergreen tree native to Southeast Asia, has nutraceuticals which possess multiple beneficial properties, especially neuroprotective, anti-oxidative, and anti-inflammatory effects. Treatment with mangosteen based nutraceuticals decreased cell death and increased brain-derived levels of neurotrophic factor. Moreover, mice fed a mangosteen supplemented diet showed improved inflammation related cognitive function, demonstrating its promise in treating AD [22]. Another nutraceutical that has garnered interest as an antioxidant is quercetin. Oxidative stress plays a role in the progression of various diseases and conditions including AD. Thus quercetin has received attention as a food derived antioxidant for its promising biological effects and ability to prevent oxidative damage [23].

9. Traditional Medicine and AD

Traditional Chinese Medicine has accumulated many experiences in the treatment of dementia during thousands of years of practice; modern pharmacological studies have confirmed the therapeutic effects of many active components derived from Chinese herbal medicines

(CHM). *Ginsenoside Rg1*, extracted from Radix Ginseng can inhibit the apoptosis of neuron cells. Tanshinone IIA, extracted from Radix *Salviaemiltiorrhizae*, and baicalin, extracted from Radix Scutellariae can inhibit the oxidative stress injury in neuronal cells. Icariin, extracted from *Epimediumbrevicornum*, can decrease the hyperphosphorylation of tau protein, and can also inhibit oxidative stress and apoptosis. Huperzine A, extracted from *Huperziaserrata*, exerts a cholinesterase inhibitor effect. Evodiamine, extracted from FructusEvodiae, and curcumin, extracted from Rhizoma*Curcumaelongae*, exert anti-inflammatory actions. Due to the advantages of multi-target effects and fewer side effects, Chinese medicine is more appropriate for long-term use [4].

Despite increasing prevalence of AD worldwide, in addition to extensive research efforts to find a cure, we still have no long term solution. Effective therapeutic and preventative treatments are urgently needed to combat the devastating cognitive decline observed in patients with AD. This is especially pertinent as many potential remedies and medications for neurodegenerative diseases have been derived from traditional medicine [4].

According to Liu et al. [24] Traditional Chinese Medicine (TCM) has been in use for more than 2,000 years. Recently herbal medicines employed to treat AD in China are based on TCM or modern pharmacological theories; this approach has resonated with respect to etiology and pathogenesis of AD, TCM therapy, and herbal extracts useful in treating AD. Evidence suggests that TCM therapy may offer certain complementary cognitive benefits for treating AD. Moreover, Chinese herbs may be advantageous if we consider multiple target regulation especially compared with single-target antagonist [24].

10. Polyphenols and AD

Despite modern medicine's incredible innovation and resulting accumulation of valuable knowledge, many of the world's most problematic diseases such as Alzheimer Disease (AD) still lack effective cures and treatments. Western medicine has revealed many genetic, cellular, and molecular processes that characterize AD such as protein aggregation and inflammation. As the need for novel and effective treatments increases, researchers have turned towards traditional medicine as a resource. Modern, evidence based research examining traditional and complementary remedies for AD has generated promising results within the last decade. Animal based products inhibiting cellular toxicity, anti-inflammatory nutraceuticals such as omega-3 fatty acids, and plant based compounds derived from herbal medicine demonstrate viability as neuroprotective treatments and possible application in developing pharmaceuticals. Analysis of antioxidant, anti-inflammatory, and neuroprotective phytochemicals used in various traditional medicines around the world reveal potential to ameliorate and prevent the devastating neurodegeneration observed in AD [4].

A large number of polyphenolic compounds showing promising results against AD

pathologies have been identified and described in the past decade. Many efforts have been made to unravel the molecular mechanisms and the specific interactions of polyphenols with their targets in the pathway. The diet related chronic diseases of modern society are now the single largest cause of death encompassing diabetes, cardiovascular disease, hypertension, obesity and cognitive decline. To sustain healthy aging requires dietary restraint, a reduction of the consumption of processed foods and fatty diets, with negative nutritional attributes such as high energy refined sugars, saturated fats, high sodium content and an increasing affinity and tendency to consume those with positive health attributes including nutraceuticals, phytochemicals and micronutrient rich foods. Carbohydrates, lipids and proteins are the primary dietary fuels that yield metabolic energy providing body function and performance, whereas dietary phytochemicals and herbal medicines rich in polyphenols [25] are associated with a decreased risk of several human chronic diseases, sustain the cellular molecular machinery, preventing the development of disorders, gain of toxic function and disease conditions. For example, by stimulating lipid metabolism in rats, the green tea flavanolepigallocatechingallate (EGCG) reversed the high-fat diet induced hypercholesterolemic levels in rats and provided protection for the cardiovascular system. The enzymatic and non-enzymatic antioxidant levels were improved, activated sirtuin 1, endothelial nitric oxide synthase and adenosine monophosphate-activated protein kinase α, are all indicators that the protective effect of EGCG and other catechins in green tea act as strong activating agents through stimulation of the metabolism of high-level fats that may lead to a lower risk of developing heart disease [26].

Natural products utilized in folk medicine have demonstrated safety profiles since they have already been utilized for decades for the treatment of disease in humans and animals, we use them as templates for the generation of analogues for the development of therapeutic compounds, and probing molecular mechanisms underlying cellular dysfunction. The major liabilities of herbal medicinal products have poor biocompatibility, pharmacokinetic profiles and BBB permeability [26].

Alkaloids, terpenes, polyphenolic compounds represent the most prevalent classes of herbal constituents with anti-dementia benefit. It is unclear to what extent many of these bioactive phytochemicals, utilized in single or herbal formulae doses can reach the brain in sufficient concentrations and in a biologically active form to exert their neuroprotective effects [27]. For AD therapy, herbal products offer a wider range of brain-targets, nutritional benefits, safer dosage, long-term applications and efficacious treatment of AD pathology. For *in vivo* and large epidemiological studies, the quality assurance of herbal bioactives and production of mass quantities is another challenge for the translation of natural products into therapeutic agents. The majority of herbs are consumed as aqueous extracts so their formulation has to provide increased bioavailability and blood brain barrier permeability. Strategies for enhancing polyphenol bioavailability include encapsulation as phospholipid nanoparticles;

incorporation with biodegradable polymers; use of bioactive analogues; modifications to improve pharmacokinetics, use of adjuvants as absorption enhancers.

Dementia is a multifactorial disease, linked to aging, environmental impacts and is different in each patient. Herbs and food supplements are readily available so it is imperative that the molecular mechanisms of their significant health benefits are determined so herbs or formulations are able to complement approved drugs and provide the best therapeutic treatment against Aβ toxicity [28].

Polyphenols are found in a wide variety of foodstuffs and beverages and the high intake of fruits, vegetables, herbs and many plant foods is inversely related to the incidence of several degenerative diseases, highlighting the increased consumer attention to the importance of a balanced diet in relation to human health. It has been estimated that a balanced diet may provide around 1 g of polyphenols daily. Polyphenols are able to (a) react with free radicals blocking their activity, (b) modulate the expression of genes (epigenetics) involved in metabolism, act as signaling molecules increasing antioxidant defense, and (c) protect and repair DNA damage. Our research efforts focus on the molecular mechanisms that correlate the health benefits of polyphenols against the most common diseases related to oxidative stress driven pathologies, including neurodegenerative, cancer, cardiovascular diseases, inflammation, type II diabetes and metabolic syndrome diseases [26].

Another characteristic feature of polyphenols is their interactions with peptides and proteins. Animal and human studies have demonstrated that dietary flavonoids from chocolate, including (-)epicatechin, promote cardiovascular health, the result of antioxidant and antithrombotic mechanisms. The consumption of dark chocolate increases blood plasma (-)epicatechin, these effects are diminished when consumed with milk/milk chocolate. This indicates not only milk proteins, but also other dietary foods may interact/impair/reduce bioavailability and the absorption of flavonoids from chocolate *in vivo* negating the potential health benefits from dark chocolate. A high cocoa flavanol intervention enhances dentate gyrus function, improving cognition in older adults, most likely by the improved vascular function of (-)-epicatechin. Fortunately the ubiquitous polyphenol-protein interactions also have beneficial effects. The propensity of certain natural polyphenols to interact with $A\beta_{42}$ monomers, blocks their rapid self-association to form low molecular weight oligomers, enables polyphenols to function as $A\beta_{42}$ inhibitors. One of our challenges in designing $A\beta_{42}$ inhibitors is to find the chemical 'Polyphenol Lipinskinisation' changes necessary, that is, to modify polyphenol structures to improve their pharmacokinetics and efficacy. $A\beta_{42}$ peptides misfold into soluble oligomers and protofibrils associated with AD. The mechanism of Aβ inhibition is driven by hydrophobic interactions that involve π-π bonding between the planar faces of the polyphenol structure and the aromatic residues of $A\beta_{42}$. Additionally, hydrogen bonding occurs between the peptide and the phenolic hydroxyl groups. The polyphenols intercede/impose between two

β_{42}-amyloid aromatic residues prevents their stacking, blocking the amyloid self-assembly-β-oligomer-sheet-fibril formation and gain of toxic function. Herbal polyphenols are known to also modulate Aβ production by stimulating the α-secretase and inhibiting the β-site amyloid precursor protein cleaving enzyme-1 (BACE1), γ-protease pathways. (−)-Epicatechin, epigallocatechin are potent inhibitors of amyloid precursor protein processing (APP). Some phenolics show both, a strong inhibition of APP-Aβ generation and anti-amyloidogenic binding [28]. The flavonoids quercetin and myricetin inhibit BACE1 activity, dose-dependently inhibit amyloid fibril formation with myricetin>quercetin>catechin = epicatechin. Similarly, EGCG, resveratrol, curcumin, oleuropein, pentagalloylglucose inhibit β-amyloid misfolding and aggregation by forming nontoxic complexes with the peptide, they also have other benefits against the onset of neurodegeneration. EGCG directly interacts with β-sheet structures in amyloid fibrils leading to an decrease in the binding of Aβ to the fluorescent dye thioflavin T and promotes the assembly of large, spherical oligomersintosafe species, unable to seed fibrillogenesis; remodels Aβ mature fibrils into smaller, amorphous protein aggregates by direct binding to the β-sheet-rich aggregates and mediating a conformational change without generating potentially toxic oligomers [28]. Curcumin is the main constituent of the spice turmeric, whose extensive use apparently accounts for the lower prevalence of AD in the Indian population. *In vitro*curcumin inhibits fibril formation and also destabilizes preformed fibrils, binds to plaques and reduces amyloid levels *in vivo*. Curcumin and resveratrol bind to the N-terminus (residues 5−20) of $A\beta_{42}$monomers. Many *in vitro* studies have demonstrated the multiple potential therapeutic effects of resveratrol, found in herbs, red wine, but it'sin *vivo* efficacy is controversial. The beneficial effects of resveratrol may contribute to its protective effects on cognitive function; however the volume of red wine to be consumed for resveratrol therapy is not practicable. Danshen constituents, salvianolic acid B, rosmarinic acid, tanshinones inhibit $A\beta_{42}$, disaggregate fibrils, and protect cultured cells. Oleuropein in olive oilpreventsformation of β-amyloid oligomers, and dietary oleuropeinaglycone improves the cognitive performance of young/middle-aged mice [26].

One neuroprotective plant that is widely used in Traditional China Medicine (TCM) as herbal medicine is *Evodiarutaecarpa* Bentham. Evodiamine (Evo), an extract of *E. rutaecarpa* Bentham, presents an extensive array of beneficial properties. First, Evo exhibits anti-AD and anti-inflammatory functions. Second, a surfeit exists. There are anticancer, antiobesity, antinomic, antinociceptive, and antimetastatic functions. These results are enormously positive for treating neurodegenerative disorders [29]. Moreover, TCM has also given us compounds that require improvement or alternative substitutes for existing AD. Retinoid X Receptor (RXR) agonist, Targetin, is an effective treatment for AD in mouse models. The TCM compounds β -lipoic acid and sulfanilic acid are also strong candidates as RXR agonists. Forming viable bonds with the RXR protein receptors, these TCM compounds exhibit potential to be developed into anti-dementia drugs [30].

Research on the promising compound Nobiletin employed in Traditional Japanese Medicine (Kampo) has yielded promising results. Nobiletin, a citrus flavonoid, exhibited memory improving functions when tested in several animal models. Demonstrating beneficial effects against oxidative stress, cholinergic neurodegeneration, dysfunction of synaptic plasticity-related signaling, and formation of plaques, nobiletin shows potential as a natural anti-dementia compound. In addition, nobiletin exerts certain possible novel pharmaceutical and preventative applications. When tested in mice, Nobiletin enhanced learning and memory, reversing the impairment inflicted on short-term memory and recognition memory [31,32]. Further investigation of Kampo treatments has revealed another herbal medicine *ninjin'yoeito* (NYT). NYT has been analyzed in clinical trials for its long term effects on cognitive function and mood. Twenty-three patients at varying stages of disease progression who had all shown insufficient responses to treatment with donzepil alone were tested. Eleven patients were treated with donzepil alone, whereas twelve received a combined combination of donzepil supplemented with NYT for two years. After this period, patients receiving donzepil and NYT showed both cognitive improvement and a reduction of AD related depression [33].

Ayurveda, Traditional Indian Medicine, has also contributed to the growing list of valuable compounds. Results have validated ancient remedies for nervous system disorders, including memory related conditions such as dementia. Efforts have been made to analyze Ayurvedic medicine experimentally and to understand its effects on geriatric diseases such as AlzhiemerDisease [34]. Traditionally used as Ayurvedic brain tonic medicine, the plant *Centellaasiatica* (L.) Urban, native to Southeast Asia, exerts various neuroprotective effects. Reducing oxidative stress, inhibiting enzymes, and preventing the formation of amyloid plaques in AD, *C. asiatica* exhibits comprehensive neuroprotection, a potential phytopharmaceutical [35].

11. Application of Integrative Approaches

Modern biomedicine mostly utilizes a Western approach towards solving current medical problems, and has revealed many of the genetic, cellular, and molecular processes that characterize AD such as protein aggregation and inflammation. The unmistakable influence and value of primitive animal models in advancing our understanding of neurodegenerative disease is evident in studies that continue to analyze Drosophila and *C. elegans*. Animal models serve as cost-effective genetic sources for evaluating treatments, enhancing our understanding of conserved biochemical pathways. However, in spite recent insights into mechanisms that underlie AD pathology, current treatments lack efficacy and adequacy, and a significant cure is yet to be found-still, we persevere [4].

As the need for novel and effective treatments increases, researchers have turned towards ancient knowledge and alternative practices. Using a rigorous, evidence based approach to

analyze compounds attributed in CAM and various traditional medicines, researchers revealed viable alternate treatments that show promising anti-inflammatory, memory improving, and neuroprotective effects. They utilize similar animal models to evaluate their potency and value, a confirmation of their still largely untapped utility. Most products are animal or plant based, and their compounds have possible applications whose development into pharmaceuticals reveals promising cures and potential prevention. Acceptance and practice of theory based medicines in Western biomedicine may require significant time to first become integrated or even totally accepted. However, utilizing Western methodology and approaches to medicine to investigate Traditional Medicine and CAM is a more pragmatic approach to integration. Evidence based research and successful application of alternative and traditional compounds have shed light on potential methods and cures for preventing AD, yielding encouraging results that point towards progress and even beyond [4].

12. Future Treatment Breakthroughs

Researchers are looking for new ways to treat Alzheimer's. Current drugs help mask the symptoms of Alzheimer's, but do not treat the underlying disease or delay its progression. A breakthrough Alzheimer's drug would treat the underlying disease and stop or delay the cell damage that eventually leads to the worsening of symptoms. There are several promising drugs in development and testing, but we need more volunteers to complete clinical trials of those drugs and increased federal funding of research to ensure that fresh ideas continue to fill the pipeline.

13. Special Remarks

• The global population is growing, therefore also aging and dementia increases in almost all parts of the world.

• Studies related to autosomal dominant AD indicate that the disease process begins around 20 years prior to onset of dementia [36].

• Recent research provides evidence that life style factors and environmental stresses that increase blood pressure may also increase the risk of AD through Angiotensin II-angiotensin type1a pathway [37].

• There is a need to generate more healthy consumer food products, and with public passion, encourage early adoption of healthy lifestyle-better diets and regular exercise as preventive strategies to reduce cognitive impairment.

• To explain the extended AD pathogenic process over 2 decades, amyloidosis, the incremental neuronal damage caused by non-sequestered β-oligomers is the early pathological event and accumulates over time eventually resulting in neurodegeneration (hypometabolism)

and then widespread cognitive impairment [38, 39].

• (−)Epicatechin and other flavonoid inhibitors of BACE1 proteolysis/cleavage of APP could be protective against early amyloidosis events of AD provided they are included in the diet. Studies suggest the efficacy of orally delivered (−)-epicatechin in a transgenic model of AD in reducing $A\beta_{42}$ production and pathology *via* modulation of BACE1 as a risk reduction strategy, supporting the positive effects of flavonoid rich diets against the development of cognitive impairment [40,41].

• The increase intake of flavonoids and polyphenols is a dietary preventative strategy to (a) reduce β-amyloid formation and (b) competitively prevent β-amyloid misfolding and toxicity against development of AD. A key question is: can these findings translate into preventative benefit for healthy humans?

• The protective effects of flavonoid rich diets against the development of dementia needs to be translated into clinical trials to directly test their efficacy in at risk individuals or those with mild cognitive impairment.

14. Conclusion

Despite extensive knowledge about how diet and nutrition has advanced beyond understanding cellular energy status, diet related chronic diseases of modern society are now the single largest cause of death. Epidemiological investigations indicate that nutrition and dietary patterns are modifiable risk factors that can help limit and prevent chronic diseases, enabling the achievement of the overall objective in slowing human aging diseases such as AD and thereby improving the quality of healthspan of everyone.

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Chapter 3

Alzheimer's Disease and Occupational Exposures: A Systematic Literature Review and Meta-Analyses

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Abstract

Six systematic literature reviews together with meta-analyses have been published on the associations between Alzheimer's disease and occupational risk factors. Our meta-analyses were based only on studies fulfilling good standards of scientific quality. We scrutinized the 54 relevant original publications found using a checklist proposed by the MOOSE-group together with a new elaborated protocol. Thus our results are not hampered by bias from studies of lower scientific quality. Thirty publications fulfilled good scientific standards and were thus used in our meta-analyses. Exposures to electromagnetic fields were concerned in 12 publications. The weighted relative risk estimate was 1.35 (95% confidence interval: 1.08-1.70). Exposure to pesticides or other chemicals resulted in the statistically significant relative risk 1.5 while exposure to metals involved no increase of risk. A high degree of work complexity (especially in relation to people) and long education were both protective against Alzheimer's disease. Based on ten studies the weighted relative risk was 0.47 (95% CI: 0.35-0.63). Both work-related risk factors and protective factors are discussed in relation to possible pathophysiological mechanisms.

Key words: Epidemiology; Electromagnetic fields; Pesticides; Chemicals, Metals; Work complexity; Education.

1. Introduction

Alzheimer's disease is both the predominant type of dementia and the most prevalent of the degenerative disorders. The disease is present in less than 1% of the population at age 65, but after that the prevalence doubles every fifth year [1]. Since the degenerative process starts decades before the onset of clinical disease, it is relevant to examine whether exposures at the workplace are risk or protective factors. There is evidence that some lifestyle factors are important to consider with regard to risk of Alzheimer's disease. Smoking exerts almost a doubled risk [2], while the effects of alcohol and diet are smaller and more complex [3,4].

Six systematic literature reviews have been published on associations between Alzheimer's disease and occupational risk factors [1,5-9]. Two of these publications also included meta-analyses with a focus on exposure to electromagnetic fields, and both studies indicated that exposure might involve an increased risk of disease [8,9] The results were quite heterogenic, which might be explained by methodological weaknesses in some of the included studies with regard to validity of diagnoses and exposures, statistical methods, and recall bias.

Our study originated in a commission from one of the biggest public insurance companies on the Swedish labor market (AFA Insurance), which needed a scientifically-based standard for evaluating work-related disease. The commission involved creating an updated foundation for decisions regarding prevention of and compensation for damage. Taking into consideration all work-related exposures with regard to Alzheimer's disease,we conducted a systematic review of the published literature, scrutinized relevant publications, and carried out meta-analyses using only studies that fulfilled good scientific standards. Our report was published in year 2015 in a Swedish peer-review scrutinized series of publications https://gupea.ub.gu.se/handle/2077/40542. The aim of the present chapter is to update our previous review and meta-analyses and to make the results available to the international public.

2. Methods

2.1. Literature Search

We identified relevant published articles using bibliographic search engines in PubMed, Embase, and Arbline prior to 1 February 2016. Our search criteria were MeSH terms for study design (*cohort, epidemiol*, epidemiologic studies*) in combination with MeSH terms for exposure (*employment, workplace, professions, career, career choice, job, occupations, employment, occupational health, occupational medicine, occupational exposure, occupational injuries, occupational diseases, electromagnetic field*) in combination with any of the MeSH terms for disease (*Alzheimer, Alzheimer disease*). This search produced 919 articles. After we scrutinized the titles and/or abstracts and excluded a few duplicates and excluded all articles

which were not based on original data on exposures related to occupation 89 potentially relevant articles remained.

2.2. Quality Classification

We assessed all relevant publications according to the checklist proposed by the MOOSE-group. [10] We considered selection bias and 'falling off [11], as well as occurrence of dose-response effects [12] and used a system for grading observational epidemiologic articles into a global class I-V as proposed by Armon. [13] Based on these documents, we constructed a decision protocol involving the categories Diagnosis, Exposure, Study group (selection, controls, missing data), Methods and analyses, Armon class, Funding and Exposures, see table 1 and 2. Armon's check-list and our decision protocol are presented in an appendix to a recent publication [14].

An appropriate diagnosis is a basic criterion for classification and preferably should fulfil the NINCDS-ADRDA consensus standards (National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association) used for a clinical diagnosis of Alzheimer's disease. [15] The NINCDS-ADRDA standards are compatible with the diagnostic criteria used both in Diagnostic and Statistical Manual of Mental Disorders (DSM III) and International Classification of Diseases (ICD 9).

The quality of the diagnosis was graded with scores from 1 to 4: 1=the Alzheimer's disease diagnosis from a specialist (neurologist/psychiatrist), 2=diagnosis from a hospital (as an in-patient), 3=diagnosis from a doctor (also including mortality registers), and 4=dementia without separation of Alzheimer's disease. The other categories were graded also with scores from 1 to 4: 1=good, 2=sufficient, 3=uncertain/insufficient, or 4=unacceptable. Sometimes a category was graded in between, and thus was given an interval, for example 2-3. The reason for this was usually lack of sufficient information to obtain a unique score.

The prerequisites for accepting a publication as fulfilling good scientific standards (Armon class II or III) were that the diagnosis score should be 1, 2 or 3 and all the other categories should be scored as 1-2, or 2-3. Articles not qualifying for classes II-III were impaired by serious weaknesses (Armon class IV) or should not be paid attention to (Armon class V). None of the publications fulfilled Armon class I, which almost requires an experimental design.

Only publications [5, 16-44] fulfilling good scientific standards (Armon class II or III) were used in our meta-analyses; see Table 1. Relevant publications not fulfilling good scientific standards regarding the exposure of interest [45-68] are summarized in Table 2.

2.3. Statistical Analysis

Risk estimates from the selected studies are reported as relative risks (RR), as the

outcome is rare, and so odds ratios (OR) and hazard ratios (HR) can be considered equivalent to the RR. When both unadjusted and multivariable-adjusted risk estimates were reported, we only considered the adjusted estimates. Studies which reported stratified estimates for sex were considered as separate studies, and included with both estimates. When exposure was categorized into different levels, the risk rate for the highest level was used according to the principle of dose-response, [69] provided a sufficient number of exposed cases was observed (usually around 30 or more). Estimates based on an extremely small number of individuals were not included, as their effect on the combined estimate could only be of an extremely small magnitude.

We examined the fixed effects model as well as the random effects model by considering statistical heterogeneity. To this end, we used the I^2 statistic and considered the recommended cut-offs of 25%, 50%, and 75% degrees of heterogeneity. We also used a meta-regression approach to stratify on study characteristics, selected a priori, and to evaluate the significance of the stratification variable. The I^2 criterion was applied to examine heterogeneity for each strata. As both these tests indicated a random effects model as the most appropriate choice in almost all studies, the results are reported with random effects estimates. Another reason to choose the random effects model was that the results were drawn from observational studies in different contexts, such as different countries and industries. The weights used for pooling the risk estimates were equal to the inverse-variance weighting. We also performed leave-one-out analysis for each study, to check the influence of each study on the combined estimate.

Publication bias was analyzed by inspection of the funnel plot, which in the absence of such bias would show the RR estimates distributed symmetrically around the weighted RR. The rank correlation test proposed by Begg & Mazumdar [70] was used to supplement the interpretation of the funnel plot. Statistical analyses were conducted using procedures for different aspects of meta-analysis available in STATA software (version 14.2, www.Stata.com), and described in articles from the STATA journal [71].

3. Results

3.1. Electromagnetic Fields and Work With Electricity.

Occupational exposure to electromagnetic fields and work with electricity has been studied extensively with regard to neurodegenerative diseases. Based on 14 publications of sufficient scientific standards, the weighted risk estimate for Alzheimer's disease was 1.25 with a 95% confidence interval (CI) of 1.07-1.46 **(Figure 1)** and for electromagnetic fields in particular the estimate from 12 publications was 1.35 (95% CI: 1.08 – 1.70). The estimate remained firm irrespective of different stratifications (study quality in grade II or III, design in case-control or cohort and funding in public funding or not). Between 1998 and 2014 the estimated cumulative risk rate gradually decreased from 2.70 to 1.25. The funnel plot **(Figure**

2) showed an asymmetric distribution which was especially evident for smaller studies with increased risk, but the risk estimates from more recent studies involving more cases were arranged fairly symmetrically around the combined estimate RR=1.25. Begg's test gave p=0.13, indicating some publication bias, but not to a very pronounced degree.

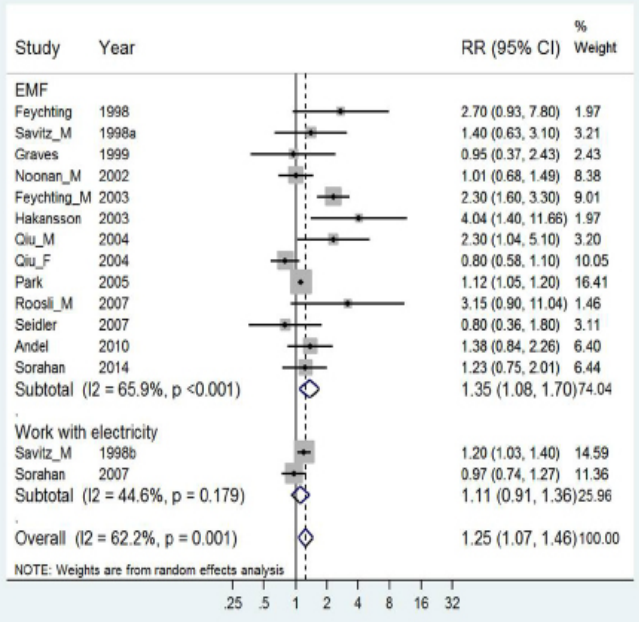

Figure 1: Forest plot for studies assessing the association between Alzheimer's disease and occupational exposure to electromagnetic fields and work with electricity. Results for men only are indicated by M and those for women only by F; otherwise the results concern both sexes. Random effect models were used, with stratification for different exposure categories. Heterogeneity was tested by the I^2 statistic, with $p<0.05$ indicating rejection of homogeneity.

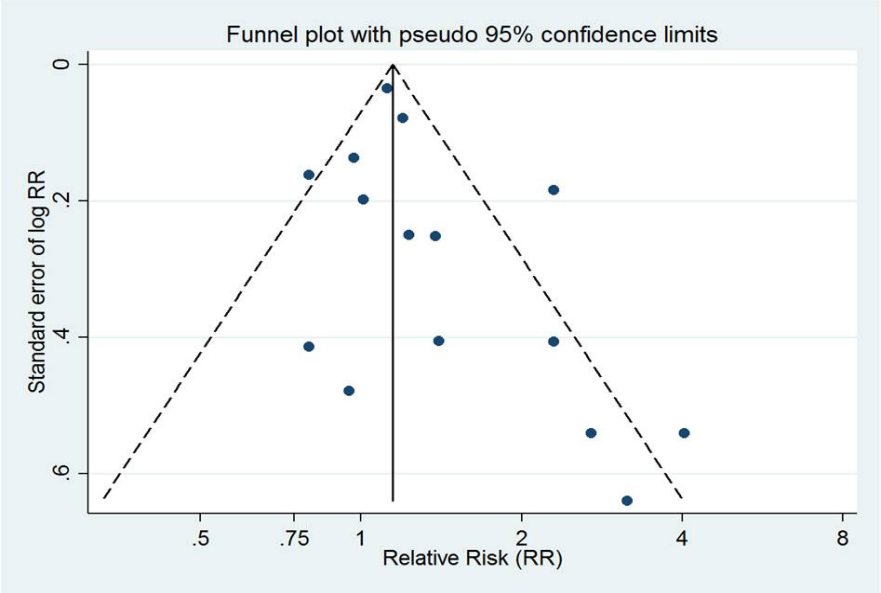

Figure 2: Funnel plot for the RR estimates of the association between Alzheimer's disease and exposure to electromagnetic fields and work with electricity.

The leave-one-out test showed that excluding the result from Feychting [20] for males had the greatest impact on the combined estimate, lowering it to 1.15 (95% CI: 1.01–1.31), and that excluding the large study by Park [32] raised the estimate to 1.32 (95% CI: 1.06–1.63). Also the heterogeneity as indicated by I^2 decreased from 62.2% to 41.8% when the result for Feychting was excluded, and a fixed effect estimate for RR was 1.13 (95% CI: 1.06 – 1.19),

thus somewhat lower risk and a smaller confidence interval.

3.2. Chemicals and Metals

The four publications on associations between Alzheimer's disease and exposure to chemicals [19,22,29,32] gave a weighted risk estimate of 1.52 (95% CI: 1.00–2.31) **(Figure 3)**. In one of these publications, the information on exposure was less specific, being based only on occupation as registered in the census [32]. Exclusion of this study yielded a weighted risk estimate of 1.93 (95% CI: 1.30-2.87) and no heterogeneity. The four publications concerning the effect of pesticide exposure [19, 25, 32, 35] gave a risk estimate of 1.50 (95% CI: 0.98–2.29) **(Figure 4)**. Excluding the study by Park [32] increased the estimate to 1.85 (95% CI: 1.12–3.05). Regarding chemicals and pesticides there were too few studies to make the tests of publication bias trustworthy. Three studies examined exposure to metals; aluminium [22] and welding [32, 43]. The weighted risk estimate for these diverse metal exposures was 0.95 (95% CI: 0.90–1.00) **(Figure 5)**. There was no heterogeneity and the study by Graves [22] had almost no influence on the weighted risk estimate.

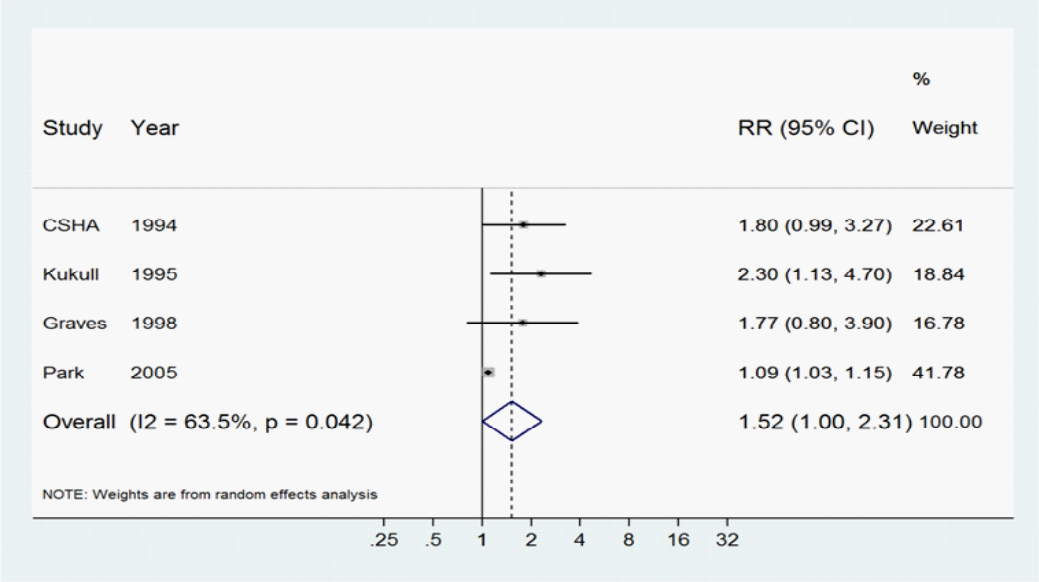

Figure 3: Forest plot for studies assessing the association between Alzheimer's disease and occupational exposure to chemicals. A random effect model was used. Heterogeneity was tested by the I^2 statistic, with $p<0.05$ indicating rejection of homogeneity.

3.3. Work complexity and education

Education level and work complexity were studied in twelve publications. [5,16,18,19,26-28,30,33,39,40,44] One of these publications was excluded from the meta-analyses, since it only stated that Alzheimer's disease was not associated with any specific occupation and did not present numeric risk estimates. [26] However, that study showed that the relative risk in the group with dementia (including Alzheimer's disease) was 0.66 (95% CI: 0.48-0.91) comparing white-collar work with blue-collar work. Another publication [40] was also excluded from the meta-analyses since the statistical methods were not analogous to the methods in the remaining

ten publications. However, that article stated that overall the cases showed significantly lower mental occupational demands and significantly higher physical occupational demands, in comparison to controls.

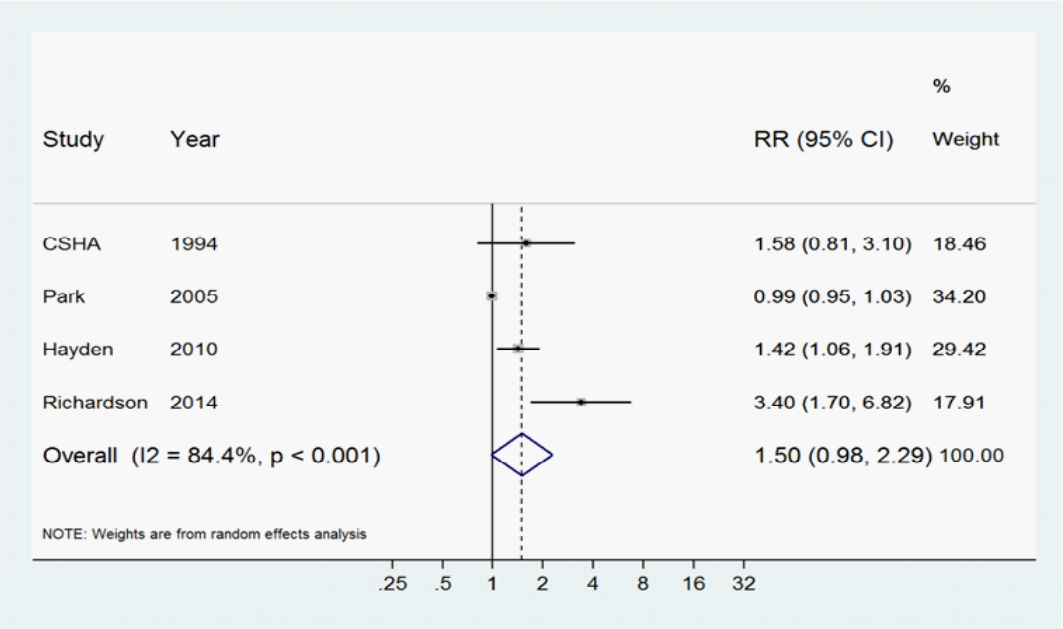

Figure 4: Forest plot for studies assessing the association between Alzheimer's disease and occupational exposure to pesticides. A random effect model was used. Heterogeneity was tested by the I^2 statistic, with $p<0.05$ indicating rejection of homogeneity.

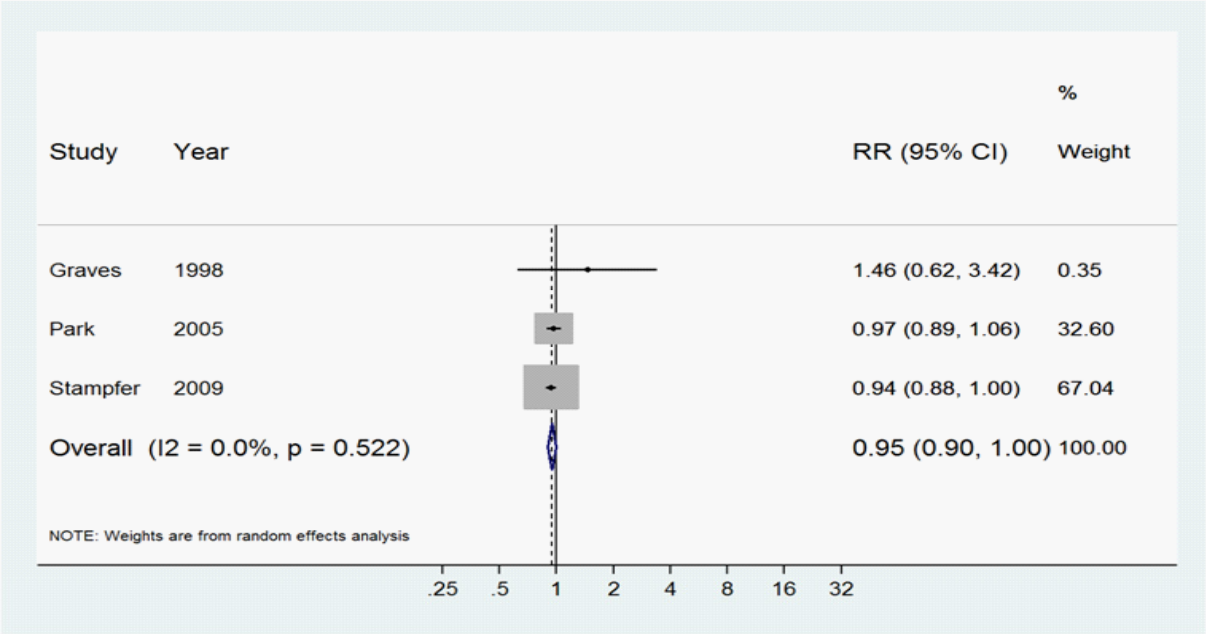

Figure 5: Forest plot for studies assessing the association between Alzheimer's disease and occupational exposure to metals. A random effect model was used. Heterogeneity was tested by the I^2 statistic, with $p<0.05$ indicating rejection of homogeneity

The risk estimates in the remaining ten studies were harmonized/inverted with low work complexity, low education and/or low job control as reference category **(Figure 6)**. The weighted risk estimate was 0.47 (95% CI: 0.35–0.63); after exclusion of an extreme outlier [18], this increased to 0.52 (95% CI: 0.40–0.68), and exclusion of the Canadian study [19] had a similar effect (RR: 0.52, 95% CI: 0.39–0.68). For cognitive work in particular the RR

increased from 0.46 to 0.72 (95% CI: 0.53 – 0.91) when Bickel [18] was excluded and the heterogeneity decreased to 27.6%. Overall, education had the highest impact, reducing the risk of Alzheimer's disease to one third while white versus blue collar works had the smallest impact on the risk. There were no clear indications of publication bias when the extreme outlier [18] was excluded, but the inclusion of this study influenced the test of publication bias towards significance.

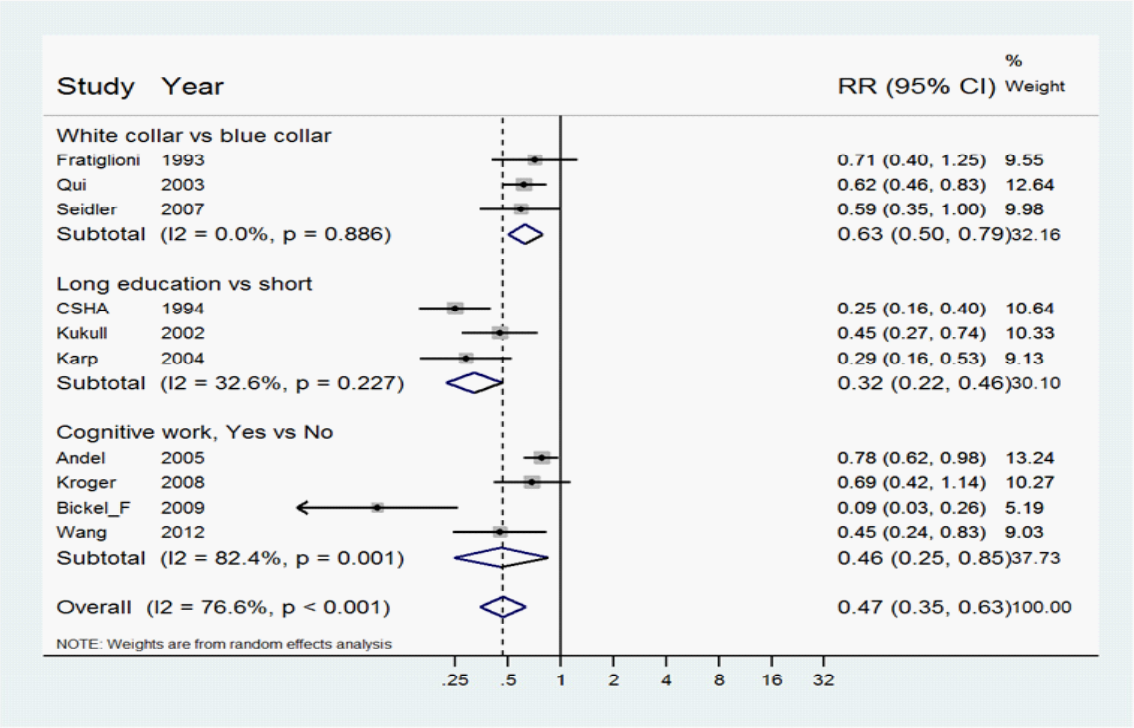

Figure 6: Forest plot for studies assessing the association between Alzheimer's disease and white-collar work versus blue-collar work, length of education, and cognitive demands at work. Results for women only are indicated by F; otherwise the results concern both sexes. Random effect models were used, with stratification for work and education. The heterogeneity was tested by the I^2 statistic, with $p<0.05$ indicating rejection of homogeneity.

4. Discussion

Weighted risk estimates based on scientifically high quality epidemiologic publications indicate that the risk of Alzheimer's disease is elevated after exposure to chemicals and possibly also after exposure to electromagnetic fields. The latter exposure might at least partially be explained by publication bias. However, the highest impact from occupation was related to a high degree of work complexity, which could reduce the risk of disease to less than half.

Meta-analyses have the general limitation that the calculations can only be based on published data and will reflect any inherent weaknesses of design in the studies included. Furthermore, all previously published meta-analyses of Alzheimer's disease have been based on all relevant publications identified, irrespective of the quality of the study design.

One strength of our study is that the meta-analyses were based on a systematic literature review including only studies fulfilling high scientific standards. In order to make a standardized examination of the publications we used an elaborated protocol [14] that was based on the

detailed check-list proposed by Armon. [13] For every publication the authors individually filled in the scores of the protocol and our inter-observer agreement was high; however if our scores were divergent, we rescrutinized the publication and found consensus. However, there is always room for a reader's own discretion when judging a publication. Before adapting our protocol we blindly tested it on articles graded in Armon [13] and also here we found very high agreement between our grading and that of Armon's quality assignment.

Another strength of our meta-analyses is that we focused heavily on finding all possible sources of bias, using stratification of data with regard to possible confounders such as study design, gender, and funding. We also looked for publication bias using both funnel plots and tests for publication bias.

Although much research in recent decades has focused on the role of the amyloid cascade in the degenerative processes of Alzheimer's disease, this hypothesis has failed to identify the mechanisms causing the neurodegenerative process. Another approach is to study the amyloid precursor protein (APP) from which the much smaller amyloid protein emanates. [72] APP is a transmembrane big protein that belongs to the group of 'housekeeping' proteins. Outside the cell, APP has several receptors for different external products, and the protein might be regarded as a 'lodge-keeper' transmitting information from the outside of the cell to the nucleus. Since APP is such a big molecule, the folding and turnover might be highly influenced by toxins such as pesticides as well as by ultra-fine particles. The latter exposure can come from combustion and smoking, and smoking almost doubles the risk of Alzheimer's disease [2].

Considering the available evidence, no biological pathways have been identified by which exposure to electromagnetic fields might precipitate pathological changes leading to Alzheimer's disease [9]. Publication bias and methodological shortcomings might explain the slightly elevated risk estimates found [8,73], a conclusion also supported by our meta-analyses. The funnel plot **(Figure 2)** showed that the risk estimates were not elevated in studies based on bigger study groups. The almost doubled risk caused by exposure to pesticides is of the same magnitude as that caused by exposure to tobacco smoking [2].

The highest protection against Alzheimer's disease was found for cognitive work and long education. The Bavarian School Sisters study, which included 442 female members of a religious order with an average of 54 years of membership of the order, had 60 participants diagnosed with Alzheimer's disease.[18] Those with longer education, with vocational training, and/or who had been appointed to leading positions were at much lower risk. Cognitive work can also be described in terms of work complexity, with regard to work with data, people, and things [74]. Two epidemiologic studies evaluating work complexity in relation to Alzheimer's disease [16,28] both found that the protective effect was most evident for complexity in relation to people, which can be seen as equivalent to having had leading positions in the

Bavarian School Sisters study [18]. The latter study also showed an evident protective effect of education.

There has been some discussion of whether the underlying protective effect is education, rather than work complexity per se. A twin study among 2 622 pairs of twins, including 146 individuals diagnosed with Alzheimer’s disease [16], reported that the level of education was quite similar within each pair and would thus not convey bias in the analyses; the author concluded that education had its own protective effect independent of work complexity. Moreover, in the Bavarian School Sisters study, the protective effects of education, vocational training, and leading positions were additive and potentiated instead of linear [18].

Figure 7 provides a timeline illustrating the relationship between the protective factors. The individual starts with a certain degree of cerebral complexity predestined by their genes and early life experiences. The brain is then exposed first to education and later to cognitive tasks such as vocational training and work complexity, both of which factors increase the complexity of the neural network. In other words, exposure to challenging tasks improves the development of the brain. The factors are independent and have additive protective effects, although they are related; individuals who start with a high degree of cerebral complexity more frequently apply for higher education and those with higher education are more likely to work in leading positions and perform complex work.

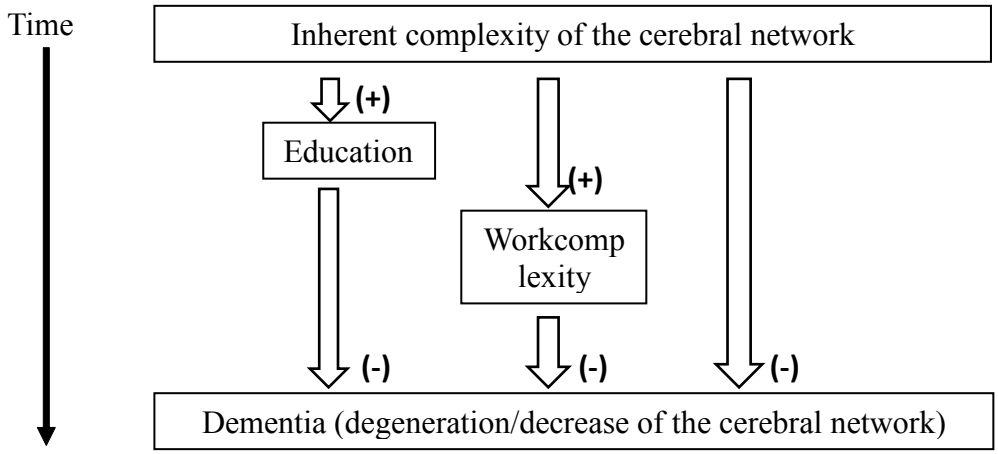

Figure 7: A graphical illustration of the interaction between different preventive factors and dementia. The arrow on the far left starts at birth and ends at death. The direction of each association is indicated within brackets, where (+) indicates a positive association (i.e. an enhanced ability to manage education and work complexity) and (-) indicates a negative association entailing an increased risk of development of dementia.

In a recent study, 323 middle-aged persons diagnosed with Alzheimer’s disease underwent structural magnetic resonance imaging, cognitive evaluation, and work history assessment. [75] The results indicate that brain degeneration had a less harmful effect on cognition among those exposed to higher work complexity, although the brain atrophy was inexorably progressive. Thus, in people at risk of Alzheimer’s disease, occupational complexity may confer resilience of cognition against progressive neurodegeneration. Additive protective effects can be expected

from inherent high brain complexity, further improved by the beneficial brain plasticity effects of a long education.

Table 1: Publications fulfilling good scientific standards (Armon class II or III) defined in an appendix (www.sjweh.fi/index.php?page=data-repository). [14] Diagnosis was graded at least score 3 (diagnosis from neurologist/psychiatrist, in-patient care or mortality register). The other categories involved at least a single score 2 or included in the interval 2-3 (1=Good, 2=Sufficient, 3=Uncertain/Insufficient).[EMF=electromagnetic fields, I=industry, PA=patient association, PU=public,?=funding is not possible to classify based on information in source text]

Publication	Year	Diagnosis	Exposure	Study group	Methods, analysis	Armon's global class [13]	Funding:	Exposures
Andel [16]	2005	1	1	2-3	1	III	PU	Occupation (work complexity)
Andel [17]	2010	1	1	2	1	II	PA, PU	Occupation (EMF)
Bickel [18]	2009	1	2	1	1	II	PU	Work complexity
CSHA [19]	1994	1	2	2	2	II	PU	Pesticides, chemicals, education
Feychting [20]	1998	1	1	2	1-2	II	PU	Occupation (EMF)
Feychting [21]	2003	3	2-3	1	1	III	PU	Occupation (EMF)
Fratiglioni [5]	1993	1	2	2-3	2	III	PU	Manual work
Graves [22]	1998	2	2	2-3	2-3	III	PU	Chemicals including metals
Graves [23]	1999	2	2	2-3	2-3	III	PU	Occupation (EMF)
Hakansson [24]	2003	3	2-3	2	1	III	I	Occupation (EMF)
Hayden [25]	2010	1	2	2-3	2	III	PU	Pesticides
Helmer [26]	2001	1	2-3	2-3	1	III	PU, I	Occupation (blue-collar work)
Karp [27]	2004	1	1	2-3	1	III	PU	Education
Kroger [28]	2008	1	2-3	2	1	III	PU	Work complexity
Kukull [29]	1995	1	2	2-3	1	III	PU	Solvents
Kukull [30]	2002	1	2	2-3	1	III	PU	Education
Noonan [31]	2002	3	2-3	2	1-2	III	?	Occupation (EMF)
Park [32]	2005	3	2-3	2	2	III	PU	Occupation (EMF, pesticides, metals, chemicals)
Qui [33]	2003	1	1	2-3	1	III	PA, PU	Occupation (blue-collar work)
Qui [34]	2004	1	1-2	2-3	1-2	III	PA, PU	Occupation (EMF)
Richardson [35]	2014	1	2	2-3	2	III	PU	Pesticides (DDT)
Roosli [36]	2007	3	1	2	2	III	PU	Occupation (EMF)
Savitz [37]	1998	3	2-3	2	2	III	I, ?	Occupation (EMF)

Savitz [38]	1998	3	2-3	2	2	III	?	Occupation (work with electricity)
Seidler [39]	2007	1	2	2-3	2	III	?	Occupation (EMF, blue-collar work)
Smyth [40]	2004	1	1	2-3	2	III	PU, I	Occupations (work complexity)
Sorahan [41]	2007	3	2	2	2	III	?	Power station workers
Sorahan [42]	2014	3	2	2	2	III	?	Power station workers (EMF)
Stampfer [43]	2009	3	2-3	2	2	III	I	Occupation (welding, metals)
Wang [44]	2012	1	1	2-3	1-2	III	PA, PU	Psychosocial stress (cognitive work)

Table 2: Publications not fulfilling good scientific standards (Armon global class IV or V) defined in an appendix (www.sjweh.fi/index.php?page=data-repository) [14]. The category Diagnosis was graded as 4 (dementia without separation of Alzheimer's disease) or any other category involved a single score 3 or 4 (3=Uncertain/Insufficient, 4=Unacceptable). [EMF=electromagnetic fields, I=industry, PA=patient association, PU=public,?=funding is not possible to classify based on information in source text]

Publication	year	Diagnosis	Exposure	Study group: selection, controls, missing data	Methods and analysis	Armon global class[13]	Funding:	Exposures
Amaducci [45]	1986	2	2-3	3-4	3	IV	PU	Possible risk factors
Baldi [46]	2003	2	2-3	3	1	IV	PU	Occupation (pesticides)
Beard [47]	1992	2-3	3-4	2-3	2	IV	PU	Medical journals (education)
Chandra [48]	1987	2	2-3	3	3	IV	?	Possible risk factors
Davanipour [49]	2007	1	2-3	3	2-3	IV	PU	Occupation (EMF)
French [50]	1985	2	3	3	3	IV	PU	Possible risk factors
Gauthier [51]	2001	2	2-3	3-4	2	IV	PU, I	Pesticides, chemicals and metals
Gun [52]	1997	2-3	3	3	2	IV	PU	Chemicals + vibrations
Harmanci [53]	2003	2	3	3	2-3	IV	I	Possible risk factors
Heyman [54]	1984	2-3	2	2-3	3	IV	PU	Animals
Johansen [55]	1998	4	2	2	2-3	V	PU, I	Occupation (EMF)

Johansen [56]	2000	4	2	2	2	V	PU, I	Occupation (EMF)
Lehman [57]	2012	4	1	2	3	V	PU	Professional American Football
Li [58]	1992	1	2-3	3-4	2-3	IV	?	Pesticides, chemicals and metals
O´Flynn [59]	1987	3	3	2	2-3	IV	?	Occupation (solvents)
Peters [60]	2013	3	3	2-3	2-3	IV	?	Company register (aluminum)
Ravaglia [61)	2002	1	2-3	3	2-3	IV	PU	Occupation (education, agricultural work)
Rovio (62)	2007	2	2	2-3	3	IV	PU	Physical activity
Salib (63)	1996	1	3	3	2	IV	?	Aluminum
Schulte (64)	1996	3	3	2-3	3-4	IV	PU	Occupation
Shalat (65)	1988	2	2	3	2-3	IV	PU	Occupation (lead, solvents)
Sobel (66)	1995	1	2	4	2-3	V	PU	Occupation (EMF)
Sobel (67)	1995	1-2	2-3	3-4	2-3	IV	PU	Occupation (EMF)
Tyas (68)	2001	1	2-3	3	2-3	IV	PU	Chemicals

5. Acknowledgements and Funding

This study was supported by grants from AFA Insurance (which is owned by Sweden's labour market parties) and the Department of Occupational and Environmental Medicine, Sahlgrenska Academy, University of Gothenburg. There are no conflicts of interest.

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Chapter 4

Fruit Fly (*Drosophila melanogaster*): A Viable Model for Screening Tropical Functional Foods for Neuroprotective Properties

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Abstract

In the prevention of chronic disease such as neurodegenerative diseases, nutrition is critical; not just to meet nutritional requirements but more importantly to contribute to the total wellness of the consumer by either preventing and/or managing such disease conditions. This has further promoted the concept of functional foods and nutraceuticals. However, while several studies abound on the huge abundance and diversity of functional foods especially in tropical parts of the world, there is a still serious limitation to rapid and high throughput experimental screening for neuroprotective properties of several functional foods especially in developing nations. These limitations include modern, effective and accessible experimental models for rapid screening, cost of research and ethical issues with animal use among others. Fruit fly (*Drosophila melanogaster*) has emerged as a very useful model of neurodegenerative disease and could be more effective for therapeutic screening for neuroprotective properties of functional food and nutraceuticals especially from developing countries of tropical Africa. This

model organism has such advantages as short life span, high fecundity, low cost of maintenance, ease of handling and small genome size already sequenced and easy to manipulate. Therefore, this chapter review recent trends in functional food research especially of tropical African origin and how *D. melanogaster* can help optimize the effective screening of their neuroprotective properties.

1. Introduction

The saying "Let food be thy medicine and medicine be thy food" of Hippocrates about 2,500 year ago has attracted much scientific attention. This is clearly seen in the interest shown by scientists from various relevant fields in the role of specific food in enhancing health and well- being. While all foods could be said to have functionality mainly in terms of their nutritive values, however, the idea of functional food is not to be viewed as only necessary for living but is to also contribute to the total wellness of the consumer which involves prevention and reduction of disease risk factors, thereby enhancing the overall physiological function. In 1989, Stephen DeFelice, MD, (founder and chairman of the Foundation for Innovation in Medicine (FIM), Cranford) coined the word 'nutraceutical' from nutrition and pharmaceutical [1]. Nutraceutical can be described as a food (or part of a food) that provides medical or health benefits including the prevention and or treatment of a diseases. Nutraceuticals are often said to be products that are extracted or purified from animal, plant or marine sources which have shown physiological benefit or known to protect against chronic diseases.

Neurodegenerative diseases are pathologies with many ethology. Studies have shown that impairments in neurochemistry, oxidative stress and elevated metal ions deposits in the brain are few of the factors that contribute to the progression of neurodegenerative disease [2]. In order to set the key etiological factors as a focal point, it is very important to develop a multidimensional therapy that will prevent and manage these diseases. Most drugs designed all have short live span and side effects [3]. In order to overcome this limitation, dietary interventions as a complementary approach in management/prevention of neurodegenerative disease becomes imperative for holistic management.

Many studies have been published on therapeutic properties of functional foods especially of tropical African origins. In the last few decades, many interesting research publications have originated from Africa on therapeutic properties of several tropical functional foods. However, one major limitation to full evolution of functional food research in Africa has been adequate screening models. Many of published data on functional food from Africa has been from *in vitro* research with a good number on *in vivo* animal (usually mouse and rats) models. However, the current advocacy on ethical controls on laboratory rodent use is gradually challenging biomedical research generally in Africa. Therefore, it has become imperative to explore alternative models. *Drosophila melanogaster* also called fruit fly has emerged to the fore when it comes to therapeutic screening of functional foods, nutraceuticals, chemotherapeutic drugs

and lots more. Screening a large pool of therapeutics in search of few lead compounds is cost effective using Drosophila.

2. Functional Foods and Nutraceuticals: An Overview

The term functional foods originates from Japan in the mid-1980s and it often referred to processed foods that possess active ingredients that aids specific function in the body in addition to its nutritional values. Around the world, Japan is foremost in creating specific regulatory approval process for functional foods. Functional food possesses a wide range of definition, one of which was published in European consensus publication as: "a food is said to be functional if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond its adequate nutritional effects, in a way which is relevant to either the state of well-being and health or the reduction of the risk of disease". Another definition is given by the Institute of Medicine of the National Academy of Sciences "as any food or food ingredient that may provide health benefit beyond the traditional nutrients it contains" [4]. Another simpler definition states that functional food are foods that are similar in appearance to conventional food and are often regarded as normal diet, but have been modified to provide physiological benefits apart from the basic nutrients they provide [5].

Functional food and conventional foods are often similar in appearance or even closely related. But they differ slightly in that functional foods has physiological benefits and are capable of reducing of the risk of chronic diseases beyond provision of nutritional values [6]. Functional food components are potentially beneficial components present in naturally occurring foods or functional ingredients added. These components include carotenoids, dietary fibres, fatty acids, flavonoids, phenolics acids, plant sterol, prebiotics and probiotics, soy proteins, vitamins and minerals, isothiocyanates. The concept of functional food and nutraceuticals are often used interchangeably but they can be distinguished. Functional food is a broad term used to describe food or part of food with specific function [7], whereas nutraceuticals deals with the expected result of the products which could be prevention or treatment of diseases. While functional food can also be food products that are required to be taken as part of usual diet so as to elicit beneficial effect (therapeutic effect) that goes beyond the known traditional nutritional benefit [8], nutraceuticals on the other hand could be described as purified products from plant or animal functional foods.

3. Functional Foods are Beyond Nutrient Sources

A lot of scientific findings have proven that functional foods possess a wide range of therapeutic potential in the prevention and management of several diseases afflicting humans. Since functional food is mostly of plant origin the bioactive components present therein are majorly phytochemicals. These includes phenolic acids, flavonoids, alkaloids, ascorbic acids and vitamin E. These components have been reported upon to possess antioxidant property

which is one of the proposed mechanism through which they bring forth their therapeutic effects [9]. Antioxidants are essential molecules which are needed to counteract the deleterious effect caused by free radicals in biological tissues. Although, every biological organism has endogenous antioxidant systems to effectively manage the oxidative damages of free radicals, an overwhelming amount of free radicals could cause a tilt in this check and balance system which could lead to extensive tissue damage; this phenomenon is called oxidative stress. Oxidative stress often necessitates sourcing antioxidants from exogenous sources such as found in functional food. Furthermore, oxidative stress has been linked to the pathogenesis and progression of many human diseases such as diabetes, cardiovascular disease, inflammation, cancer and dementia among others. Interestingly, reports have shown potentials in the management of these diseases such as ginger and turmeric for the management of hypertension [10,11], green leafy vegetables for the management of dementia [12,13] and legume seeds for the management of type 2 diabetes [14,15]. The health benefits of fruits and vegetables in preventing chronic diseases including type 2 diabetes are attributed to their antioxidant constituents including polyphenols, carotenoids and ascorbic acid which could help prevent or ameliorate oxidative stress [16]. In addition to ameliorating oxidative stress, the bioactive component of fruits and vegetables can elicit their antidiabetic effect by stimulating insulin secretion and inhibiting carbohydrate absorption in the small intestine ultimately resulting in lower blood glucose level [17, 18]. In the past decades, fruits and vegetables have gained much interest in the management of cancer [19]. Although, most functional foods are of plant sources but some are also of animal origin that is quite interesting. Examples of such are (n-3) fatty acids from fish and conjugated linolenic acid present in milk and meat products [20]. In the management of cardiovascular disease, (n-3) fatty acid which are essential class polyunsaturated fatty acids have shown potential.

4. Tropical Functional Foods for the Brain

Functional foods that have neuroprotective properties are of higher focus due to the increased incidence of neurodegenerative diseases. A brief outline of research findings on some tropical functional foods with neuroprotective properties is given in table 1. Nevertheless, it is still clear that many outstanding *in vitro* findings do not often get to *in vivo* levels due to among others factors of cost and ethical issues with the use of mammalian models.

Table1: A brief outline of research findings on some tropical functional foods with neuroprotective properties

Class	Common name	Botanical name	Plant part	Major findings	Nature of study	Reference
Fruits and nuts	Citrus (orange, grape fruit and shaddock)	*Citrus spp.*	Peel	1. AChE, BChE and MAO inhibitory properties 2. Antioxidant properties	*In vitro*	[21]
	African star apple	*Chrysophyllum albidum*	Fruit			[22]
	Mangosteen	*Garcinia mangostana* Linn.	Fruit	1. Antioxidant properties 2. Protect against H_2O_2-induced oxidative stress in NG 108-15 neuroblatoma cells.	*In vitro*	[23]
Spices	Tumeric	*Curcuma longa*	Rhizome	1. Chronic dietary inclusion of turmeric rhizome protects against MPTP-induced PD in mice 2. Dietary inclusion of turmeric rhizome ameliorates alterations in activities of major neuronal enzymes in synaptosomes from the cerebral cortex of hypertensive rats	*In vivo*	[24, 25]
			Curcumin isolated from turmeric rhizome	1. Protect against ethanol-induced brain damage in rats 2. Protects blood-brain barrier integrity in cerebral ischemic rats 3.Synergize antiamnesic and anticholinesterase properties of Donepezil in rats	*In vivo*	[26, 27]
	Pepper	*Capsicum* spp.	Fruit	1. AChE and BChE inhibitory properties 2. Antioxidant properties	*In vitro*	[28, 29]
				Ameliorate cyclophosphamide induced oxidative stress in rat brain	*In vivo*	[30]
			Capsaicin isolated from red pepper	Ameliorate biochemical markers in MPTP-induced PD in male C57BL/6J mice	*In vivo*	[31]

	Ginger	*Zingiber officinale* Roscoe	Rhizome	Enhance cognitive function and protect against neurochemical alterations in experimentally induced mammalian models of cognitive dysfunction	*In vivo*	[32]
			Essential oil	Prevents oxotremorine-inducedtremors and increased the latency of pilocarpine-induced seizures, as well as survival at 50 and 100 mg/kg in male swiss mice. However, higher dose of 100 mg/kg presents some cognitive impairments		[33]
	Onion	*Allium cepa*	Bulb	Attenuation of ischemia-induced oedema and elevation in MDA level in mice brain	*In vivo*	[34]
				Protection against ischemic neuronal damage in Gerbil hippocampus		[35]
Vegetable	Tomato	(*Lycopersicon esculentum* Mill. var. *Esculentum*	Fruit	1. AChE inhibitory property 2. Inhibit Fe^{2+} and QA-induced lipid peroxidation in rat brain	*In vitro*	[36]
		Lycopersicon esculentum Mill. var. *Cerasiforme*)				
	African Jointfir	*Gnetum africanum*	Green leafy vegetable	AChE, BChE and MAO inhibitory properties	*In vitro*	[37]
	Fluted pumpkin	*Telfairia occidentalis*)	Green leafy vegetable	1. AChE inhibitory property 2. Inhibit Fe^{2+} SNP and QA-induced lipid peroxidation in rat brain	*In vitro*	[12]
	Bitter leaf	*Vernonia amygdalina*	Green leafy vegetable	Antioxidant properties and Inhibit H_2O_2-induced lipid peroxidation in rat brain	*In vitro*	[38]
	Horseradish	*Moringa oleifera*	Green leafy vegetable	Ameliorate cognitive dysfunction in STZ-treated rats treated with acarbose	*In vivo*	[39]
	Black nightshade	*Solanum nigrum* L	Green leafy vegetable	Ameliorate cognitive dysfunction and neurochemical impairments in scopolamine-treated rats	*In vivo*	[13]
	African eggplant	*Solanum macrocarpon* L		AChE inhibitory and antioxidant properties	*In vitro*	[40]

AChE= Acetylcholinesterase. BChE= Butyrylcholinesterase, MAO=Monoamine oxidase, MDA=Malondialdehyde, MPRP=1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, PD= Parkinsons's disease, QA= Quinonilic acid, SNP= Sodium nitroprusside, STZ= Sterptozotocin

5. Fruit Fly (*Drosophila Melanogaster*) as a Therapeutic Screening Model for Functional Foods

5.1. Life cycle of *drosophila melanogaster* in brief

Drosophila has a very speedy life cycle **(Figure 1).** A single mating pair that is fertile can produce hundreds of offspring that are similar genetically in a period of 10- 12 days at room temperature. This is highly contrasting to the laboratory rodents that produce just a few offspring every 3- 4 months. The development of this fly occurs in stages (complete metamorphosis): the egg (embryo), larva, pupae and the full grown adult. The larva stage is actually in three phases; the first instar larva, second instar larva and the third instar larva. The third instar larva often wander in the culture media and it is at this phase the pupa develops. A great morphological changes often characterize the development of the third instar larva into pupae and eventually to the full grown adult. Under optimal condition life span of an adult fly is approximately 120 days. (For further reading, see Abolaji et al., [41] for more detailed review on drosophila development and husbandry).

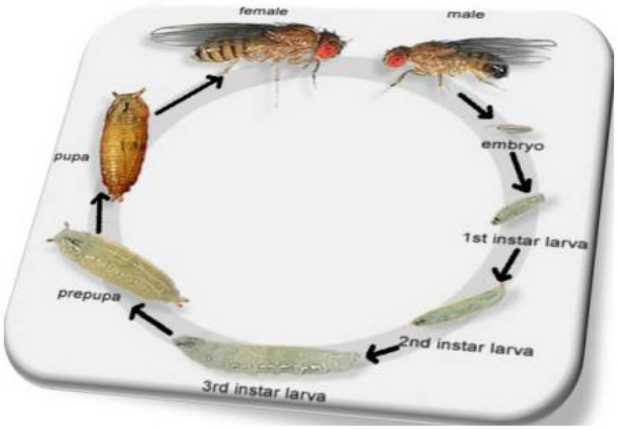

Figure 1: Life cycle of fruit fly (*Drosophila melanogaster*) (Abolaji et al., [42])

5.2. Potentials of *Drosophila melanogaster* as Model Organism

As stated earlier, functional food and nutraceuticals are of great interest to humans. Therefore, it becomes imperative to screen large pool of potential sources of functional foods and nutraceuticals for their perceived therapeutic functions; obviously, using humans as a screening model will be ambiguous, the extreme number of rats to be used will be unethical and *in vitro* studies will be insufficient. In search of a way out, the use of *Drosophila melanogaster* as a model animal comes to fore. Although, it might seem ironic that a 'tiny' fruit fly serving as a model for human therapeutic screening and physiologically spealing, there exist a wide range of differences between humans and the fruit fly. However, there is genetic homology between them which is the striking factor that makes research using *D. melanogaster* unique. Approximately 75% of diseases causing genes in human are conserved in *D. melanogaster* [43]. Another striking fact about *D. melanogaster* is the ease and cost effectiveness to manipulate and create a transgenic fly using high throughput genetic procedures [44]. With the availability

of these procedures, it is easier to generate models of human disease rapidly through various genetic engineering processes including mutation, genetic inactivation, or mis-expression of fly homologs of human disease genes and protein themselves.

Drosophila can also be used to monitor various pathological indices of neurodegeneration (figure 2). The wide varieties of these indices spanning biochemical, anatomical, molecular and behavioural aspects of neurodegeneration makes this organism quite useful as a research model. The flies present several anatomical features that can be monitored as indices of incidence and progression of neurodegeneration. Such features as simple as wing shape, eye colour and shape, fly size, larva size, and as complex as neuronal integrity, microtubule formation, synaptic formation and function could be used as markers of neurodegeneration as well as to monitor the potentials of any test therapeutic agent [45-48]. Such intrinsic anatomical alterations are often the phenotypic realities of several biochemical and molecular changes which ultimately reflect in their behavioural patterns such as their ability to climb (negative geotaxis), sleep-wake behaviour (circadian rhythm), copulation (aggressive behaviour) and their spatial orientation (movement pattern). Usually, fly models of neurodegeneration with marked anatomical and behavioural changes display concomitant biochemical and molecular modulations such as elevated level of reactive oxygen and nitrogen species, impaired cholinesterase activity, alterations in activities of antioxidant enzymes and levels of neurotransmitters, as well as modulation of gene expression levels of proteins of therapeutic importance; all these offers several useful points of evaluating therapeutic potentials of test compounds [46, 49-54]. Furthermore, the short and completely sequenced genome, coupled with the availability of relevant and accessible bioinformatics tools for Drosophila such as 'fly base' (http://flybase.org) makes investigations using Drosophila as model organism at the molecular level more achievable and attractive.

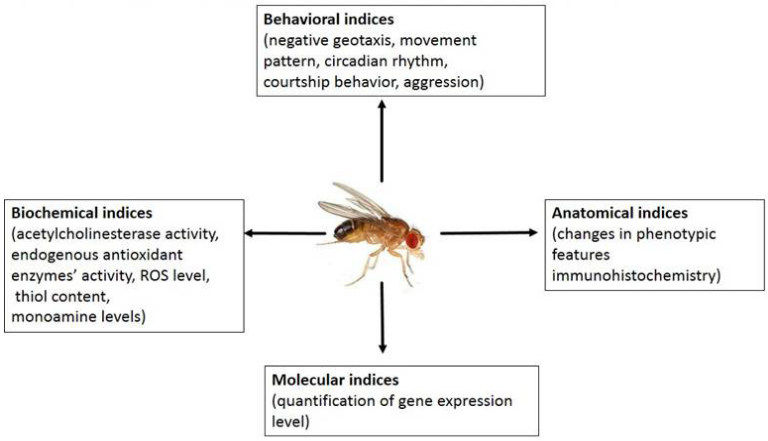

Figure 2: Chart showing the various pathological indices that *Drosophila melanogaster* can be used to monitor.

5.3. Current Possibilities

While the use of *D. melanogaster* for biomedical research in general and functional food research in particular in developing parts of the world such as Africa is still at its nascent

stage, it is interesting to note that a few interesting findings are already being published. One of such study accessed the toxicological implications of the popular condiment-monosodium glutamate and reported the toxicological implications of its consumption [55]. In this study, the author reported that feeding flies with monosodium glutamate up to 2.5 g/kg diet for five days significantly reduced their longevity, induced production of reactive oxygen and nitrogen species, hydrogen peroxide production and impaired activities of catalase and glutathione-S transferase antioxidant enzymes. Another study by Farombi et al., [56] recently used *D. melanogaster* as a model organism to show the ameliorative effect of kolaviron (the biflavonoid from bitter kola) on rotenone-induced toxicity. The biflavonoid was able to ameliorate the impaired locomotor performance, reduced life span, altered enzymes' activities and ROS/RONS production induced by rotenone. Another biflavonoid (hesperidin), a nutraceutical from citrus has been investigated for its therapeutic properties using *D. melanogaster* as model organism [57, 58,54]. Furthermore, curcumin which is adjudged the main polyphenol in turmeric was also shown to modulate acetylcholine gene expression level in *D. melanogaster* [53]; in this study, the authors reported that curcumin-supplemented diet improves survival ability increased antioxidant enzymes' activities but decreased AChE activities. The mRNA expression levels of AChE was similarly supressed; the authors proposed this as one of the major mechanism behind the neuroprotective properties of this compound as previously reported [59-62]. Just recently, Abolaji et al., [63] studied the ameliorative effect of resveratrol on oxidative damage induced by 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) in *D. melanogaster*. MPTP has been extensive used to model Parkinson's disease in various animal models and as noted by the authors, this was the first time MPTP-induced toxicity will be studied in drosophila. These few are a testament to the possibilities of using *D. melanogaster* for functional food research especially in developing world. (For further reading, see 'Notes on Recent History of Neuroscience in Africa', by Russell, [64])

5.4. Limitations

Although fly models can have high degrees of conservation and validity, providing opportunity for rapid screening and interpretation of results, however, modelling multifactorial human disease may be a bit complicated mainly due to the fact that such fly models usually express only certain aspects of the disease, making result interpretation more complex. Furthermore, while there seems to be a strong correlation of toxicity between the two organisms, nevertheless, due to metabolic differences, it is possible to observe that some drugs toxic to flies might not be in humans and vice versa [65]. In view of these limitations, it should be emphasized that D. melanogaster could be a useful model for rapid screening of a pool of functional foods and isolated nutraceuticals, as well as post screening validations to narrow down potential therapeutic candidates to a much smaller pool of lead substances/compounds, which could still be necessary to validate using conventional mammalian experimental

procedures. Nevertheless, with increasing attention being given to the use of *D. melanogaster* for biomedical research globally, which is attracting more sophisticated experimental protocols and re-validated research findings, these limitations might soon be overcome and a 'fly-to-bed' research approach, allowing direct clinical trials of validated fly-based research findings might not be too far away.

6. Acknowledgment

This chapter is written as part of a research funded by The World Academy of Science (TWAS) Grant No: 16-500 RG/CHE/AF/AC_G – FR3240293300.

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Alzheimer's Disease & Treatment

Chapter 5

Drug Targets and Therapeutic Approaches of Alzheimer's Disease

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Abstract

Dementia is an emerging global clinical complication and it is associated with a variety of distinct pathological and intellectual functions. Alzheimer's disease (AD) is major part (70%–80%) of the dementia. AD is a progressive multifarious neurodegenerative disorder which affects the routine life of the patients with several complications like memory loss and difficulty in communication skills. The pathophysiological conditions of AD are influenced by a variety of genetic and environmental factors. Effective management of AD and other types of dementia is essential for better healthy longevity of patients. In the past two decades, advances in the field of pathogenesis have enthused the research community for investigation of novel pharmacological therapeutics with known targets of the disease. Currently available therapeutic methods are targeted indirectly, like acetylcholinesterase inhibitors and N-methyl d-aspartate receptor antagonist, which contribute to a minimal impact on the disease. The identification of a number of new targets and specific small molecules is also essential for the significant therapeutic management of AD. In this study, we report on the potential targets and recent developments in the discovery of inhibitors against the disease. Brief in silico studies are also included, to address the possibility of a theoretical lead in the drug designing for AD.

1. Introduction and Epidemiology of Alzheimer's Disease

Alzheimer's is a neurological disorder in which the death of brain cells causes memory loss and cognitive decline. It is a type of dementia, accounting for 60%–80% of cases of dementia in the world population. Alzheimer forms a major part of dementia, and is caused by genetic and environmental factors. In 2013, 6.8 million people in the US were diagnosed with dementia. Of these, 5 million were diagnosed with Alzheimer's Disease (AD). By 2050, the diagnosis of AD is expected to double [1]. It is rare for AD to develop in the 30–50 age group; however, early development of AD can be seen in individuals with mutation in one of the three inherited genes that cause the disease.

In 2016, published research findings suggested that a change in the sense of humor might be an early stage of Alzheimer's. Recent research has indicated that the features of Alzheimer's, such as brain lesions, may already be present in midlife, though the symptoms of the disease do not appear until many years later [2].

The early symptoms of dementia are identified as the reduced ability to take in and remember new information, which can lead to repetitive questioning or conversations, misplacing personal belongings, forgetting events, or getting lost on a familiar route. The most common symptoms observed are as follows:

1. Impairments in reasoning, complex tasking, and exercising judgment, for example, poor understanding of safety risks, inability to manage finances, poor-decision-making ability, and inability to plan complex or sequential activities.

2. Impaired visuospatial abilities due to eyesight problems, inability to recognize faces, common objects, or to find objects in direct view.

3. Impaired speaking, reading, and writing, difficulty in recollecting common words while speaking, hesitations, and spelling and writing errors.

4. Changes in personality and behavior, such as out-of-character, mood change, including agitation, apathy, social withdrawal or a lack of interest, motivation, or initiative, loss of empathy, and compulsive or socially unacceptable behavior.

If the number and severity of symptoms confirm dementia, the following can confirm Alzheimer's. (1) A gradual onset, over the months to years, rather than hours or days. (2) A marked worsening of the individual's normal level of cognition in partial areas [3].

A number of proteins have been found to have a regulatory role in the pathogenesis of AD. Many of these proteins are involved in the morphogenesis, development, and embryogenesis of the organism. Among these, tau and Amyloid Precursor Protein (APP) are the key proteins

in the pathogenesis of sporadic and inherited AD. Thus, developing ways to inhibit the production of these proteins is of increasing research and therapeutic interest. The selective silencing of mutant alleles, moreover, represents an attractive strategy for treating inherited dementias and other dominantly inherited disorders. Here, using tau and APP as model targets is described as an efficient method for producing Small Interfering RNA (siRNA) against any essentially targeted region of a gene. This approach was utilized to develop siRNAs that display optimal allele-specific silencing against a well-characterized tau mutation (V337M) and the most widely studied APP mutation (APPsw). The allele-specific RNA duplexes identified by this method then served as templates for constructing short hairpin RNA (shRNA) plasmids that successfully silenced mutant tau or APP alleles. These plasmids should prove useful in experimental and therapeutic studies of AD. Our results suggest guiding principles for the production of allele-specific siRNA, and the general method described here should facilitate the production of gene-specific siRNAs [4].

2. Causes of AD

AD is caused due to a combination of genetic, lifestyle, and environmental factors that affect the brain over time. Less than 5% of AD is caused by specific genetic changes that virtually guarantee that a person will develop the disease. Although the causes of Alzheimer's are not fully understood yet, its effect on the brain is clear. AD damages and kills the brain cells. A brain affected by AD has fewer cells and much fewer connections among surviving cells than does a healthy brain. As more and more brain cells die, Alzheimer's leads to significant brain shrinkage. When physicians examine an Alzheimer's brain tissue under the microscope, they see two types of abnormalities that are considered to be the hallmarks of the disease:

- **Plaques:** These clumps of a protein called beta-amyloid may damage and destroy brain cells in several ways, including interfering with cell-to-cell communication. Although the ultimate cause of brain-cell death in Alzheimer's is not known, the collection of beta-amyloid on the outside of brain cells is a prime suspect.

- **Tangles:** Brain cells depend on an internal support and transport system to carry nutrients and other essential materials throughout their long extensions. This system requires the normal structure and functioning of a protein called tau. In AD, threads of the tau protein twist into abnormal tangles inside the brain cells, leading to failure of the transport system. This failure is also strongly implicated in the decline and death of brain cells [1,4]. The plaque formation and tangles AD are illustrated diagrammatically in the figure.

3. Diagnosis of Alzheimer's Disease

There is no single test for AD, so physicians look at the signs and symptoms, obtain the medical history, and rule out other conditions before making a diagnosis. They may also check

the individual neurological function, for example, by testing the patient's balance, senses, and reflexes. Other assessments include a blood and urine test, a CT, or a magnetic resonance imaging (MRI) scan of the brain, and screening for depression. Sometimes the symptoms of dementia are related to an inherited disorder such as Huntington's disease, so genetic testing and molecular diagnosis are strongly recommended for the confirmation of Alzheimer's [5].

4. Genes and Genetics of Alzheimer

A gene known as APOE-e4 is associated with higher chances of people above the age of 55 to develop AD [6]. Using this test early could show the symptoms of someone having or developing the disease. However, the test is controversial, and the results are not entirely reliable. In the future, emerging biological tests may make it possible to assess for biomarkers in people who may be at risk of AD.

5. Signaling Mechanism of AD

AD is well studied with its molecular mechanism and detailed signaling topology of the gene network is illustrated. Here, the potential targets were discussed that are essential for the pathogenesis of AD **(Figure 1).** Apart from the general view, a few more genes were also focused to be better therapeutic target.

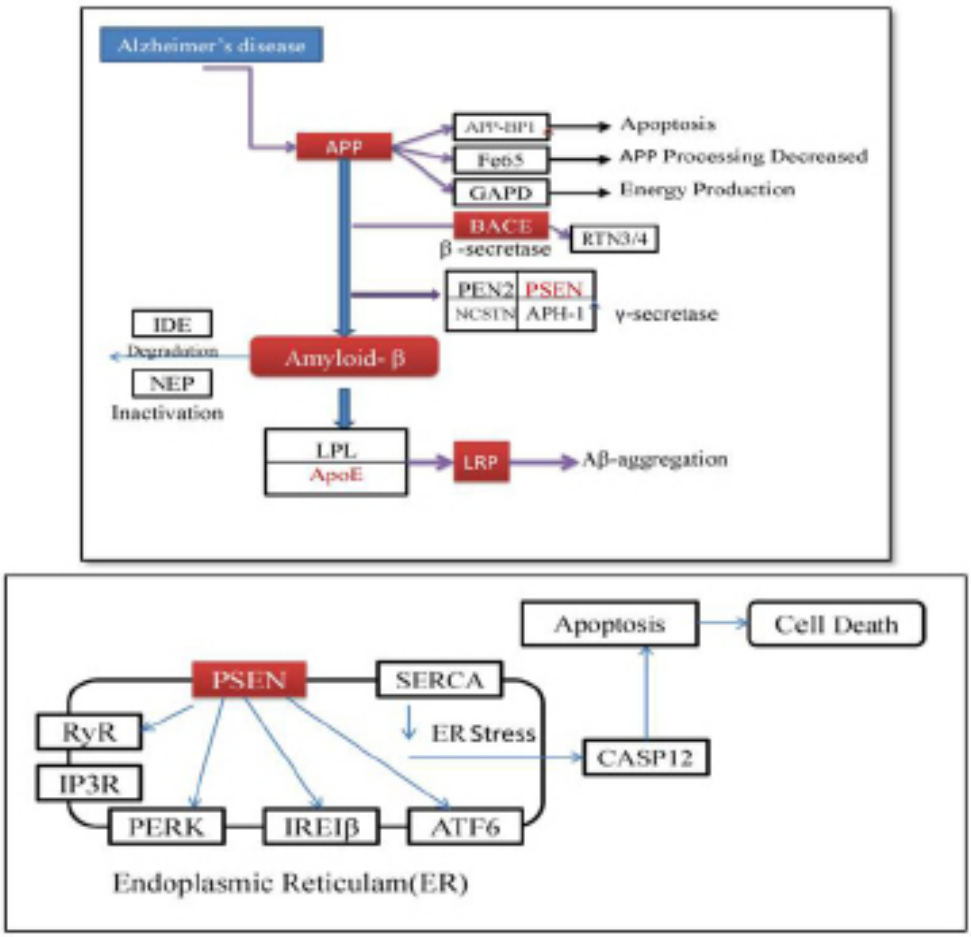

Figure 1: Molecular signaling pathway of the Alzheimer's Disease. The potential gene targets are highlighted in red.

6. Molecular Targets of Alzheimer's

Beta-amyloid (Abeta) protein signaling mechanism is a well-studied pathway for AD. and has a vital role in the pathogenesis of the disease. Data accumulated for well over a decade have implicated the Abeta peptide to be a central player in the pathogenesis of AD **(Figure 1).** Amyloid plaques, composed primarily of Abeta progressively form in the brain of AD patients, and mutations in three genes (APP and presenilin 1 and 2 [PS1 and PS2]) cause early-onset familial AD (FAD) by directly increasing production of the toxic, plaque-promoting Abeta42 peptide. Given the strong association between Abeta and AD, it is likely that therapeutic strategies to lower the levels of Abeta in the brain should prove beneficial for the treatment of AD [7]. One such strategy could involve inhibiting the enzymes that generate Abeta. The Abeta is a product of catabolism of the large type-I membrane protein APP. Two proteases, beta- and gamma-secretase, endoproteolyze APP to liberate the Abeta peptide. Recently, the molecules responsible for these proteolytic activities have been identified. Several lines of evidence suggest that the PS1 and PS2 proteins are gamma-secretase, and the identity of beta-secretase have been shown to be the novel transmembrane aspartic protease, beta-site APP-cleaving enzyme 1 (BACE1; also called Asp2 and memapsin 2). BACE2, a protease homologous to BACE1, was also identified, and together the two enzymes define a new family of transmembrane aspartic proteases. BACE1 exhibits all the functional properties of beta-secretase, and as the key enzyme that initiates the formation of Abeta, it is an attractive drug target for AD. The identification and initial characterization of BACE1 and BACE2, and summaries of recent studies of BACE1 knockout mice have validated BACE1 as the authentic beta-secretase *in vivo* [42]. High throughput screening and various computational studies have been conducted to identify BACE-1 inhibitors [8].

Evidence suggests that the beta-amyloid peptide (Abeta) is central to the pathophysiology of AD. Amyloid plaques, primarily composed of Abeta, progressively develop in the brains of AD patients, and mutations in three genes (APP, PS1, and PS2) cause early onset FAD by directly increasing synthesis of the toxic, plaque-promoting Abeta42 peptide. Given the strong association between Abeta and AD, therapeutic strategies to lower the concentration of Abeta in the brain should prove beneficial for the treatment of AD. One such strategy would involve inhibiting the enzymes that generate Abeta. Abeta is a product of catabolism of the large Type l membrane protein, APP. Two proteases, called beta- and gamma-secretase, mediate the endoproteolysis of APP to liberate the Abeta peptide [9]. For over a decade, the molecular identities of these proteases were unknown. Recently, the gamma-secretase has been tentatively identified as the presenilin proteins, PS1 and PS2, and the identity of the beta-secretase have been shown to be the novel transmembrane aspartic protease, beta-site APP cleaving enzyme 1 (BACE1; also called Asp2 and memapsin2). BACE2, a novel protease homologous to BACE1, was also identified, and together the two enzymes define a new family of transmembrane

aspartic proteases. BACE1 exhibits all the properties of the beta-secretase, and as the key rate-limiting enzyme that initiates the formation of Abeta, BACE1 is an attractive drug target for AD. Here, the identification and initial characterization of BACE1 and BACE2 summarize our current understanding of BACE1 post-translational processing and intracellular trafficking. In addition, recent studies of BACE1 knockout mice and the BACE1 X-ray structure relate implications for BACE1 drug development [10].

6.1. BACE1 Structure and Function in Health and AD

Amyloid plaques, the hallmark neuropathological lesions in the AD brain, are composed of Abeta. Much evidence suggests that Abeta is central to the pathophysiology of AD and is likely to play an early role in this intractable neurodegenerative disorder. Given the strong correlation between Abeta and AD, therapeutic strategies to lower cerebral Abeta levels should prove beneficial for AD treatment. Abeta is derived from APP via cleavage by two proteases, beta- and gamma-secretase [11]. The beta-secretase have been identified as a novel aspartic protease named BACE1 (beta-site APP Cleaving Enzyme (1) that initiates Abeta formation. Importantly, BACE1 appears to be dysregulated in AD. As the rate-limiting enzyme in Abeta generation, BACE1, in principle, is an excellent therapeutic target for strategies to reduce the production of Abeta in AD. While BACE1 knockout (BACE1-/-) mice have been instrumental in validating BACE1 as the authentic beta-secretase *in vivo*, data indicate that complete abolishment of BACE1 may be associated with specific behavioral and physiological alterations [44]. Recently a number of non-APP BACE1 substrates have been identified. It is plausible that failure to process certain BACE1 substrates may underlie some of the reported abnormalities in BACE1-/- mice. Here, they reviewed the basic biology of BACE1, focusing on the regulation, structure, and function of this enzyme. Special attention is given to the putative function of BACE1 during normal conditions and it is discussed in detail the relationship that exists between key risk factors for AD and the pathogenic alterations in BACE1 that are observed in the diseased state [12].

6.1.2. BACE With Small Inhibitory Nucleic Acids

Beta-secretase, a beta-site APP cleaving enzyme (BACE), participates in the secretion of beta-amyloid peptides (Abeta), the major components of the toxic amyloid plaques found in the brains of patients with AD. According to the amyloid hypothesis, accumulation of Abeta is the primary influence driving AD pathogenesis. Lowering of Abeta secretion can be achieved by decreasing BACE activity rather than by down-regulation of the APP substrate protein. Therefore, beta-secretase is a primary target for anti-amyloid therapeutic drug design. Several approaches have been undertaken to find an effective inhibitor of human beta-secretase activity, mostly in the field of peptidomimetic, non-cleavable substrate analogs [41,42]. This review describes strategies targeting BACE mRNA recognition and its down-regulation based

on the antisense action of small inhibitory nucleic acids (siNAs). These include antisense oligonucleotides, catalytic nucleic acids ribozymes and deoxyribozymes as well as small interfering RNAs (siRNAs). While antisense oligonucleotides were first used to identify an aspartyl protease with beta-secretase activity, all the strategies have now demonstrated that siNAs are able to inhibit BACE gene expression in a sequence-specific manner, measured both at the level of its mRNA and at the level of protein. Moreover, knockdown of BACE reduces the intra- and extracellular population of Abeta 40 and Abeta 42 peptides. An anti-amyloid effect of siNAs is observed in a wide spectrum of cell lines as well as in primary cortical neurons. Thus, targeting BACE with small inhibitory nucleic acids may be beneficial for the treatment of AD and for the future drug design [15].

6.2. Amyloid Precursor Protein

APP is a single-pass transmembrane protein expressed at high levels in the brain and metabolized in a rapid and highly complex fashion by a series of sequential proteases, including the intramembranous γ-secretase complex, which also process other key regulatory molecules. The Aβ accumulation in the brains of elderly individuals is unclear, but could relate to changes in APP metabolism or Aβ elimination. Lessons learned from biochemical and genetic studies of APP processing will be crucial for the development of better therapeutic targets for the treatment of AD [14].

6.2.1. Structure And Function of APP

APP is a member of a family of related proteins that include the amyloid precursor-like proteins (APLP1 and APLP2) in mammals and the amyloid precursor protein-like (APPL) in Drosophila. All are single-pass transmembrane proteins with large extracellular domains (Figure 2), and they are all processed in a manner similar to APP. Only APP generates an amyloidogenic fragment owing to sequence divergence at the internal Aβ site [13]. Alternate splicing of the APP transcript generates 8 isoforms, of which 3 are the most common: the 695 amino acid form, expressed predominantly in the CNS and the 751 and 770 amino acid forms. Sequential cleavage of the APP occurs by two pathways. (1) The APP family of proteins has large, biologically active, N-terminal ectodomains as well as a shorter C-terminus that contains a crucial Tyrosine–Glutamic Acid-Asparagine-Proline-Threonine-Tyrosine (YENPTY) protein-sorting domain to which the adaptor proteins X11 and Fe65 bind. The Aβ peptide starts within the ectodomain and continues into the transmembrane region (red). (2) Nonamyloidogenic processing of APP involving α-secretase followed by γ-secretase is shown. (3) Amyloidogenic processing of APP involving BACE1 followed by γ-secretase is shown. Both processes generate soluble ectodomains (sAPPα and sAPPβ) and identical intracellular C-terminal fragments (AICD) [14].

6.2.2. Regulatory role of App in AD

Mutations at codon 717 in exon 17 of the β–APP gene have previously been shown to segregate with early onset AD in some families. The mutation occurs at codons 670 and 671 (APP 770 transcript), the amino terminal of β–amyloid and may be pathogenic because it occurs at or close to the endosomal/lysosomal cleavage site of the molecule. Thus, pathogenic mutations in APP frame the β-amyloid sequence [9].

The APP gene protects against AD and cognitive decline in the elderly without Alzheimer's disease.The coding variants in APP in a set of whole-genome sequence data from 1,795 Icelanders found a coding mutation (A673T) in the APP gene that protects against AD and cognitive decline in the elderly without Alzheimer's disease.The strong protective effect of the A673T substitution against AD provides proof of principle for the hypothesis that reducing the β-cleavage of APP may protect against the disease. Furthermore, as the A673T allele also protects against cognitive decline in the elderly without AD, the two may be mediated through the same or similar mechanisms [10].

The primal role that the amyloid-β (Aβ) peptide has in the development of AD is now almost universally accepted. It is also well recognized that Aβ exists in multiple assembly states, which have different physiological or pathophysiological effects. Although the classical view is that Aβ is deposited extracellularly, emerging evidence from transgenic mice and human patients indicates that this peptide can also accumulate intraneuronally, which may contribute to disease progression [11].

Recent therapeutic investigations of AD have been guided by two seemingly opposed hypotheses: the amyloid cascade theory, which favors the amyloid plaques as the cause of AD; and the cholinergic theory, which favors cholinergic neuron loss as the cause. New investigations indicate that the synthesis and processing of APP are linked to the trophic actions of the nerve growth factor. A pathological cascade in both AD- and Down's syndrome-related memory loss could be triggered by alterations in APP processing or ACh-mediated neuronal function, or both, which in turn trigger the overexpression of amyloid β, synaptic malfunction, and trophic factor loss in target regions. This eventually leads to synaptic and dendritic loss with age [12].

AD is the most common cause of age-related dementia. Pathologically, AD is characterized by the deposition of amyloid-β peptides in the brain, derived from proteolysis of APP by β-site APP cleaving enzyme 1 (BACE1) and γ-secretase. A growing body of evidence implicates cholesterol and cholesterol-rich membrane microdomains in the amyloidogenic processing of APP. Here, we review the recent findings regarding the association of BACE1, γ-secretase, and APP in lipid rafts, and discuss the potential therapeutic strategies for AD that are based on knowledge gleaned from the membrane environment that fosters APP processing.

6.3. Gamma secretase activating protein

The regulator of gamma-secretase activity specifically activates the production of amyloid-beta protein (amyloid-beta protein 40 and amyloid-beta protein 42), without affecting the cleavage of other gamma-secretase targets such as Notch. The gamma-secretase complex is an endoprotease complex that catalyzes the intramembrane cleavage of integral membrane proteins such as Notch receptors and APP (amyloid-beta precursor protein). Specifically it promotes the gamma-cleavage of APP CTF-alpha (also called APP-CTF) by the gamma-secretase complex to generate amyloid-beta, while it reduces the epsilon-cleavage of APP CTF-alpha, leading to a low production of AICD. The gamma-secretase regulator activity is specifically inhibited by imatinib (also known as STI571 or Gleevec), an anticancer drug that selectively decreases amyloid-beta protein production. Imatinib binds PION/GSAP and acts by preventing PION/GSAP interaction with the gamma-secretase substrate. Its role as an activator of amyloid-beta protein production makes it a promising therapeutic target for the treatment of AD [17]. Activation of the γ-secretase complex is required for the final formation of Aβ peptides, and decreasing Aβ production by blocking this complex as a disease modifying approach for the treatment of AD has received intense investigation [18]. However, γ-secretase is known to process multiple substrates in addition to APP, most notably Notch, and this fact has severely limited the clinical development of inhibitors directly and irreversibly targeting this enzyme. The recent discovery of a γ-secretase activating protein (GSAP) which interacts with this protease to facilitate Aβ formation without affecting Notch has established it as a relevant target for a viable and safer anti-Aβ therapy. GSAP is increased in postmortem brain tissues of AD patients, and its pharmacological or genetic inhibition results in an amelioration of the AD-like amyloidotic phenotype in transgenic mouse models of the disease [19,57].

6.3.1. Role of GSAP in AD

Gamma-secretase is a large enzyme complex comprising presenilin, nicastrin, presenilin enhancer 2, and anterior pharynx-defective that mediates the intramembrane proteolysis of a large number of proteins including APP and Notch. Recently, a novel GSAP was identified that interacts with Gamma-secretase and the C-terminal fragment of APP to selectively increase amyloid-beta production. In this study, the role of endogenous and exogenous GSAP in the regulation of Gamma-secretase activity and amyloid-Beta production *in vitro* is further characterized. Knockdown of GSAP expression in N2a cells decreases amyloid-beta levels. In contrast, overexpression of GSAP in HEK cells expressing APP or in N2a cells had no effect on amyloid-beta generation. Likewise, purified recombinant GSAP had no effect on amyloid-β generation in two distinct *in vitro* Gamma-secretase assays. In subsequent cellular studies with imatinib, a kinase inhibitor that reportedly prevents the interaction of GSAP with the C-terminal fragment of APP, a concentration-dependent decrease in amyloid-beta levels was observed. However, no interaction between GSAP and the C-terminal fragment of APP was

evident in co-immunoprecipitation studies. In addition, sub-chronic administration of imatinib on rats had no effect on the brain amyloid-beta levels. In summary, these findings suggest that the roles of GSAP and imatinib in the regulation of Gamma secretase activity and amyloid-Beta generation are uncertain [20].

6.4. Tau protein

Tau is a major MAP in the brain, but in this regard it is not more or less interesting than other MAPs that have been discovered and classified over the years (MAP1, MAP2, MAP4, etc.). The major interest in tau stems from its aggregation in AD and other tauopathies. There has been a debate on whether tau is causal to the disease or just a byproduct of some disease process. In the case of AD the case is still open, and changes in tau are mostly viewed as a consequence of Aβ pathology. However, the discovery of mutations in the tau gene causing frontotemporal dementias has confirmed a causative role of tau in neurodegeneration, as well as the identification of Tau as one of the risk factors in PSP, PD, and others. Even in the context of AD, the active contribution of Tau was highlighted by animal models, suggesting that tau is required for the induction of Aβ-induced toxicity. In fact, an increased Tau level alone suffices as a risk factor, as demonstrated for the H1c haplotype. This provides a rationale for the quest for tau-lowering drugs [21].

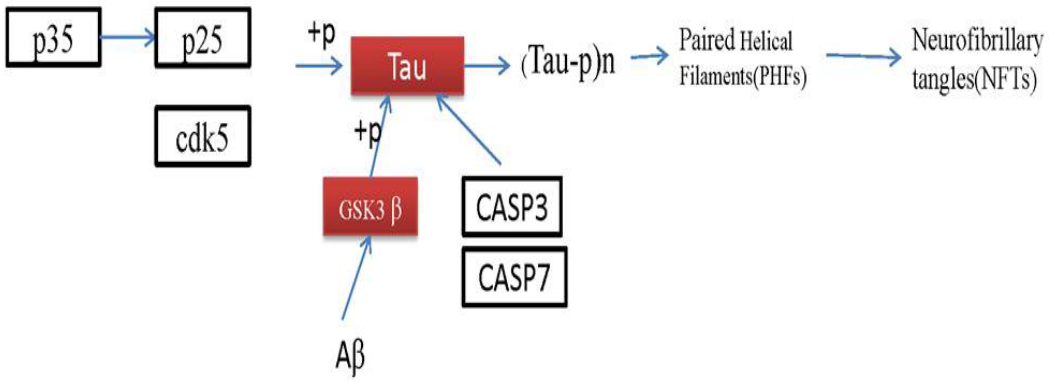

Figure 2: Signaling mechanism of tau protein with characteristic pathological features.

6.4.1. Tau protein isolation and localization

The tau (tubulin-associated unit) protein was isolated from porcine brain extracts as a heat-stable, highly soluble protein essential for microtubule (MT) assembly. Following the initial discovery of tau, two studies reported the process of tau purification and its physical and chemical properties, including the ability of tau to become phosphorylated **(Figure 2)**. In 1983, it was discovered that tau could be phosphorylated at multiple sites by various protein kinases, including cyclic-AMP-dependent protein kinases and casein kinase type-1. Further studies showed that tau is a phosphoprotein and that phosphorylation negatively regulates its ability to stimulate MT assembly. An immunohistochemical study that compared the localization of

tau using the tau-1 antibody (that recognizes all isoforms of tau) with that of microtubule-associated protein 2 (MAP2) and tubulin in human postmortem brain tissue demonstrated that tau protein was primarily localized to axons. Using the same tau-1 monoclonal antibody and electron microscopy with colloidal gold-labeled secondary antibodies, tau were also found in very low amounts in astrocytes and oligodendrocytes, and this was confirmed by tau mRNA expression analysis in the mouse brain [22].

6.4.2. Regulatory role of tau protein

Functions of the tau protein is most abundantly expressed in axons of central nervous system neurons, but can also be found in the somatodendritic compartment of neurons, oligodendrocytes, and non-neural tissues. Probably the most important role of the tau protein is to promote the assembly and stability of MT, although this function is complemented by other MAP (especially by MAP1B), as tau knockout mice are viable, fertile, and relatively normal, with no signs of neurodegeneration. Also, knockdown of tau with small interfering RNA does not kill primary neurons in culture or prevent axon formation. Additionally, MAP1B is probably more important for MT stability than tau itself, because knockout of MAP1B results in abnormal brain development and early death, and concurrent knockout of both MAP1B and *MAPT* worsens the phenotype. The most common post-translational modifications of tau proteins are phosphorylation and O-glycosylation. Phosphorylation changes the shape of tau molecule and regulates its biological activity. Most of the phosphorylation sites are on Ser-Pro and Thr-Pro motives, but a number of sites on other residues have also been reported. The majority of tau-based therapeutic strategies against neurodegeneration have focused on modulating tau phosphorylation, given that tau species present within NFT are hyperphosphorylated. O-glycosylation is characterized by the addition of an *O*-linked *N*-acetylglucosamine(*O*-GlcNAc) on Ser or Thr residues in the vicinity of Pro residues. It is presumed that glycosylation may have a role in subcellular localization and degradation of tau proteins. The recent discovery that tau is also modified by acetylation requires additional research to provide greater insight into the physiological and pathological consequences of tau acetylation.

Tau protein can be divided into two main functional domains: the basic MT binding domain (toward the C-terminus) and the acidic projection domain (toward the N-terminus). The MT binding domain regulates the rate of MT polymerization through highly conserved repetitive domains R1–R4 encoded by exons 9–12. Adult tau isoforms with 4R (R1–R4) are about 40-fold more efficient at promoting MT assembly than the fetal isoform that lacks exon 10 and thus has only 3R. The absence of expression of the R1–R2 inter-repeat region during fetal development allows for the cytoskeletal plasticity required of growing immature neurons and their elongating axons. Apart from binding to MT, the repeat domains of tau also bind to tubulin deacetylase, histone deacetylase 6 (HDAC6), and apolipoprotein E (apoE, more with

the ε3 than the ε4 isovariant) [24].

6.4.3. Role of tau protein in AD

The sequences of isoforms of human tau protein differ from previously reported forms by insertions of 29 or 58 amino acids in the amino-terminal region. Complementary DNA cloning shows that the insertions occur in combination with both three and four tandem repeats. RNAase protection assays indicate that transcripts encoding isoforms with the insertions are expressed in an adult-specific manner. Transcripts encoding four tandem repeats are also expressed in an adult-specific manner, whereas mRNAs encoding three tandem repeats are expressed throughout life, including in fetal brain. The levels of transcripts encoding the 29 or 58 amino acid inserts were not significantly changed in the cerebral cortex of patients with AD. Antisera raised against synthetic peptides corresponding to these different human tau isoforms demonstrate that multiple tau protein isoforms are incorporated into the neurofibrillary tangles of AD [22].

Glycogen synthase kinase-3 (GSK-3) reduced the mobility of human tau on SDS-PAGE, prevented binding of the monoclonal antibody (mAb), Tau.1, and induced binding of the mAb 8D8. Recombinant tau phosphorylated by GSK-3 aligned on SDS-PAGE with the abnormally phosphorylated tau (PHF-tau) associated with the paired helical filaments in AD brain. Phosphorylated serine^{396} (numbering of the large human brain tau isoform) was identified as a binding site on tau for mAb 8D8. The localization of GSK-3 within granular structures in pyramidal cells indicates that GSK-3α and GSK-3β may have a role in the production of PHF-tau in AD [24]. Paired helical filaments (PHFs) are a characteristic pathological feature of AD; their principal component is the microtubule-associated protein tau. The tau in PHFs (PHF-tau) is hyperphosphorylated, but the cellular mechanisms responsible for this hyperphosphorylation have yet to be elucidated. A number of kinases, including mitogen-activated protein (MAP) kinase, glycogen synthase kinase (GSK)-3*α*, GSK-3*β* and cyclin-dependent kinase-5, phosphorylate recombinant tau *in vitro* so that it resembles PHF-tau as judged by its reactivity with a panel of antibodies capable of discriminating between normal tau and PHF-tau, and by a reduced electrophoretic mobility that is characteristic of PHF-tau. To determine whether MAP kinase, GSK-3*α* and GSK-3*β* can also induce AD -like phosphorylation of tau in mammalian cells, the phosphorylation status of tau in primary neuronal cultures and transfected COS cells following changes in the activities of MAP kinase and GSK-3 was studied. Activating MAP kinase in cultures of primary neurons or transfected COS cells expressing tau isoforms did not increase the level of phosphorylation for any PHF-tau epitope investigated. However, elevating GSK-3 activity in the COS cells by co-transfection with GSK-3α or GSK-3*β* decreased the electrophoretic mobility of tau so that it resembled that of PHF-tau, and induced reactivity with eight PHF-tau-selective monoclonal antibodies.The data indicate that GSK-3*α* and/or GSK-3*β*, but not MAP kinase, are good candidates for generating PHF-type phosphorylation

of tau in AD. The involvement of other kinases in the generation of PHFs cannot, however, be eliminated. Our results suggest that aberrant regulation of GSK-3 may be a pathogenic mechanism in AD [25].

6.5 APOE Protein

AD affects over 30 million people worldwide and one in nine people above 65 years of age [26]. AD is characterized clinically by brain shrinkage accompanied by progressive memory loss and cognitive decline as well as personality changes later in the disease course. Pathologically, AD is characterized by the progressive accumulation of neuritic plaques of amyloid-beta (Ab) followed by neurofibrillary tangles of hyperphosphorylated tau. Overt clinical symptoms typically do not appear until the underlying pathology is well developed [27]; however, functional imaging studies suggest that changes in synaptic function occur several years before outward signs of the disease are apparent [26]. Moreover, rising Ab levels may in part be responsible for the subtle, but also progressive, reduction in cognitive ability that occurs during normal aging, and patients with "subjective cognitive decline" (i.e., patients who perform normally on standardized memory tests, but nevertheless report subjective memory impairment) have generally higher levels of Ab deposition on positron emission tomography (PET) [27].

Over two decades ago, ApoE4 was identified as a major genetic risk factor for late-onset AD (i.e., after 60 years of age) [26]. Possession of one copy of ApoE4 triples the risk of developing AD, while individuals with two copies have a 90% lifetime risk of developing the disease. The allele frequency ApoE4 is 15%–20%, with some variation in incidence between populations. Conversely, ApoE2 is considered to be protective against AD, while ApoE3 is considered risk neutral because it is by far the most common of the three isoforms and, thus, is considered the standard for the general population [26].

Since its identification as an important risk factor, great strides have been made in understanding the role that ApoE4 plays in synapse function and AD. One important role of ApoE is the clearance of Ab, with ApoE4 hindering Ab clearance significantly over ApoE3 and ApoE2 and thus directly increasing amyloid pathology. Additional roles for ApoE4 have been indicated by noninvasive imaging studies, which have shown that older individuals who are ApoE4 carriers have structural and functional alterations in AD-affected areas in the absence of cognitive dysfunction. Moreover, some of these changes are present much earlier in life, indicating a role for ApoE in neuronal function before amyloid deposition. There is an enormous literature exploring the interaction between ApoE4 and Ab, which has been reviewed in-depth recently; therefore, this review focuses on the roles of ApoE and its receptors at the synapse.

6.5.1 Role of APOE in AD

As the population ages, neurodegenerative diseases such as AD are becoming a significant burden on patients, their families, and healthcare systems. Neurodegenerative processes may start up to 15 years before outward signs and symptoms of AD, as evidenced by data from AD patients and mouse models. A major genetic risk factor for late-onset AD is the e4 isoform of apolipoprotein E (ApoE4), which is present in almost 20% of the population. The contribution of ApoE receptor signaling to the function of each component of the tripartite synapse, the axon terminal postsynaptic dendritic spine, and the astrocyte and examine how these systems fail in the contexts of ApoE4 and AD [27].

Among other metabolic functions, the apolipoprotein E (APOE) plays a crucial role in neuroinflammation. Aiming at assessing whether *APOE* ε4 modulates levels of glial cerebrospinal fluid (CSF) biomarkers and their structural cerebral correlates along the continuum of AD, brain MRI scans were acquired in 110 participants (49 control; 19 preclinical; 27 mild cognitive impairment [MCI] due to AD; 15 mild AD dementia) and CSF concentrations of YKL-40 and sTREM2 were determined. Differences in CSF biomarker concentrations and interactions in their association with gray-matter volume, according to *APOE* ε4 status were sought after. Preclinical and MCI carriers showed higher YKL-40 levels. There was a significant interaction in the association between YKL-40 levels and gray-matter volume according to ε4 status. No similar effects could be detected at sTREM2 levels, an indication of increased astroglial activation in APOE ε4 carriers while both groups displayed similar levels of CSF AD core biomarkers.

APOE4, identified in 1993, is the greatest genetic risk factor for sporadic AD, increasing the risk by up to 15-fold compared with *APOE3*, with *APOE2* decreasing the AD risk. However, the functional effects of *APOE4* on AD pathology remain unclear and, in some cases, controversial. *In vivo* progress to understand how the human (h)-*APOE* genotypes affect AD pathology has been limited by the lack of a tractable familial AD-transgenic (FAD-Tg) mouse model expressing h-*APOE* rather than mouse (m)-*APOE.* The disparity between m- and h-apoE is relevant for virtually every AD-relevant pathway, including amyloid-β (Aβ) deposition and clearance, neuroinflammation, tau pathology, neural plasticity, and cerebrovascular deficits. EFAD mice were designed as temporally useful preclinical FAD-Tg-mouse models expressing the h-*APOE* genotypes for identifying mechanisms underlying *APOE*-modulated symptoms of AD pathology. From their first description in 2012, EFAD mice have enabled critical basic and therapeutic research. Here, we review insights gleaned from the EFAD mice and summarize the future directions [28]. In 2012, we studied computationally to identify the potential ApoE4 inhibitor from plant compounds. Rigid docking study was performed for 18 plant compounds and 11 cholinesterase inhibitors. Based on the docking score, binding energy and number of hydrogen bonding curcumin possess the best scoring function. For further validation, induced

fit docking was performed which also showed that curcumin binds to the same binding pocket of ApoE4 protein. Biological activity prediction reveals that curcumin has a potential therapeutic activity against AD. Pharmacokinetic properties of this compound are under the acceptable range. From the results obtained, we concluded that the plant compound curcumin could be a potential inhibitor of ApoE4 and it can control the AD [28].

6.6. Presenilin (PSEN) protein

The first clue to the role of presenilins in APP processing came from observations that AD-causing mutations in *PSEN1* and *PSEN2* (more than 150 different mutations in these genes have been identified) affect the generation of Aβ peptides, changing the relative amount of Aβ42 peptide (Aβ containing 42 amino acid residues) versus the shorter Aβ40 (the more abundantly generated peptide, containing 40 amino acid residues; [51]. This was shown in fibroblasts derived from patients [52], by overexpressing the mutant presenilins in cell lines [53,55] and by experiments in living mice, either overexpressing the mutant presenilin in the brain using various promoters [53,54,55] or by knocking in mutations in the endogenous mouse presenilin gene [56].

The function of presenilin in the γ-secretase proteolytic activity became apparent when neurons were derived from *PSEN1* knockout mice and were used to show that PSEN1 was critically involved in the generation of all Aβ peptides [57]. This experiment established presenilin as an important AD drug target. The central role of presenilin in the γ-secretase processing of Notch was established a year later in mouse and Drosophila [57,58]. Furthermore, because a γ-secretase inhibitor was shown to block not only APP processing, but also Notch cleavage, it was suggested that a presenilin-dependent protease was responsible for both cleavages, and that blocking this enzyme would cause major side effects in patients. Notch is indeed not only involved in embryogenesis and development, but also in differentiation of immune cells, the goblet cells in the intestine, and others [58].

At the same time, other studies suggest that presenilin was actually the catalytic subunit of γ-secretase. Site-directed mutagenesis of two aspartyl residues embedded in the TMDs VI and VII of PSEN1 resulted in a dominant-negative effect on γ-secretase activity, suggesting that presenilin was a protease, specifically of the aspartyl type. These mutations did not affect the expression or the incorporation of presenilin into the γ-secretase complex, and are in a conserved region of the presenilin proteins [59]. They are found in a family of related intramembrane-cleaving proteases, the signal peptide peptidases (SPP) [60]. Finally, transition-state analog (i.e., active site-directed) γ-secretase inhibitors were shown to directly bind to the presenilin subunit of the γ-secretase complex, providing convincing evidence that presenilin is indeed a protease.

In mammals, two homologous proteins exist, PSEN1 and PSEN2. They are both synthesized as precursor proteins of 50 kDa with nine TMDs, and are cleaved into a 30 kDa amino-terminal fragment (NTF) and a 20 kDa carboxy-terminal fragment (CTF) during maturation, probably by autocatalysis [35].

6.6.1. Role of PSEN in AD

Genetic causes of AD include mutations in the *APP*, *presenilin1* (PS1), and *presenilin2* (P52) genes. The mutant *APP k670N,M67M* transgenic line, Tg2576, shows markedly elevated amyloid β-protein (AP) levels at an early age and, by 9–12 months, develops extracellular AD-type Ap deposits in the cortex and hippocampus. Mutant *PS1* transgenic mice do not show abnormal pathology, but display subtly elevated levels of the highly amyloidogenic 42- or 43-amino acid peptide Aβ342(43). The doubly transgenic progeny from a cross between line Tg2576 and a mutant *PS1 M46L* transgenic line develop large numbers of fibrillar Aβ deposits in the cerebral cortex and hippocampus far earlier than their singly transgenic Tg2576 littermates. In the period preceding overt Aβ deposition, the doubly transgenic mice show a selective 41% increase in Aβ42(43) in their brains. Thus, the development of AD-like pathology is substantially enhanced when a P51 mutation, which causes a modest increase in Aβ42(43), is introduced into Tg2576-derived mice. Remarkably, both doubly and singly transgenic mice showed reduced spontaneous alternation performance in a "Y" maze before the substantial Aβ deposition was apparent. This suggests that some aspects of the behavioral phenotype in these mice may be related to an event that precedes plaque formation [36].

Presenilin-1 and -2 (PS1 and PS2) mutations, the major cause of FAD, have been causally implicated in the pathogenesis of neuronal cell death through a perturbation of cellular Ca^{2+} homeostasis. They have recently shown that, at variance with previous suggestions obtained in cells expressing other FAD-linked PS mutations, PS2-M239I and PS2-T122R cause a reduction and not an increase in cytosolic Ca^{2+} rises induced by Ca^{2+} release from the stores. In this study, we have used different cell models: human fibroblasts from controls and FAD patients, cell lines (SH-SY5Y, HeLa, HEK293, MEFs) and rat primary neurons expressing a number of PS mutations, e.g. P117L, M146L, L286V, and A246E in PS1 and M239I, T122R, and N141I in PS2. The effects of FAD-linked PS mutations on cytosolic Ca^{2+} changes have been monitored either by using fura-2 or recombinant cytosolic aequorin as the probe. Independently of the cell model or the employed probe, the cytosolic Ca^{2+} increases, caused by agonist stimulation or full store depletion by drug treatment, were reduced or unchanged in cells expressing the PS mutations. Using aequorins, targeted to the endoplasmic reticulum or the Golgi apparatus, shows that FAD-linked PS mutants lower the Ca^{2+} content of intracellular stores. The phenomenon was most prominent in cells expressing PS2 mutants, and was also observed in cells expressing the nonpathogenic, "loss-of-function" PS2-D366A mutation, while confirming the capability of presenilins to modify Ca^{2+} homeostasis suggests a reevaluation of the "Ca^{2+}overload"

hypothesis in AD and a new working hypothesis is presented [37].

The BRCA1 protein, one of the major players responsible for DNA damage response has recently been linked to AD. Using primary fibroblasts and neurons reprogrammed from induced pluripotent stem cells (iPSC) derived from FAD patients the role of the BRCA1 protein underlying molecular neurodegeneration. By whole-transcriptome approach, there was wide range of disturbances in cell cycle and DNA damage response in FAD fibroblasts. This was manifested by the significantly increased content of BRCA1 phosphorylated on Ser1524 and the abnormal ubiquitination and subcellular distribution of presenilin 1 (PS1). Accordingly, the iPSC-derived FAD neurons showed increased content of BRCA1(Ser1524) co-localized with degraded PS1, accompanied by an enhanced immunostaining pattern of amyloid-β. Finally, overactivation of BRCA1 was followed by an increased content of Cdc25C phosphorylated on Ser216, likely triggering cell cycle re-entry in FAD neurons. This study suggests that overactivated BRCA1 could both influence PS1 turnover leading to amyloid-β pathology and promote cell cycle re-entry-driven cell death of postmitotic neurons in AD [38].

6.7. LDL Receptor-Related Protein

The LDL receptor-related protein (LRP) is larger than but structurally similar to other members of the LDL receptor gene family, an ancient family of endocytic receptors.Whereas the LDL receptor, the founding member of this family, appears to act solely in lipoprotein metabolism, the LRP and other members of this family appear to have other distinct functions. The diverse biological roles of the LRP, include functions in lipid metabolism, and also in the homeostasis of proteinases and proteinase inhibitors, cellular entry of viruses and toxins, activation of lysosomal enzymes, cellular signal transduction, and neurotransmission.

6.7.1. Structural organization of LRP

LRP, like all members of the LDL receptor gene family, consists of five common structural units: (1) ligand-binding (complement) type cysteine-rich repeats, (2) epidermal growth factor (EGF) receptor–like cysteine-rich repeats, (3) YWTD domains, (4) a single membrane-spanning segment, and (5) a cytoplasmic tail that harbors between one and three NPxY motifs (Figure 3). Ligand-binding-type repeats in LRP occur in clusters containing between 2 and 11 individual repeats. Most of the known ligands for LRP, for which the binding sites have been mapped, interact with these ligand-binding-type domains. These are followed by EGF precursor homology domains, which consist of the two EGF repeats, six YWTD repeats that are arranged in a propeller-like structure, and another EGF repeat. Six EGF repeats precede the single membrane-spanning segment. NPxY motifs that serve as docking sites for the endocytosis machinery and forcytoplasmic adaptor and scaffolding proteins involved in signaling events [39].

Binding of LRP ligands to the different clusters of ligand-binding repeats. Cysteine-rich ligand-binding repeats (red ovals) in LRP are arranged in four clusters containing 2, 8, 10, and 11 repeats, respectively. Each cluster is followed by 1–4 EGF homology domains (blue), which consist of cysteine-rich EGF repeats (blue circles) and YWTD domains (wavy line). NPxY motifs in the cytoplasmic tail are indicated by the asterisks. No ligand interactions have been mapped to cluster I. Clusters II and IV bind most of the currently mapped known ligands of LRP binding of α_2M to clusters II and IV found by surface plasmon resonance, although cells transfected with minireceptors containing these clusters do not bind and internalize α_2M. ApoE was found to bind to clusters II, III, and IV by ligand blotting. LPL, lipoprotein lipase [39].

6.7.2. LRP Potential Role in AD

A number of findings suggest that LRP contributes to the pathobiology of AD. LRP serves as a receptor for APP, apoE, and α_2M, all of which have been genetically linked to AD. Furthermore, the levels of LRP decrease substantially with age, the major risk factor for nonfamilial AD. The contribution of LRP to AD is complex, and studies demonstrate that LRP has the capacity to influence both the production and the clearance of Aβ. The association of LRP with forms of APP that contain a Kunitz-type proteinase inhibitor (KPI) domain alters APP processing, leading to increased Aβ production. At the same time, the Aβ peptide binds avidly to LRP ligands, such as α2M and apoE, and LRP-mediated clearance of these ligands complexed to Aβ contributes to a reduction in Aβ levels. Interestingly, a silent polymorphism in exon 3 of the LRP gene (C776T) is associated with an altered risk for late-onset AD and significantly lower levels of LRP in the brain have been reported in AD patients with the C/C genotype compared with patients with the C/T or T/T genotype. Decreased LRP expression at clearance sites (perhaps at neurons or at sites along the capillary membranes) could lead to decreased α2M* and/or apoE-promoted Aβ catabolism, resulting in increased Aβ deposition.

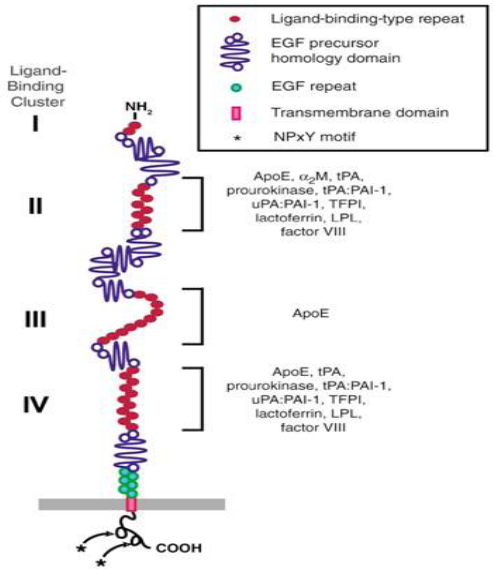

Figure 3: Structural organization of LRP. Binding of LRP ligands to the different clusters of ligand-binding repeats [39].

At the same time, increased expression of LRP in activated glia in the AD brain is well documented and could lead to increased Aβ production by these cells, also leading to increased Aβ deposition. Finally, the signaling roles of LRP in response to ligand binding may be important for normal synaptic plasticity, and loss of LRP function or levels may impair these processes and lead to neuronal degeneration[39].

6.7.3. Role of hydrophobic patches in LRP6

LRP6 protein is found in the senile plaques of AD patients. Inducing the disassociation/ inhibition of the LRP6–DKK1 complex is a vital mechanism for the treatment of AD. LRP6 is an important receptor in the Wnt/β catenin canonical pathway, where DKK1 binds in the normal state as a natural antagonist, while in the extreme level of disease, DKK1 unbinds from the LRP6, giving privilege for Wnt signaling pathway to activate the TCF/LEF genes. In 2016, we published a research article on the role of hydrophobic pathches in LRP6. Inorder to check the effectiveness of the ligands, docking was performed on the active site of DKK1 and on the hydrophobic patch of LRP6 where DKK1 binds. Ligands interacting on the active site of DKK1 and residues interacting on the hydrophobic patch of LRP6 were confirmed based on good Glide score and Glide energy. Ligands can bind to the active site of the hydrophobic patch on LRP6 with the same efficacy as DKK1 binds to LRP6 as a natural antagonist. This study accomplished its goal of targeting potent inhibitors against LRP6 by molecular modeling techniques such as high throughput virtual screening and molecular dynamics simulations [61].

6.8. Pkcδ as a target for AD

The β-amyloid protein (Aβ) plays a central role in the pathogenesis of AD. Aβ is generated from the sequential cleavage of APP by β-site APP-cleaving enzyme 1 (BACE1) and the γ-secretase complex. Although activation of some protein kinase C (PKC) isoforms such as PKCα and ε has been shown to regulate nonamyloidogenic pathways and Aβ degradation, it is unclear whether other PKC isoforms are involved in APP processing/AD pathogenesis. In this study, we report that increased PKCδ levels correlate with BACE1 expression in the AD brain. PKCδ knockdown reduces BACE1 expression, BACE1-mediated APP processing, and Aβ production. Conversely, overexpression of PKCδ increases BACE1 expression and Aβ generation. Importantly, inhibition of PKCδ by rottlerin markedly reduces BACE1 expression, Aβ levels, and neuritic plaque formation and rescues cognitive deficits in APP Swedish mutations K594N/M595L/presenilin-1 with an exon 9 deletion-transgenic AD mouse model. The PKCδ plays an important role in aggravating AD pathogenesis, and PKCδ may be a potential target in AD therapeutics [41].

7. Molecular Pathogenesis of AD

Accumulation of neurotoxic βamyloid (Aβ) is a major hallmark of AD. Formation of Aβ is catalyzed by γsecretase, a protease with numerous substrates 2,3. Little is known about the molecular mechanisms that confer substrate specificity on this potentially promiscuous enzyme. Knowledge of the mechanisms underlying its selectivity is critical for the development of clinically effective γ-secretase inhibitors that can reduce Aβ formation without impairing cleavage of other γ-secretase substrates, especially Notch, which is essential for normal biological functions 3,4. Here we report the discovery of a novel γ-secretase activating protein (gSAP), which dramatically and selectively increases Aβ production through a mechanism involving its interactions with both γsecretase and its substrate, the APP C-terminal fragment (APP-CTF). The gSAP does not interact with Notch nor does it affect its cleavage. Recombinant gSAP stimulates Aβ production *in vitro*. Reducing gSAP levels in cell lines decreases Aβ levels. Knockdown of gSAP in a mouse model of AD reduces the levels of Aβ and plaque development. gSAP represents a new type of γ-secretase regulator that directs enzyme specificity by interacting with a specific substrate, demonstrating that imatinib, as an anticancer drug previously found to inhibit Aβ formation without affecting Notch cleavage5, achieves its Aβ-lowering effect by preventing gSAP interaction with the γ-secretase substrate, APP-CTF. Thus, gSAP can serve as an Aβ-lowering therapeutic target without affecting other key functions of γ-secretase [21].

The γ-Secretase-mediated cleavage of APP results in the production of AD -related amyloid-β (Aβ) peptides. The Aβ42 peptide in particular plays a pivotal role in AD pathogenesis and represents a major drug target. Several γ-secretase modulators (GSMs), such as the nonsteroidal anti-inflammatory drugs (*R*)-flurbiprofen and sulindac sulfide, have been suggested to modulate the Alzheimer-related Aβ production by targeting the APP. The novelty of GSMs is that they are selective for Aβ modulation and do not impair processing of Notch, EphB2, or EphA4. The GSMs modulate Aβ both in cell and cell-free systems as well as lower amyloidogenic Aβ42 levels in the mouse brain. Both radioligand binding and cellular cross-competition experiments reveal a competitive relationship between the AstraZeneca (AZ) GSMs and the established second-generation GSM, E2012, but a noncompetitive interaction between AZ GSMs and the first-generation GSMs (*R*)-flurbiprofen and sulindac sulfide. The binding of a ^{3}H-labeled AZ GSM analog does not co-localize with APP but overlaps anatomically with a γ-secretase targeting inhibitor in rodent brains. Combined, these data provide compelling evidence of a growing class of *in vivo* active GSMs, which are selective for Aβ modulation and have a different mechanism of action compared with the original class of GSMs described [22].

8. Computational Studies in AD

Several targets/signaling mechanisms were reported in the pathogenesis of AD. Among these signaling mechanisms, they are not together possible to test in *in vitro, in vivo* studies. Therefore, these targets can be targeted and validated by computational studies. Through a series of computational studies, we were able to find several inhibitors, theoretically checked against all the possible targets for AD. Further, the screened compounds can be experimentally validated for clinical applications. Such kind of model proteins studied through computational studies were discussed.

9. Recent Developments in the Drug Treatment of AD

Several pharmacological approaches to enhance the cholinergic function have been developed for symptomatic or palliative therapy of AD. Although these strategies have resulted in modest cognitive and behavioral improvements in patients with AD, they do not address the underlying progression of the disease. New strategies will be required to slow, stop, or reverse the effects of neuro-degeneration in AD. A number of potential therapies are currently under investigation, including estrogen replacement, anti-inflammatory agents, free radical scavengers and antioxidants, and monoamine oxidase-B (MAO-B) inhibitors. The evidence for a protective effect of estrogens or nonsteroidal anti-inflammatory drugs (NSAIDs) is controversial, and is largely based on retrospective studies. More controlled prospective studies are needed to definitively demonstrate the benefits of long-term estrogen or NS AID use in the prevention of AD. Free radical scavengers/antioxidants such as idebenone, and selective prevention MAO-B inhibitors such as lazabemide are well tolerated, but require additional studies to demonstrate their preventative effects. In addition, other approaches, such as anti-amyloid treatments that affect beta-amylase secretion, aggregation, and toxicity, appear promising; treatments that hinder neurofibrillary tangle construction and nerve growth factor (NGF) induction are in the very early stages of development [13–15].

Numerous epidemiological studies have shown a significantly higher risk for development of AD in patients affected by type 2 diabetes (T2D), but the molecular mechanism responsible for this association is presently unknown. Both diseases are considered protein misfolding disorders associated with the accumulation of protein aggregates; amyloid-beta (Aβ) and tau in the brain during AD, and islet amyloid polypeptide (IAPP) in pancreatic islets in T2D. The formation and accumulation of these proteins follow a seeding-nucleation model, where a misfolded aggregate or "seed" promotes the rapid misfolding and aggregation of the native protein. Our underlying hypothesis is that misfolded IAPP produced in T2D potentiates AD pathology by cross-seeding Aβ, providing a molecular explanation for the link between these diseases. Here, we examined how misfolded IAPP affects Aβ aggregation and AD pathology *in vitro* and *in vivo*. We observed that addition of IAPP seeds accelerates Aβ aggregation *in vitro*

in a seeding-like manner and the resulting fibrils are composed of both peptides. Transgenic animals expressing both human proteins exhibited exacerbated AD-like pathology compared with AD transgenic mice or AD transgenic animals with type 1 diabetes (T1D). Remarkably, IAPP co-localized with amyloid plaques in brain parenchymal deposits, suggesting that these peptides may directly interact and aggravate the disease. Furthermore, inoculation of pancreatic IAPP aggregates into the brains of AD transgenic mice resulted in more severe AD pathology and significantly greater memory impairments than untreated animals. These data provide a proof-of-concept for a new disease mechanism involving the interaction of misfolded proteins through cross-seeding events which may contribute to accelerate or exacerbate disease pathogenesis. Our findings could shed light on understanding the linkage between T2D and AD, two of the most prevalent protein misfolding disorders [28].

Critical review studies that used electroencephalography (EEG) or event-related potential (ERP) indices as a biomarker of AD are discussed. In the first part studies that relied on visual inspection of EEG traces and spectral characteristics of EEG. Second, the survey analysis methods motivated by dynamical systems theory (DST) as well as more recent network connectivity approaches. The third part contains studies of sleep. Next, compared the utility of early and late ERP components in dementia research. The section on mismatch negativity (MMN) studies summarize their results and limitations and outline the emerging field of computational neurology. In the following overview are the use of EEG in the differential diagnosis of the most common neurocognitive disorders. Finally, the summary of the state of the field and the conclusion that several promising EEG/ERP indices of synaptic neurotransmission are worth considering as potential biomarkers. Furthermore, some practical issues are highlighted with discussion of future challenges as well [29].

Therapeutic treatments for AD include the cholinesterase inhibitors donepezil, galantamine, and rivastigmine. A review of the evidence by searching MEDLINE, Embase, The Cochrane Library and the International Pharmaceutical Abstracts from 1980 through 2007 (July) for placebo-controlled and comparative trials assessing cognition, function, behavior, global change, and safety was made. Thirty-three articles on 26 studies were included in the review of meta-analyses of placebo-controlled data, supporting drugs with modest overall benefits for stabilizing or slowing decline in cognition, function, behavior and clinical global change. Three open-label trials and one double-blind randomized trial directly compared donepezil with galantamine and rivastigmine. The results are conflicting: two studies suggest no differences in efficacy between the compared drugs, while one study found donepezil to be more efficacious than galantamine and the other study found rivastigmine to be more efficacious than donepezil. Adjusted indirect comparison of placebo-controlled data did not find statistically significant differences among drugs with regard to cognition, but found the relative risk of the global response to be better with donepezil and rivastigmine compared with

galantamine. Indirect comparisons also favored donepezil over galantamine with regard to behavior. Across trials, the incidence of adverse events was generally lower for donepezil and the highest for rivastigmine. These studies are discussed in this presentation [30].

Reelin signaling through apolipoprotein E (ApoE) receptors activates a signaling cascade that protects against amyloid-beta (Aβ) at the level of *N*-methyl-d-aspartate receptor (NMDAR) endocytosis, actin polymerization, and tau phosphorylation. ApoE4 induces neuronal resistance to Reelin by impairing the recycling of vesicles containing ApoE receptors, which results in reduced surface expression of the receptors.ApoE receptors on the presynaptic neuron affect spontaneous vesicle release by increasing the mobilization of vesicle-associated membrane protein 7 (VAMP7)-containing vesicles. Astrocytes express ApoE receptors, which may play a role in gliotransmission and synaptic pruning [31]. Utilizing the structure-based drug discovery approach [48], several potent ApoE4 inhibitors from the plant source were identified. The identified compounds were validated computationally and their activity determined against the protein.

The conventional pharmacotherapy of AD employs the use of compounds that inhibit the enzyme acetylcholinesterase (e.g. donepezil, rivastigmine), thereby elevating the levels of Acetylcholine in the nervous tissue of the brain. Lately, another drug has come into the picture for treatment of AD, i.e., memantine. It is a glutamatergic antagonist that protects the nervous tissue against glutamate-mediated excitotoxicity. However, both these classes of drugs provide only the symptomatic relief. There has been a desperate need arising since the past few decades of evolution for a drug that could treat the underlying causes of AD and thereby halt its development in susceptible individuals. There are several plants and derived products that have been employed for their benefits against the symptoms and complications of AD. Some novel drugs having the potential to moderate AD are under clinical trial. This review presents a comprehensive overview of the existing and the upcoming potential treatments for AD [33].

In industrialized countries, AD represents the most devastating neurodegenerative disorder in elderly people and the search for a disease modifying agent is still justified by this unmet need. Several possible targets have been explored to find an appropriate drug therapy, and in this review, dual inhibitors of beta secretase and glycogen synthase kinase 3, recently reported in the literature, will be appraised. Applying a ligand-based approach, the triazinone core emerged as a suitable scaffold to simultaneously bind the aspartic dyad of BACE-1 and the ATP site of GSK-3β, leading to a series of small molecules endowed with a balanced micromolar affinity and a promising pharmacokinetic profile. Differently, by means of a structure-based approach, a series of well-balanced dual binding molecules were designed, taking advantage of the versatility of the curcumin scaffold. For some of these new compounds a potential neuroprotective effect was also observed, due to their ability to counteract the oxidative stress through the inhibition of NQO1 enzyme. Finally, different virtual screening

analyses were performed, leading to the identification of new potential scaffolds deserving further development [43].

10. Drugs, Pharmacotherapy and Pharmaocgenomics of AD

No disease modifying drugs are available for AD, but some options may reduce the symptoms and help improve the quality of life. Cholinesterase inhibitors that are FDA approved drugs for symptomatic relief help individuals to carry out the activities of daily living by maintaining thinking, memory, or speaking skills. They can also help with some of the behavioral and personality changes associated with AD, however, they will not stop or reverse AD and appear to help individuals for only a few months to a few years. The following drugs are prescribed to treat mild to moderate AD symptoms.

- Donepezil (Aricept)
- Rivastigmine (Exelon)
- Galantamine (Razadyne)

10.1. Cholinesterase Inhibitors

⇧ Preventing the breakdown of acetylcholine (a-SEA-til-KOH-lean), a chemical messenger important for learning and memory. This supports communication among nerve cells by keeping acetylcholine levels high.

⇧ Delay or slow worsening of symptoms. Effectiveness varies from person to person.

⇧ If side effects occur, they commonly include nausea, vomiting, loss of appetite, and increased frequency of bowel movements [7].

A different kind of drug, Memantine (Namenda), an NMDA receptor antagonist may also be used, alone or in combination with a cholinesterase inhibitor which is prescribed to treat moderate to severe AD symptoms [1].

10.2. Treatment Using Memantine

This drug regulates the activity of glutamate, a chemical involved in information processing, storage and retrieval. It improves the mental function and ability to perform daily activities for some people. It can cause side effects, including headache, constipation, confusion, and dizziness. Many people are in the hope that supplements such as Vitamin E, Coenzyme Q10, Coral Calcium, Ginko biloba and Huperzine A might work well as treatments for this disease [8].

10.3. Pharmacogenomic Approaches for AD

Pharmacogenomics will be the future therapeutic tool for most genetic disorder worldwide. Screening the individual difference in the genome and finding the suitable drug treatment could produce better recovery. For a decade, the pharmacogenomic research has proven its efficiency in treating various diseases. AD patients have to be screened worldwide to prepare a frequency data and genotyping may prove helpful for the discovery of individualized medicines for the treatment of AD.

11. Conclusion

AD is a neurodegenerative disorder related to aging, characterized by progressive memory loss, cognitive impairment, and the inability to carry out functional activities of daily living. It is characterized pathologically by the presence of cerebral β-amyloid (Aβ), neurofibrillary tangles composed of hyperphosphorylated tau and neurodegeneration. The presence of the apolipoprotein E (ApoE) ε4 allele is the main genetic risk factor associated with sporadic disease, which is the predominant form of AD. Other factors that have been reported to influence the onset of AD include diet as well as physical and mental activity. Significant research attention has focused on the identification of anti-Alzheimer agents for the prevention of AD and preservation in the elderly. In this study, several targets were discussed to conclude the significant targets available to target AD. Each target was highlighted in several molecular pathogeneses that cause AD. Among them, APP, BACE, GSAP, Tau, APOE, PSEN, LRP, and PKCα are the most studied targets and have been considered to have an important role in the pathogenesis of AD. The review has provided the mechanism, the structural organization of each target with their role in AD, and listed out the available inhibitors. Finally, the study concludes by emphasizing that its findings may be beneficial for the treatment of Alzheimer's disease and for future drug designing.

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Alzheimer's Disease & Treatment

Chapter 6

The Pivotal Role of Neuroinflammation in the Genesis and Evolution of Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) is a neurodegenerative disease with progressive and irreversible clinical course. Without effective treatments, Alzheimer's disease could reach epidemic proportions. This means that a global approach to the disease is based on the study of the interaction between three fundamental processes involved in neurodegeneration: neuroinflammation, amyloidogenesis and synaptic dysfunction.

For two years, there are two classic hypotheses to explain Neurodegenerative diseases. The hypothesis of amyloid plaques: in patients, beta-amyloid protein accumulates between neurons and forms senile plaques that compress neurons, which would be the cause of their destruction. The second hypothesis of tau protein: in patients, this protein accumulates inside the neuronal cells and causes their suicide. However, the experiments wanted to limit the formation of these compounds did not give results. The researchers have now been able to be stored in the brain.

Brain damage is caused by β-amyloid deposits (Aβ) and neurofibrillary tangles are responsible for neuronal death, particularly in the cortex and

hippocampus. These lesions are the central inflammatory reactions that participate in the process of neurodegeneration.

It is interesting to study in depth the fundamental principles that link neuroinflammation, amyloidogenesis and synaptic dysfunction in order to adopt an effective therapeutic strategy.

Abbreviations: Aβ: β amyloid; AD: Alzheimer's disease; ApoE: Apolipoprotein E; APP: Amyloid precursor protein; CNS: Central nervous system; IL-1β: Interleukin 1 beta; IL-6: Interleukin-6; IFN-γ: Interferon gamma; NFT: Neurofibrallary tangles; ROS: Reactive oxygen species; TNF-α: Tumor necrosis factor alpha. BBB: blood brain barrier; NO: Nitric oxide; LPS: lipopolysaccharide.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder, which is clinically characterized by progressive cognitive decline finally leading to the full-blown picture of dementia [1]. AD represents 50 to 70% of all dementia cases. Yet, it has no cure. Synaptic loss and dendritic loss have been observed in the hippocampus and neocortex of AD patients [2]. It is characterized by 3 stages according to the evolution and the severity of the symptoms. This disease is associated with an immune disorder, which appears to a significant rise in the inflammatory cytokines and increased production of free radicals such as nitric oxide (NO) [3]. Similar to peripheral inflammation, the process in the central nervous system (CNS) has both cellular and humoral mediated mechanisms. The primary cell of interest is the microglial cell, derived from myeloid precursors in the bone marrow during embryogenesis [4]. Under normal physiological conditions, microglia are in a resting state, evenly distributed throughout the brain with a characteristic star-like morphology. They have varied age-dependent functions, including brain development, synaptic plasticity, immune surveillance, and repair. These cells respond to a wide variety of stressors, including ischemia, trauma, and pathogens, in part via specific signaling molecules, such as proinflammatory cytokines, reactive oxygen species (ROS) and nitrogen species, chemokines, complement, and heat shock proteins, by becoming activated [5]. When so activated, they move to affected areas (such as areas of cell injury or apoptosis), and undergo morphological changes to resemble macrophages. This change herald's phagocytosis by the activated microglia, and the production of cytokines, chemokines, growth factors, and ROS [6]. The origin, fate and repletion of microglia are incompletely understood, but it is thought that certain cells (e.g., monocytes) can move from the periphery into the brain, especially in situations that disrupt the blood brain barrier (BBB), to participate in these processes, and perhaps become microglia. The other major brain cell type that responds to the same stressors is the astrocyte. Reactive astrogliosis is a common finding in areas of the brain damaged by ischemia, infection or misfolded protein deposits, focal lesions or trauma [7].

Neuroinflammatory responses can be both detrimental and beneficial [8]. On the one hand, activated microglia clear apoptotic or injured cells, dysfunctional synapses, and amyloid-β deposits, and with astrocytes, promote repair via secretion of neurotrophic factors

and produce immunoregulatory cytokines, such as interleukin-10 (IL-10). The timing and regionality of the humoral response are important to its success at protection. On the other hand, microglial activation is accompanied by an immune response and the expression of proinflammatory proteins, such as interleukin-beta and interleukin-6 (IL-1β, IL-6) and tumor necrosis factor alpha (TNF-α), whose exuberance can lead to the damage of normal neurons, and signaling processes through recruitment of other cells that generate an ROS response. The result is synaptic and neuronal dysfunction, manifest ultimately by cognitive dysfunction. Cognitive disturbance resulting from systemic inflammation alone has been well documented [9]. The balance between the beneficial and detrimental effects of neuroinflammation is crucial to the outcome, and thus factors capable of modulating aspects of the process are important to understand.

Neuroinflammatory response is primarily a protective mechanism in the brain. However, excessive and chronic inflammatory responses can lead to deleterious effects involving immune cells, brain cells and signaling molecules. Neuroinflammation induces and accelerates pathogenesis of Parkinson's disease (PD), Alzheimer's disease (AD) and Multiple sclerosis (MS). Neuroinflammatory pathways are indicated as novel therapeutic targets for these diseases [10]. Nitric oxide (NO) is a free radical messenger molecule produced by neuronal nitric oxide synthase (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS or NOS2) [11]. The inducible isoform of NOS (iNOS) generates large amounts of NO [12]. The iNOS is induced by lipopolysaccharide (LPS) and/or proinflammatory cytokines, like TNFα, IL-1β and IFN-γ. The nitric oxide can damage tissues in part by oxidative stress, the cytopathologic consequence of an imbalance between antioxidant defenses and free radical production leading to cellular death [13]. The nitric oxide has been implicated in neurodegeneration and neuronal cell death through its neurotoxicity in AD and other neurodegenerative dementias [14]. In AD patients, Aβ stimulates microglial and astrocytic NO production [15].

2. Cell mediators of Neuroinflammation in Alzheimer's Disease

2.1. Roles of glia in neuroinflammation

2.1.1. Microglia

Microglia are considered the resident immune cell of the brain. Under resting conditions, they exist primarily in a state of surveillance in the CNS and their major role is the maintenance of homeostasis within the brain microenvironment [16]. Maintenance of microglia in a relatively quiescent state is attributed in part to astrocyte and neuronal activity; for example, neurons can facilitate microglial quiescence by secreting signal factors including CD200, CX3CL1 and neurotrophins [17]. Microglia share phenotypic characteristics with peripheral monocytes cells and, during injury to the CNS, are polarized towards a pro-inflammatory phenotype (M1 state), induced mainly by exposure to pro-inflammatory cytokines, such as IFN-γ,

TNF-α and cellular or microbial debris. The M1 state is characterized by production of pro-inflammatory cytokines, including TNF-α, IL-1β and IL-6 and increased expression of inducible nitric oxide synthase (iNOS), inducing elevated production of NO and morphological change of microglia to an amoeboid shape [18]. However, in an effort at neuroprotection and repair, microglia can assume an 'alternative' activation, featured by an anti-inflammatory phenotype (M2 state). The M2 activation can be driven by anti-inflammatory cytokines, such as IL-4, IL-13 and IL-10 and is characterized by increased production of anti-inflammatory cytokines, including IL-4, IL-10, IL-13, as well as upregulation of Arginase-1 (Arg1), Chitinase-3-like-3 (Ym1, in rodents) and Mannose receptor C (MRC-1) [19]. Microglia activated towards the M2 state can also trigger inflammation resolution through the release of other anti-inflammatory factors, such as neurotrophins and growth factors (IGF-1 and TGF-β) [20]. The involvement of microglial activation has been identified in the pathophysiology of several neurodegenerative diseases, such as AD and Parkinson's Disease (PD), mainly by increasing neurotoxicity and cellular damage, thereby contributing to the degenerative process [21]. While polarization toward an M1 or M2 state can be readily induced in vitro, the complex nature of the brain microenvironment and the multiple signals that glia are exposed to makes it likely that a spectrum of intermediate transitional activation states exists in vivo. Nevertheless, the manipulation of microglial polarization is being actively investigated as a potential therapeutic strategy in a number of neurodegenerative conditions [22].

As a response to receptor ligation, microglia start to engulf Aβ fibrils by phagocytosis. As a consequence, these fibrils enter the endosomal/lysosomal pathway. In contrast to fibrillar Aβ, which is largely resistant to enzymatic degradation, soluble Aβ can be degraded by a variety of extracellular proteases [23]. In the microglial context, two proteases, neprilysin and insulin degrading enzyme (IDE) are of major importance. In sporadic cases of AD, inefficient clearance of Aβ has been identified as a major pathogenic pathway [24]. It has been suggested, that increased cytokine levels are responsible for the insufficient microglial phagocytic capacity by downregulating Aβ phagocytosis receptors [25]. Soluble oligomeric amyloid β (oAβ) increased the processing of pro-IL-1β into mature IL-1β in microglia via ROS-dependent activation of NLRP3 inflammation. The production of IL-1β depends on the activation of MAP kinases and NF-κB signaling pathways. Subsequently, that overexpression of IL-1β exacerbates tau phosphorylation and tangle formation through aberrant activation of p38-MAPK and synthase kinase 3 (GSK3) the increased expression of IL-1β, was found to impair microglial Aβ clearance functions and increase BBB permeability, which can promote the accumulation of Aβ in the brain and increased neurotoxic factors. Thus, IL-1β may play a complex role in AD pathogenesis (**Figure 1**) [26].

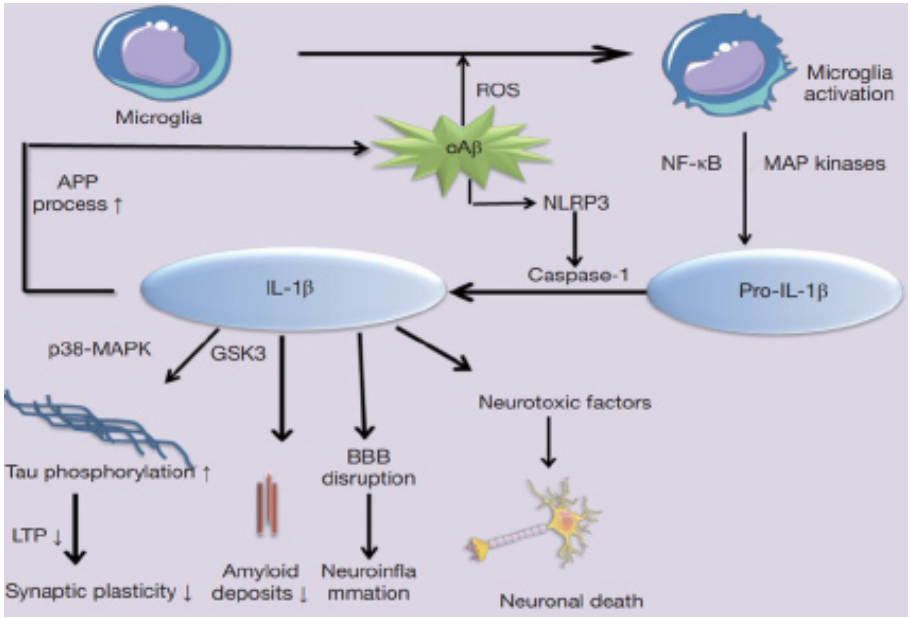

Figure 1: Hypothetical model linking the IL-1β activation to AD pathogenesis [26].

AD, Alzheimer's disease; oAβ, oligomeric amyloidβ; GSK3, glycogen synthase kinase 3; LTP, inhibiting long-term potentiation; BBB, blood-brain barrier; pro-IL-1β, pro forms IL-1β; ROS, reactive oxygen species; ↑↓, increase or decrease [26].

2.1.2. Astrocytes

Astrocytes are the most populous cells in the CNS, where they provide structural and functional support to neurons, form part of the blood brain barrier (BBB) and participate in synaptic formation [6]. While their main role is in neuronal support and brain homeostasis, it is accepted that they play an important role in neuroinflammation [27]. Similar to microglia, astrocytes can be activated from the resting state in response to insults and pathologies and this reactive astrogliosis is characterized by increased expression of GFAP [28]. Activated astrocytes have been shown to be a significant source of pro-inflammatory cytokines, including TNF-α, IL-1β and IL-6 as well as other inflammatory mediators such as iNOS [29]. Most recently, a harmful/helpful A1/A2 classification, analogous to the microglial M1/M2 phenotypes, has been suggested though it is proposed that, again similar to microglia, a continuum of activation states is likely, especially in vivo [30]. Accordingly, astrocytes exposed to IL-4 and IL-10 show typical "alternative" activation (A2 phenotype), increasing expression of Arg-1, Mrc-1 and Ym1, while activated astrocytes can also help in tissue repair by releasing IL-10, which has been reported to suppress neuronal apoptosis through TLR/NF-κB pathway activation [31]. In addition, astrocyte reactivity has been associated with several neurodegenerative diseases, including Huntingtons Disease, PD and AD and most recently, it was suggested that the normal process of ageing induces astrocytes to present A1-like astrocyte reactivity, with pro-inflammatory features [29].The ability of reactive astrocytes and microglia to influence each other's morphology and function is now being painstakingly investigated, for example it has recently been shown that the A1-type astrocyte phenotype can be induced by neuroinflammatory microglia [32]. It is hoped that investigation of the cross-talk between microglia,

astrocytes and neurons will yield insights that may inform therapeutic interventions in diseases and disorders of the brain, including AD. Aβ aggregation and accumulation associated with AD pathogenesis trigger an inflammatory response in affected areas of the brain. Thus, active microglia and astrocytes are conspicuous around neuritic plaques [33]. Both microglia and astrocytes modify their morphology to adopt a reactive morphology and undergo functional changes [10]. Chronic inflammation states, characterized by sustained reactive gliosis, have been shown to worsen the AD pathology [34].

Aβ1–42 and neurofibrillary tangles (NFTs) are the classic hallmarks of Alzheimer's disease can trigger neuroinflammatory changes, which induces the release of complement factors, cytokines and others inflammatory factors. PET uses biological surrogates for measuring neuroinflammation. Microglial activation is estimated by the expression of the 18-kDa translocator protein (TSPO), which is mainly found on the outer mitochondrial membrane of the microglial cells under inflammatory conditions. Monoamine oxidase-B (MAO-B), an enzyme usually located on the outer mitochondrial membrane of astrocytes, is proposed as an index of reactive astrocytosis. Radiolabeled arachidonic acid (AA), a phospholipid present in the cell membrane and cleaved by phospholipase A2 (PLA2), can estimate the AA metabolism. AA is the precursor of eicosanoids - prostaglandins and leukotrienes - which are potent mediators of the inflammatory response (**Figure 2**) [35].

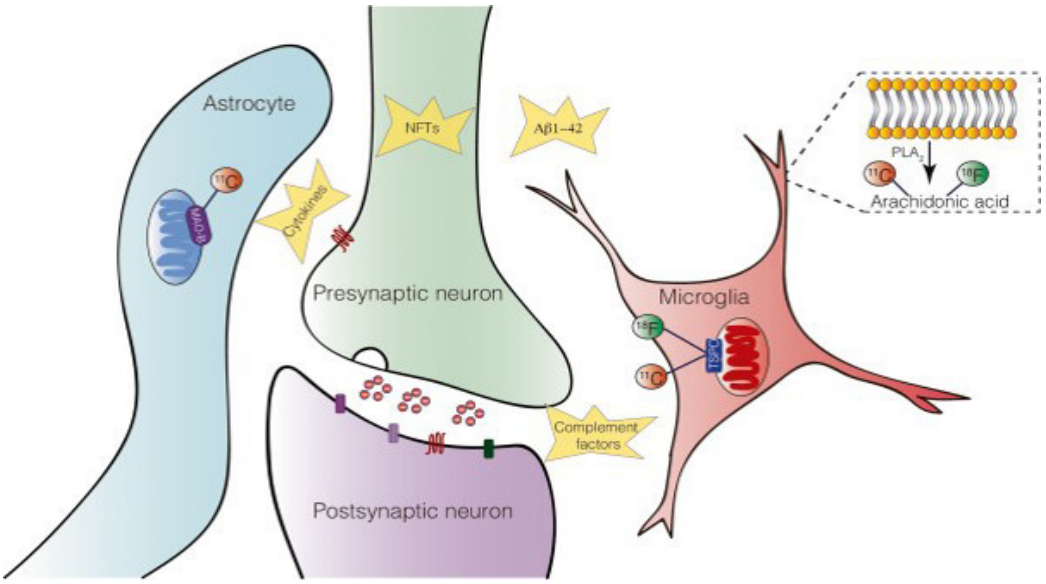

Figure 2: PET (positron emission tomography) biological targets for measuring neuroinflammation in AD [35].

Recently, a study demonstrated the involvement of microglia in synapse pathology at early stages of AD, preceding plaque formation, thus supporting the existence of a mechanism described during development and also modulating early pathological conditions during AD [36].

2.2. The Role of CD4+T Lymphocytes in Neuroinflammation

CD4+T cells are capable of activating and directing the functions of other cells. They participate in cellular mechanisms as antibody isotype switching and activation, and mobiliza-

tion of cytotoxic T lymphocytes. They also regulate phagocytic and lytic activity of mononuclear phagocytes (microglial and tissue macrophages) [37]. Activated CD4+T cells can easily cross the BBB [38]. Once they enter the damaged site, the cells exert several actions according to their phenotype [39]. Each cell subpopulation is specialized in coordinating immune responses against different types of threats and will produce particular effects. For example, in a medium where IL-12 is predominant, there will be a polarization toward the T helper 1 (Th1) phenotype, which has been associated to the elimination of intracellular microorganisms and causes neuroinflammation and neuronal damage in the CNS [40]. Th17 is another inflammatory phenotype whose differentiation is mediated by the presence of IL-23. These cells participate in intestinal immunity, autoimmune diseases and have been related to neuroinflammation and neurodegeneration mediated by the activation of apoptotic Fas/FasL pathway [41]. On the other hand, differentiation toward Th2 phenotype occurs in a microenvironment where IL-4 is predominant. This cell subpopulation directs immune response against helminths and in allergy, but it is also involved in the attenuation of neuroinflammatory processes. Tregs are a cell subpopulation that suppresses the effector function of Th cells [42]. These cells usually participate in the maintenance of peripheral tolerance to own molecules, limiting inflammatory responses against exogenous antigens. Within the CNS they attenuate neuroinflammation and, in consequence, neurodegeneration (**Figure 3**) [38].

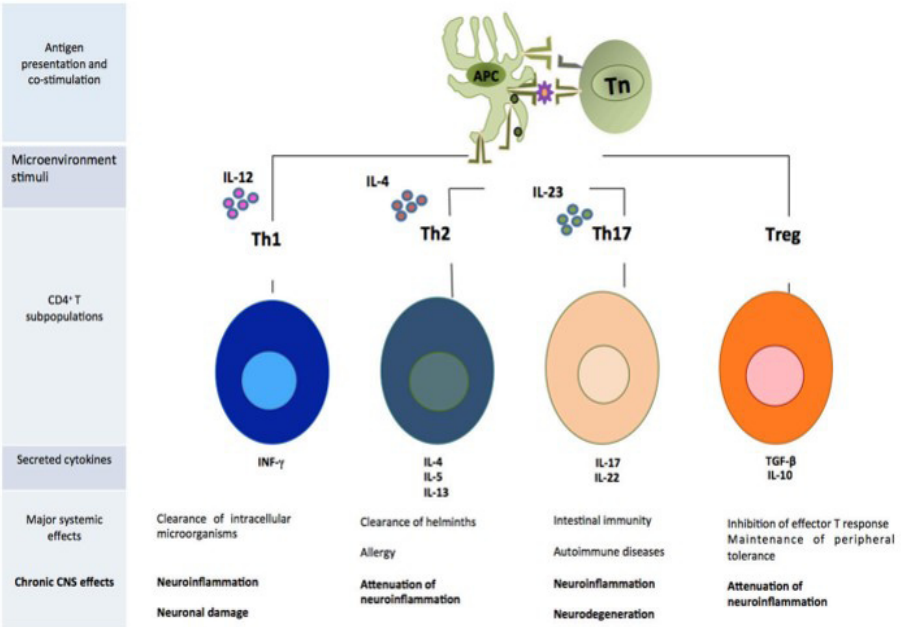

Figure 3: Subpopulations of CD4+T cells that play an important role in the development of neuroinflammation. APC, antigen presenting cell; Tn, T naive cell [9].

3. Inflammatory Mediators in Alzheimer's Disease

3.1. The complement system

The complement system is part of the innate immune system in multicellular organisms, and it is activated by three biochemical pathways. The classical complement pathway is activated when ligands bind to C1q triggering C1 complex activation. C3, a central protein

of the complement cascade, acts as downstream of C1q in the classical complement cascade and also activates the alternative pathway when ligands bind directly to it. Recent publications point towards a role played by microglia and astrocytes in early synapse pruning during development, presumably via the classical complement pathway [43]. They also showed that expression of C1q protein by retinal neurons modulated by astrocytes was a crucial event for synaptic pruning [44]. In AD, complement components have been associated with amyloid-β (Aβ) plaques [36]. It has also been reported that oligomeric/fibrillar Aβ and hyperphosphorylated tau (pTau) activate the complement pathway by binding to C1q [45]. C1q is upregulated and associated to synapses in the presence of oligomeric Aβ [36]. Under these circumstances, the classical complement pathway activates and results in synapse loss before Aβ deposition takes place [36]. C3 has been localized on reactive astrocytes in human AD cases [46] and they might contribute to synapse loss by releasing complement components themselves. In vitro and in vivo studies involving the use of mRNA expression and immunohistochemistry techniques have described the localization of C1q in neurons, both in synaptic puncta and axons during development. Also, astrocyte-secreted transforming growth factor-β (TGF-β) has been demonstrated to increase C1q expression in neurons [43]. Synapse loss is an early manifestation of pathology in Alzheimer's disease (AD) and is currently the best correlate to cognitive decline. Microglial cells are involved in synapse pruning during development via the complement pathway [47].

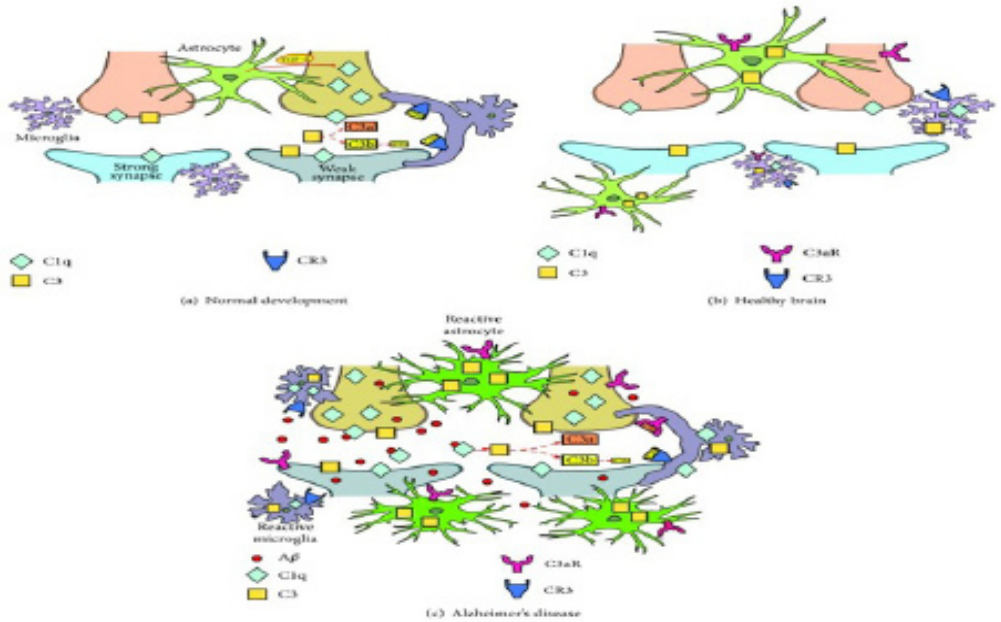

Figure 4: Model of complement-mediated synapse elimination during development, adulthood, and Alzheimer's disease [47].

(a) During early postnatal development, synaptic pruning takes place in order to eliminate excessive or weak synapses. Astrocytes induce the expression of C1q in neurons through TGF-β, and C1q colocalizes with synapses. The complement protein C3, which also colocalizes with synaptic puncta, is enzymatically cleaved to smaller fragments C3a and C3b. Finally, microglia engulf the synapse through the interaction of iC3b, the cleavage product of C3b, with its CR3 receptors [47].

(b) In the healthy brain, synaptic pruning decreases with age to basal levels and complement protein expression is reduced. Nonetheless, microglia and astrocytes continuously survey surrounding synapses [47].

(c) AD brain is characterized by progressive accumulation of extracellular and intracellular Aβ, gliosis, and neuroinflammation. Some studies have reported the role of microglia and complement pathway on synapse loss in AD models. Neuron-derived C1q and microglia-derived C1q are recruited to synapses and interact with Aβ. This triggers the activation of complement protein C3, expressed by both astrocytes and microglia. C3 is cleaved to smaller fragments such as C3b and iC3b that tag synapses and bind to CR3 on microglia. All these events lead to the removal of tagged synapses by the latter (**Figure 4**) [47].

3.2. Proinflammatory cytokines

Microglia and astrocytes are arguably the major source of cytokines in AD. Cytokines contribute to nearly every aspect of neuroinflammation, including pro- and anti-inflammatory processes, bystander neuronal injury, chemoattraction and response of microglia to Aβ deposits. Microglial activation is both characterized and modulated by cytokines. Increase in Aβ in aging TgAPPsw and PSAPP transgenic mice is associated with increased pro-inflammatory cytokines including TNF-α, IL-6, IL-1α and granulocyte macrophage-colony stimulating factor (GM-CSF) [48]. This observation suggests that pathological accumulation of Aβ is a key factor that drives neuroinflammatory responses in AD. In addition, exposure of microglia to pre-aggregated Aβ1-42 increases production of pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α), macrophage inflammatory peptide (MIP-1α) and macrophage colony-stimulating factor (M-CSF) [49]. Furthermore, MCSF levels in the plasma and CNS of AD patients are significantly elevated when compared to age-matched healthy controls or patients with mild cognitive impairment [50]. Caspase-1 activation, which is required to maturate IL-1β from its inactive pro-forms is similarly elevated in the brains of patients suffering from MCI and AD [51]. Consequently, high levels of the cardinal pro-inflammatory cytokine IL-1β are detected in microglial cells surrounding. Aβ plaques in AD patient brains and cerebrospinal fluid (CSF). In vitro, IL-1β is released by activated microglia after stimulation with Aβ [52]. IL-1β can, at least under certain circumstances, favor Aβ deposition by modulating APP expression and proteolysis. In addition to these cytokines, IL-12 and IL-23, well known from leukocytes, have been found to be produced by microglia in AD mouse models and the inhibition of IL 12/23 improves AD-like pathology [53], even so the regulation of IL-12 in human CSF is controversial [54]. There are multiple evidences suggesting that the pro-inflammatory environment present in the AD brain and in transgenic mouse models of cerebral amyloidosis assumes damaging proportions. For instance, risk for conversion from MCI to AD is higher in subjects with elevated CSF presence of the pro-inflammatory cytokine TNF-α and decreased anti-inflammatory TGF-β levels [55]. IL-1β, TNF-α and other cytokines may impair neuronal func-

tion even before leading to structural changes [56]. Multiple interactions as well as elevated expression of additional cytokines/chemokines and innate immune receptors favor an M1- like activation state in AD. For example, in neuron-microglia co cultures, the synergistic action of Aβ with either interferon-γ (IFN-γ) or CD40 ligand triggers TNF-α secretion and production of neurotoxic reactive oxygen species [57]. In addition, the innate immune toll like receptor 4 is responsible for elevated levels of TNF-α and MIP-1α in AD model mice.

Conversely, stimulation of some pro-inflammatory signaling pathways seems to be a beneficial approach in AD mouse models. Transgenic expression of IL-1β in APP/PS1 led to robust neuroinflammation and a reduction of amyloid plaque pathology [58]. These findings implicate IL-1β expression in activating a "good" form of neuroinflammation in APP/PS1 mice. In another study, (Adino Associated Virus, AAV) mediated expression of IFN-γ in the brains of the mouse model demonstrated the ability of this pro inflammatory cytokine to enhance clearance of amyloid plaques, alongside with a widespread increase astrogliosis and microgliosis [58]. In addition, these mice exhibited decreased levels of soluble Aβ and Aβ plaque burden, without altered APP processing. Similar results were obtained using AAV mediated expression of IL-6 and TNF-α [59]. Using the opposite approach, expression of the anti-inflammatory cytokine IL-4 resulted in the exacerbation of Aβ deposition [60]. These results suggest that certain "good" forms of pro-inflammatory microglial activation are potentially beneficial for reducing AD-like pathology in transgenic mouse models.

The source of the circulating cytokines in the plasma from patients is probably related to peripheral cells or endothelial cells and/or the brain [3]. Inflammatory mediators, including inflammatory cytokines, are highly expressed in the vicinity of Aβ deposits and neurofibrillary tangles. A wide range of inflammatory markers, typically absent in the normal elderly population, have been found in AD. Cytokines, such as interleukin-1β (IL-1β), tumor necrosis factor-a (TNF-α), and interleukin-6 (IL-6), when they are chronically produced, have been clearly implicated in the inflammatory process near the amyloid plaques inducing a cytotoxic effect. These cytokines could stimulate the production of Aβ peptides [61].

NO production is related to the increased levels of IFN-γ and TNF-α, in mild and severe stages of AD. Remarkably, significant IFN-γ level is only detected in mild stage of AD. NO production is IFN-γ dependent both in MCI and mild Alzheimer's patients. Further, high levels of NO are associated with an elevation of TNF-α levels in severe stage of AD. The proinflammatory cytokine production seems, in part, to be involved in neurological deleterious effects observed during the development of AD through NO pathway [3].

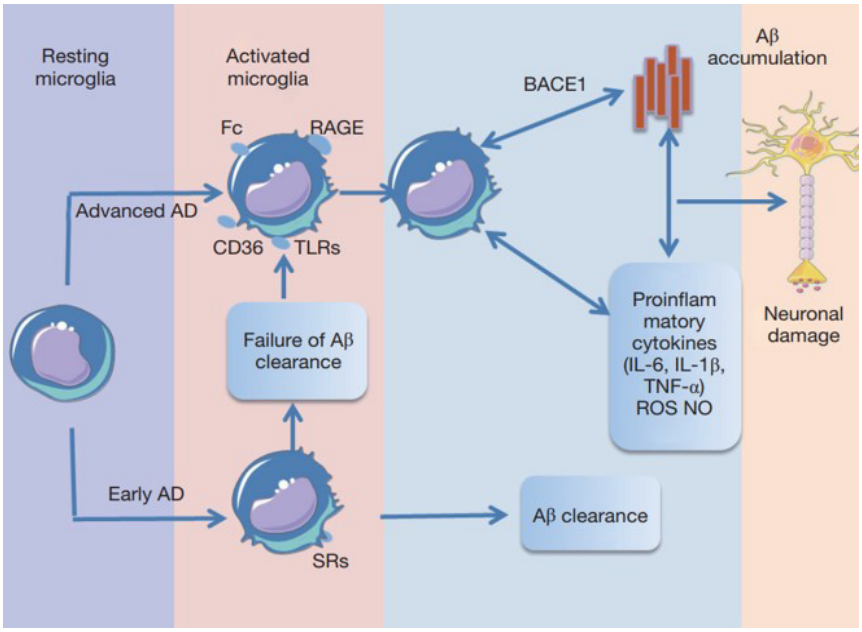

Figure 5: Possible mechanisms underlying microglial activation Aβ deposition and subsequent pro-inflammatory cytokine release contribute to AD [26].

In the early stages of AD microglial activation can promote Aβ clearance via microglia's SRs. The persistent microglial activation stimulated by Aβ via the receptor for CD36, Fc receptors, TLRs and RAGE, creating a vicious circle between microglia activation, neuroinflammation, and Aβ accumulation. A crucial role on pathogenesis of AD is an absolute culprit for both amyloid plaque and other pathologic change such as the neuronal damage. Aβ, amyloid-β; AD, Alzheimer's disease; SRs, scavenger receptors; TLRs, toll-like receptors; RAGE, complement receptors advanced glycation end products; NO, nitric oxide; ROS, reactive oxygen species (**Figure 5**) [26].

2.3. Chemokines

Chemokines in AD have been suggested to regulate microglial migration to areas of neuroinflammation, thereby enhancing local inflammation [62]. In AD up-regulation of CCL2, CCR3 and CCR5 in reactive microglia has been reported [63], whereas CCL4 has been detected in reactive astrocytes near Aβ plaques. In vitro, Aβ causes the generation of CXCL8 (IL-8), CCL2, CCL3 and CCL4 in human macrophages and astrocytes 90, and microglia cultured from autopsies of AD revealed an increased expression of CXCL8, CCL2, and CCL3 after experimental exposure to Aβ [64]. In AD mouse models a modulation of neuronal survival, plaque load, and cognition by the CX3CR1/CX3CL1 system has been shown. Further, the receptors CCR5 and CCR2 can modulate the course of the disease by influencing microglial positioning and function [65].

2.4. Nitric oxide and reactive oxygen species and oxidative stress

Next to their direct actions via surface receptors, cytokines stimulate inducible nitric oxide synthase (iNOS) in micro-and astroglia, producing high levels of NO that can be toxic to neurons. iNOS is upregulated in AD brains, and genetic knockout of iNOS is protective in

mouse models of AD [66]. Likewise, NADPH oxidase (PHOX) is highly expressed by microglia, upregulated in AD, and rapidly activated by inflammatory stimuli such as Aβ, resulting in hydrogen peroxide that further promotes microglial activation [67]. Superoxide from PHOX reacts with iNOS-derived NO forming peroxynitrite. Increased expression of iNOS in AD has also been shown to introduce NO-caused posttranslational modifications, which include nitration, S-nitrosylation and dityrosine formation, nitration of the Aβ peptide at tyrosine 10 has been recently shown to increase the propensity of Aβ to aggregate and has been identified in the core of the amyloid plaques [67]. More compelling, this modified peptide was able to initiate plaque formation in APP/PS1 mice, suggesting a central role during the early phase of AD. Nitrated Aβ suppressed hippocampal LTP more effectively when compared to non-nitrated Aβ, indicating that this posttranslational modification exerts functional as well as structural damage to the AD brain. There is evidence that oxidative stress supports the formation of this Aβ species [68]. Other NO-mediated modifications that may relevant for AD have already been reported and it is to be expected that there are more to follow. Oxidative stress has been identified as a key feature in the pathogenesis of AD, and has been associated with the deposition of Aβ. The Aβ plaques have been related to cellular effects, such as the activation of p38 MAPK signaling pathway that leads to tau hyperphosphorylation, which, in turn, lead to intracellular NFT formation; mediation of apoptotic pathways by triggering the death promoter Bcl-2, which leads to the mitochondrial release of cytochrome C, and the infiltration of T cells into the brain parenchyma [69]. On the other hand, CNS or systemic inflammation positively feedback ROS over-accumulation.

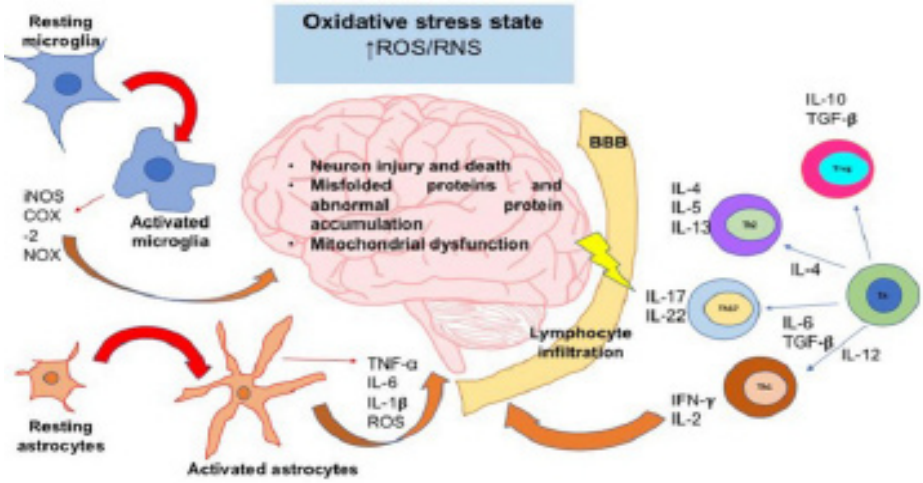

Figure 6: The oxidative stress state induces neuroinflammation and neurodegeneration [9].

In an oxidative stress state, ROS and RNS levels are augmented; these reactive species can activate signaling pathways that lead to the activation of the major glial inflammatory characters: microglia and astrocytes. These glial cells secrete proinflammatory factors which positively feedback the neuroinflammatory response. On the other hand, SNC-secreted factors and peripheral cytokines are able to disrupt the blood brain barrier (BBB) integrity; thereby, leukocytes such as T cells are able to infiltrate into SNC and take turn in the positive feedback of neuroinflammation. Inflammatory cells and secreted factors lead to neurodegeneration, in

which the most characteristic feature is the neuron injury and death. iNOS, inducible nitric oxide synthase; COX-2, cyclooxigenase-2; NOX, NADPH oxidase; IL, interleukin; Th, T helper cell; Tn, T naive cell; Treg, T regulatory cell; ROS, reactive oxygen species; RNS, reactive nitrogen species; TNF-α, tumor necrosis factor alpha; TGF-β, transforming growth factor beta (Fig 06) [9].

2.5. Autoantibodies

The etiology of AD has not been fully defined and currently there is no cure for this devastating disease. Compelling evidence suggests that the immune system plays a critical role in the pathophysiology of AD. Autoantibodies against a variety of molecules have been associated with AD. The roles of these autoantibodies in AD, however, are not well understood [70].

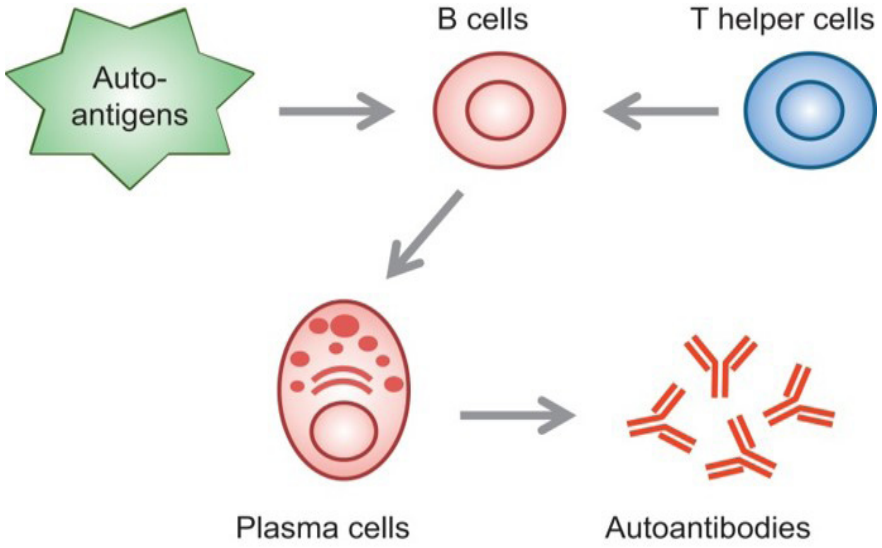

Figure 7: Schematic illustration of autoantibody production [70].

Under certain physiological and pathological conditions, B cells recognize endogenous constituents of the body as antigens (autoantigens). With the stimulation of the T helper cells, B cells differentiate into plasma cells that produce autoantibodies (**Figure 7**) [70].

Some of the autoantibodies described above are not merely markers but also contributors to the pathogenesis of AD. Early studies showed that AD brains had significantly more Ig-positive neurons, which showed neurodegenerative apoptotic features absent in Ig-negative neurons [71]. Immunoglobulin-positive neurons were frequently found in AD brains while they were rarely observed in the brains of healthy controls, suggesting a pathogenic role for autoantibodies in neuron death. Later studies reported that brain-reactive autoantibodies were nearly ubiquitous in human sera, which could contribute to neuropathology under the condition of BBB breakdown as in AD [48]. These studies confirmed the abundance of Ig-positive neurons in AD brains, and showed that treatment of cultured neurons with brain-reactive autoantibodies prevalent in human sera increased intraneuronal Aβ42 accumulation, demonstrating a potential role of brain-reactive autoantibodies in the initiation and/ or progression of AD.

Further studies suggested that protein citrullination, a post-translational protein modification that converts arginine to citrulline within proteins, may be involved in eliciting the production of brain-reactive autoantibodies [72]. The preclinical studies also support the pathogenic role of autoimmunity in AD. In a triple transgenic mouse model of AD, which develops both amyloid plaques and tau tangles, it was found that these mice exhibited manifestations of systemic autoimmune/inflammatory disease [73], including the elevation of autoantibodies. Further, the mice develop behavioral deficits in company with systemic autoimmune/inflammatory manifestations, prior to plaque and tangle pathology in the brain. These findings suggest a causal link between autoimmunity and abnormal behavioral function. The pathogenic role of some specific autoantibodies has also been investigated. For example, it has been shown that autoantibodies to ATP synthase are not only indicative of AD but also pathogenic in AD [74]. ATP synthase autoantibodies were capable of inducing the inhibition of ATP synthesis, alterations of mitochondrial homeostasis and cell death by apoptosis in SH-SY5Y neuroblastoma cell line. Further studies *in vivo* showed that intracerebroventricular administration of ATP synthase autoantibodies purified from AD patients caused poor cognitive performance and pronounced cell damage in the hippocampus in mice [75]. In addition, specific autoantibodies to ceramide were found to increase amyloid plaque burden in a transgenic mouse model of AD [35]. Natural autoantibodies against Aβ are generally considered protective in AD. Active and passive immunizations against Aβ have been explored as potential therapeutic approaches for AD. However, these immunotherapies have been associated with severe side effects related to Aβ anti-body-induced cerebral amyloid angiopathy (CAA) and perivascular inflammation [56]. Recent studies showed further evidence that Aβ autoantibodies causes CAA-related inflammation [73], similarly to what observed in Aβ-immunization trials. Thus, autoantibodies to Aβ could be pathogenic under certain conditions.

3. Neuroinflammation is the Result of the Interaction Between the Peripheral Immune System and the Central Nervous System

Further, inflammatory mediators from the brain can also enter into the peripheral system through defective BBB, recruit immune cells into the brain, and exacerbate neuroinflammation. We suggest that mast cell-associated inflammatory mediators from systemic inflammation and brain could augment neuroinflammation and neurodegeneration in the brain [10]. Systemic inflammation-derived proinflammatory cytokines/chemokines and other factors cause a breach in the blood brain-barrier (BBB) thereby allowing for the entry of immune/inflammatory cells including mast cell progenitors, mast cells and proinflammatory cytokines and chemokines into the brain. These peripheral-derived factors and intrinsically generated cytokines/chemokines, α-synuclein, corticotropin-releasing hormone (CRH), substance P (SP), beta amyloid 1–42 (Aβ1–42) peptide and amyloid precursor proteins can activate glial cells, T-cells and mast cells in the brain can induce additional release of inflammatory and neurotoxic molecules

contributing to chronic neuroinflammation and neuronal death (**Figure 8**) [10].

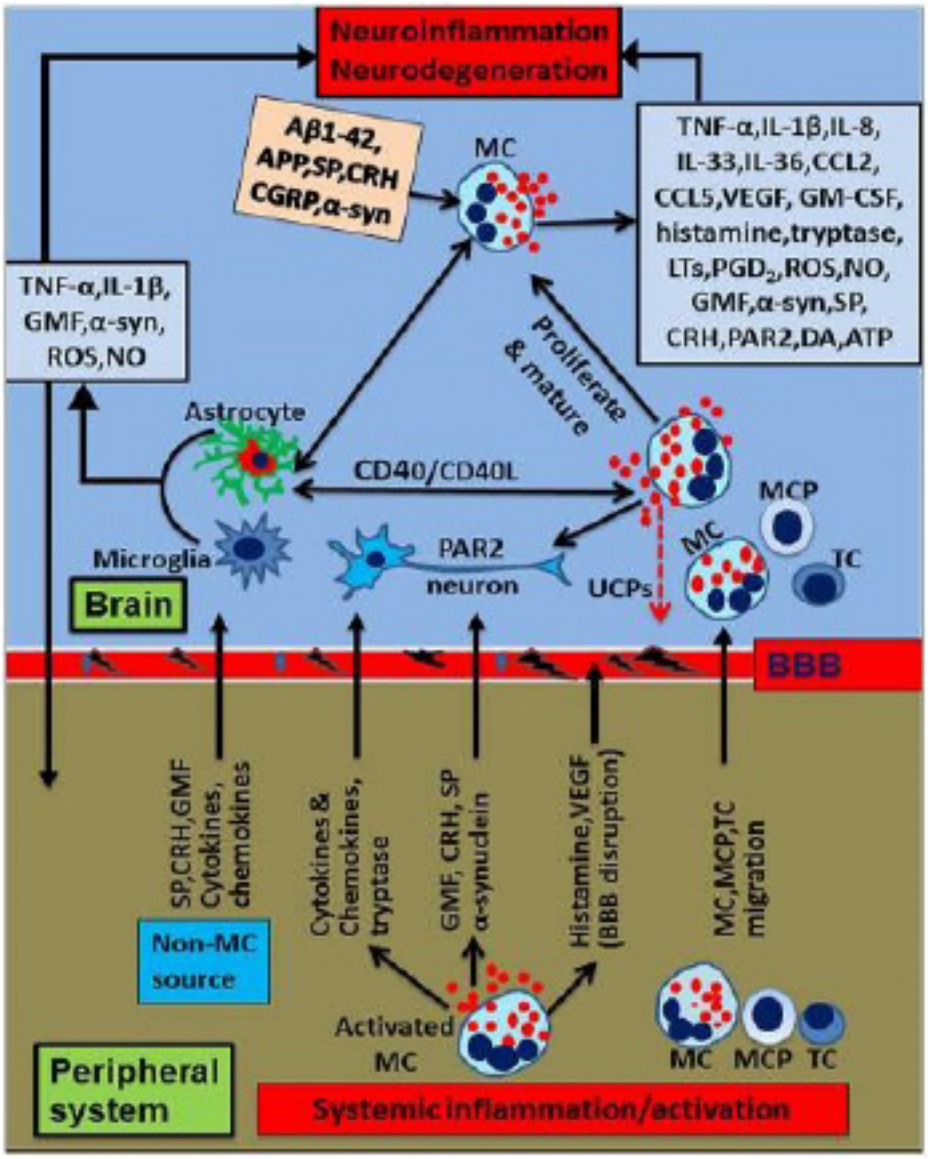

Figure 8: Schematic diagram showing peripheral inflammatory factors and cells on neuroinflammation and neurodegeneration [10]

Peripheral mast cell activation releases proinflammatory and neurotoxic mediators such as histamine, glia maturation factor (GMF), α-synuclein, corticotropin-releasing hormone (CRH), proteases, cytokines and chemokines. These mediators can induce neuroinflammation by inducing BBB breakdown, entering into the brain and activating glia and neurons to secrete various additional inflammatory mediators [10]. Peripheral mast cells and T-cells enter into the brain, proliferate and secrete proinflammatory mediators that activate glia and neurons to secrete more inflammatory mediators, reduce uncoupling protein (UCP) expression, and induce neurodegeneration. Further, glia and mast cells reactivate each other in the brain through co-stimulatory molecules CD40/CD154 or inflammatory mediators such as TNF-α, IL-1β or IL-33 [10]. Mast cell tryptase acts on the neurons through PAR2. Mast cells can reactivate by their own mediators in an autocrine and paracrine manner to exacerbate inflammatory mechanisms [10]. The α-synuclein or MPP+ from glia/neuron or extracellular Aβ1–42 can further activate mast cells to release neuroinflammatory mediators in Alzheimer's disease (AD) [10]. Additionally, several inflammatory mediators from the peripheral system can alter BBB, enter

the brain and activate the neuroinflammatory pathways. Inflammatory mediators released from activated microglia and astrocytes can enter into peripheral system through defective BBB; then, they can activate and recruit immune and inflammatory cells towards the inflammatory site in the brain [10]. MC, mast cell; MCP, mast cell progenitor; TC, T-cell; PAR2, protease activated receptor-2 [10].

MMP-9 is a secreted enzyme and member of the zinc metalloprotease (MMP) family. In general, MMPs are responsible for the degradation and maintenance of the extracellular matrix. MMP-9 has been shown to degrade compact plaques as well as soluble Aβ42 and Aβ40 [76]. In the CNS, MMP-9 is expressed by neurons, microglia, astrocytes, and infiltrating Iba+/CD45hi monocytes [77]. MMP-9 has also been shown to act as an α-secretase, favoring non-amyloidogenic processing of APP and the production of sAPPα [78]. In addition to its efficient degradation of Aβ, MMP-9 was shown to be involved in both TNFα-mediated pro-inflammatory and anti-inflammatory signaling in activated macrophages and microglia [79]. Elevated levels of MMP-9 have been correlated with BBB breakdown, demyelination, and cell death in other CNS disorders like multiple sclerosis and spinal cord injury [80]. These effects should be considered when modulating MMP-9 activity in vivo.

3.1. Mechanisms by which peripheral inflammatory factors and inflammatory cells augment neuroinflammation

The brain was originally considered as an immunologically privileged organ but now it is well known that the peripheral immune system and the brain communicate through several pathways [34]. We have previously shown that the presence of a tumor in the brain affects peripheral blood immune parameters [81]. Several peripheral inflammatory conditions could activate mast cells and release proinflammatory and neurotoxic mediators such as GMF. Systemic inflammation also increases BBB permeability in AD [82]. Normally BBB, formed by endothelial cells and astrocyte end-feet, restricts transfer of larger molecules and cells into the brain. Cytokines/chemokines and other proinflammatory molecules have been shown to cross BBB by an active transport mechanism [83] or through circumventricular organs that lack BBB [8]. The peripheral immune and inflammatory mediators can interact with brain BBB endothelial cells and induce the release of additional inflammatory molecules including PGD2 into the brain [84]. Systemic immune cells such as T cells can infiltrate into the brain through the BBB via choroid plexus or CSF that could induce neurodegeneration [85]. Peripheral inflammation is also translated to the brain through the vagus nerve by neural reflex [86]. As the BBB is disrupted in neurodegenerative diseases, the flow of immune cells and inflammatory molecules across the BBB is increased and thereby increases neuroinflammation [82]. Several previous reports indicate that mast cell activation [87], as well as peripheral inflammation influences BBB disruption to increase the permeability of inflammatory mediators and immune cell infiltration into the brain thereby spreading peripheral inflammation into the brain (Figure

09) [82].

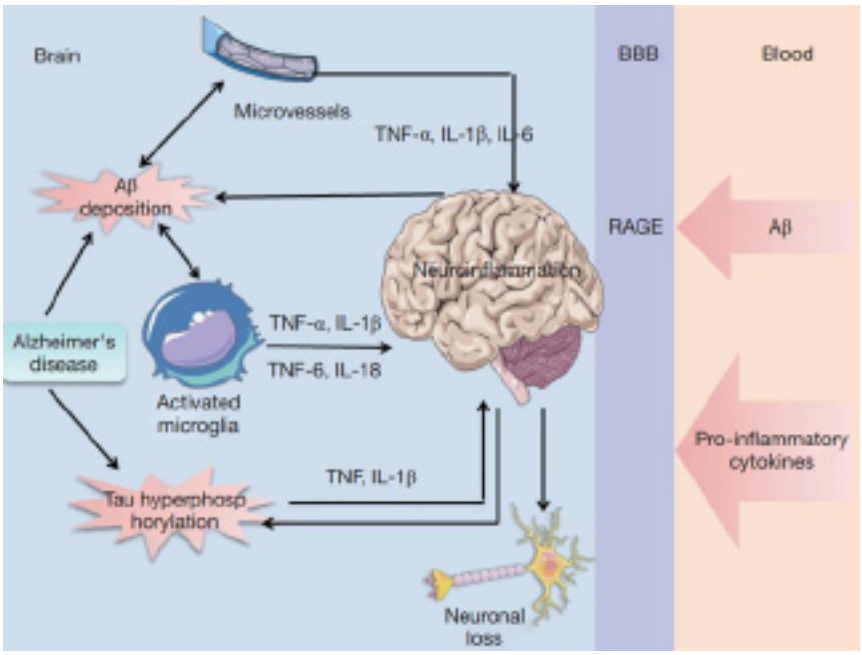

Figure 9: Speculative model of dysregulation of pro-inflammatory cytokines in the AD brain [82].

(I) A vicious circle between microglia activation, pro-inflammatory cytokines production, and Aβ, tau accumulation in AD brain; (II) AD cerebral microvessels participates in a destructive cycle of events where inflammation precedes Aβ deposition and Aβ in turn promotes release of proinflammatory cytokines; (III) pro-inflammatory cytokines and Aβ could across the BBB from the periphery into brain, the latter is mediated by RAGE. AD, Alzheimer's disease; Aβ, amyloid-β; RAGE, complement receptors advanced glycation end products; BBB, blood-brain barrier [26].

4. Conclusion

An early protective role of the immune system against Alzheimer's disease is identified in the early and even preclinical stages to the response of microglial immune cells. Increasing evidence has certified that the inflammation induced by Aβ plays a key role in AD pathogenesis. The inflammatory process itself is driven by microglial activation through the induction of pro-inflammatory molecules and related signaling pathways, thus leading to Aβ aggregation, tau formation, synaptic damage, neuronal loss, and the activation of other inflammatory participants. Thus, modulating neuroinflammation by targeting causing agents or/and trying to ameliorate their harmful effects could be of great importance to possibly, prevent AD pathology and contribute to stimulate endogenous repairing mechanisms as the formation of new neurons. A protective role of the inflammatory reaction during the disease, but only in the preclinical and asymptomatic stage, the progressive disease, inflammation that seems to have ignited. Finally, these findings underscore the importance of diagnosing the disease earlier and the new therapeutic perspective to slow down or even prevent its progression.

5. References

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