

Computational modeling and biomarker studies of pharmacological treatment of Alzheimer's disease (Review)

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Abstract. Alzheimer's disease (AD) is a complex and multifactorial disease. In order to understand the genetic influence in the progression of AD, and to identify novel pharmaceutical agents and their associated targets, the present study discusses computational modeling and biomarker evaluation approaches. Based on mechanistic signaling pathway approaches, various computational models, including biochemical and morphological models, are discussed to explore the strategies that may be used to target AD treatment. Different biomarkers are interpreted on the basis of morphological and functional features of amyloid β plaques and unstable microtubule-associated tau protein, which is involved in neurodegeneration. Furthermore, imaging and cerebrospinal fluids are also considered to be key methods in the identification of novel markers for AD. In

conclusion, the present study reviews various biochemical and morphological computational models and biomarkers to interpret novel targets and agonists for the treatment of AD. This review also highlights several therapeutic targets and their associated signaling pathways in AD, which may have potential to be used in the development of novel pharmacological agents for the treatment of patients with AD. Computational modeling approaches may aid the quest for the development of AD treatments with enhanced therapeutic efficacy and reduced toxicity.

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1. Introduction

Memory loss is naturally associated with old age (1). Specifically, dementia is the clinical condition whereby the severity of the symptoms, such as memory loss, begins to affect normal functioning and social life (2,3). Alzheimer's disease (AD) is a slow but progressive and lethal neurodegenerative disorder (4), and the risk of developing AD increases in individuals >65 years of age (5). However, cases of early-onset AD (EOAD) have been reported in individuals between 40 and 50 years of age. EOAD occurs less frequently and is classified as presenile dementia of the Alzheimer type, whereas the late-onset form of AD (LOAD) is classified as senile dementia

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Abbreviations: AChE, acetylcholinesterase; AD, Alzheimer's disease; APOE, apolipoprotein E; APP, amyloid precursor protein; A β , amyloid β ; BACE, β -secretase; BChE, butyrylcholinesterase; CaMKII/IV, calcium/calmodulin-dependent protein kinase II/IV; CREB, cAMP response element-binding protein; CSF, cerebrospinal fluid; DG, dentate gyrus; EC, entorhinal cortex; EOAD, early onset AD; ERK, extracellular signal-regulated kinase; GABA, γ -aminobutyric acid; LOAD, late onset AD; LTP, long-term potentiation; MAPK, mitogen-activated protein kinase; nAChRs, nicotinic acetylcholine receptors; NFT, neurofibrillary tangles; NMDA, N-methyl-D-aspartate; MR, muscarinic receptors; PKA, protein kinase A; RSM, runaway synaptic modification

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of the Alzheimer type and affects 7% of individuals >65 and 40% of individuals >80 years of age (6).

AD is a complex and multifactorial disorder (7), the progression of AD is influenced by genetic, environmental and dietary factors. The genetic vulnerability is also associated with AD in autosomal dominant linkage and is considered to be early onset familial AD (8). Autosomal dominant familial AD is primarily attributed to mutations in the following three protein-encoded genes: Amyloid precursor protein (APP); and presenilin (PSEN) 1 and 2 (9). Mutations in APP and PSEN genes lead to increased production of amyloid β ($A\beta$)₄₂, a small protein that is the primary component of senile plaques (10). It has been observed that environmental and genetic differences may also be risk factors for and govern sporadic AD, without following the autosomal-dominant inheritance pattern. The ϵ 4 apolipoprotein E (APOE) allele is considered to be a genetic risk factor for AD (11,12). Genome-wide association studies have demonstrated that mutations in genetic material are frequently associated with AD, and mutations in specific genes are considered to be risk factors for the development of AD (13). The genomic location and functional characteristics of these AD-associated genes are provided in Table I, whereas a graphical depiction of AD-mediated genes, i.e., genes that are altered during AD, is presented in Fig. 1. In addition, previous reports have demonstrated that environmental and dietary factors, including toxic metals, air pollutants, pesticides and diet, are also risk factors for AD (14). Various toxic compounds, including iron (Fe), zinc (Zn), copper (Cu), aluminum (Al) and lead (Pb), have been reported to alter APP expression and $A\beta$ aggregation, and a high cholesterol diet is reported to be implicated in plaque formation and subsequent (15).

2. Neuropathology and disease mechanisms

Neurobiological data have demonstrated that AD is characterized by the degeneration of neurons and disturbances in neuronal synapsis within cortical and subcortical areas (16). Amyloid plaques and neurofibrillary tangle (NFT) accumulations have been reported to be governing mechanisms of AD in humans (17). Plaques are characterized by dense deposition of $A\beta$, while NFTs are clumps of microtubules associated with tau protein. $A\beta$ consists of 39-43 amino acids, which are also found in APPs. Proteomic studies have demonstrated that APP is a transmembrane protein that aids neuron growth and post-injury repair (18,19). In AD, β - and γ -secretase are proteolytic enzymes that cleave APP into smaller fragments, which accumulate outside the neurons to form senile plaques (20,21). The basic mechanistic pathway of AD is presented in Fig 2A.

Glycogen synthase kinase 3 (GSK-3) is also associated with neuronal loss and is potentially implicated in AD, as GSK-3 forms associations with $A\beta$ and NFTs, which is considered to be a major hallmark of AD (22). GSK-3 controls various metabolic processes, including phosphorylation, protein complex formation and subcellular distribution (23). Additionally, GSK-3 is considered to increase the production of $A\beta$ and NFTs by hyperphosphorylation of tau proteins (24). Furthermore, disturbance in hippocampal volume, inflammation and oxidative stress may also be implicated in the pathology of AD.

3. Neuronal receptors and their associations with AD via downstream signaling pathways

Acetylcholine (ACh) receptors and AD. ACh receptors are the most important target proteins that specifically bind to ACh neurotransmitters. Based on the affinities and specificities with neurotransmitters, ACh receptors are divided into nicotinic ACh receptors (nAChRs) and muscarinic receptors (MRs). nAChRs are localized to skeletal neuromuscular junctions and autonomic ganglia, whilst MRs are present in the brain and parasympathetic effector organs (25), and are associated with cognition in AD (26,27). Tsang *et al* (28) identified that M1/G-protein coupling significantly decreased with the progression of AD, whereas the density of M1 receptors was not reduced. Furthermore, another *in vitro* study reported that an M1 receptor agonist, TBPB, reduced $A\beta$ production, which indicates that the M1 receptor may be used as a novel therapeutic target for the treatment of AD (29). Furthermore, in a knockout mouse study, the M3 receptor was reported to be associated with fear learning and memory conditions, which is relevant to AD symptoms (30).

Another type of receptor that has been extensively investigated is the nicotinic ACh receptor (nAChR), which consists of two subtypes, α 7 and α 4 β 2 (31). Previous studies have reported that the expression of these receptors is reduced with the progression of AD (25,32). Young *et al* (33) also investigated the role of α 7-nAChR in knockout mice and demonstrated an impairment in the attention of knockout mice compared with wild type mice (33). However, another study reported conflicting results in α 7-nAChR knockout mice by demonstrating neuroprotective effects compared with normal groups (34). However, additional studies have reported that α 7-nAChR agonists have led to improvements in cognitive deficits (35-37).

ACh receptors and signaling pathways in AD. It has been observed that ACh receptors are associated with improvements in cognitive deficits in patients with AD (38). For example, ACh receptors govern calcium signaling, which has been demonstrated to improve learning and memory in aging (38,39). Upon activation, ACh receptors trigger increases in calcium levels, which induces various intracellular processes that mediate learning and memory (40). Calcium signaling mediates three different types of effects, which include rapid, short and long-term effects. Short and long-term effects are the result of signaling cascades and changes in gene expression, respectively (41).

Specifically, following activation of calcium influx, long-term effects involve the activation of calcium/calmodulin-dependent protein kinase II/IV (CaMKII/IV), extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) and cAMP response element-binding protein (CREB). As a result, the activated enzymatic cascades alter the gene expression and may govern cognition symptoms via long-term potentiation (LTP) (42-45). An antagonistic association was observed between $A\beta$ peptides and cholinergic systems. The binding of $A\beta$ to nAChRs is also a factor in the activation of calcium, and may induce certain downstream signaling pathways that lead to a decline in cognition (Fig. 2B) (46,47).

Table I. Genes which increase the risk of AD.

Gene	Genomic location	Encoded protein	Functions
CASS4	20q13.31	Cas scaffolding protein family member 4	Axonal transport and influence the expression of APP and tau
CELF1	11p11.2	CUGBP Elav-like family member 1	Tau modifiers, and these loci have been independently validated as AD susceptibility loci
FERMT2	14q22.1	Fermitin family member 2	Tau modifiers
HLA-DRB5	6p21.3	Human leucocyte antigen DRB5	Methylation in the locus associated with A β load and with tau tangle density
INPP5D	2q37.1	Inositol polyphosphate-5-phosphatase D	Lipid metabolism, homeostasis and endocytosis, as the likely modes through which INPP5D products participate in AD
MEF2C	5q14.3	Myocyte enhancer factor 2C	Immune response and inflammation. Mutations are associated with severe mental retardation, seizure, cerebral malformation and stereotypic movements
NME8	7p14.1	NME/NM23 family member 8	rs2718058 polymorphism appears to have a role in lowering brain neurodegeneration
PTK2B	8p21.2	Protein tyrosine kinase 2 β	Acts as an early marker and <i>in vivo</i> modulator of tau toxicity
SORL1	11q24.1	Sortilin-related receptor 1	Contributes to AD through various pathways, processing of APP, involvement in A β destruction, and interaction with apolipoprotein E and tau proteins
ZCWPW1	7q22.1	Zinc finger CW-type and PWWP domain containing 1	ZCWPW1 involved in epigenetic regulation. NYAP1 gene in ZCWPW1 region is involved in brain and neural development
SLC24A4	14q32.12	Solute carrier family 24 member 4	Potassium-dependent sodium/calcium exchanger. SLC24A4 with methylation, and brain DNA methylation has a role in the pathology of AD
CLU	8p21.1	Clusterin	Clusterin levels in the blood associated with faster cognitive decline in individuals with AD
PICALM	11q14.2	Phosphatidylinositol binding clathrin assembly protein	PICALM affects AD risk primarily by modulating production, transportation, and clearance of A β peptide, but other A β -independent pathways are discussed, including tauopathy, synaptic dysfunction, disorganized lipid metabolism, immune disorder and disrupted iron homeostasis
CR1	1q32.2	Complement component 3b/4b receptor 1	Astrocyte CR1 expression levels or C1q or C3b binding activity are the cause of the genome-wide association study identified association of CR1 variants with AD
BIN1	2q14.3	Bridging integrator 1	BIN1 affects AD risk primarily by modulating tau pathology
ABCA7	19p13.3	ATP binding cassette subfamily A member 7	Has a role in the regulation of A β homeostasis in the brain, which may be associated with A β clearance by microglia
EPHA1	7q34	EPH receptor A1	EPHA1 gene product in AD may be mediated via the immune system
CD2AP	6p12.3	CD2-associated protein	CD2AP in mediating blood-brain barrier integrity and indicates that cerebrovascular roles of CD2AP may contribute to its effects on AD disease risk

AD, Alzheimer's disease; APP, amyloid precursor protein; NYAP1, neuronal tyrosine phosphorylated phosphoinositide-3-kinase adaptor 1; A β , amyloid β .

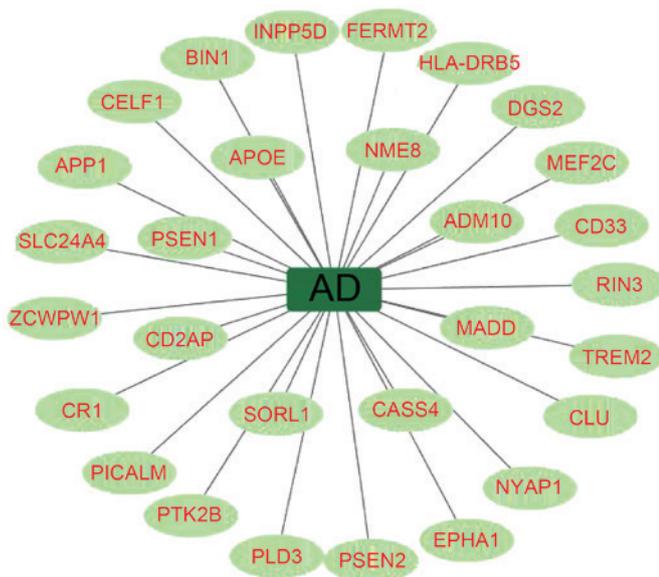


Figure 1. AD-mediated genes which are altered during AD. AD, Alzheimer's disease; APOE, apolipoprotein E; NME8, NME/NM23 family member 8; MADD, MAP kinase-activating death domain; CASS4, cas scaffolding protein family member 4; SORL1, sortilin-related receptor 1; CD2AP, CD2-associated protein; PSEN, presenilin; INPP5D, inositol polyphosphate-5-phosphatase D; FERMT2, fermitin family member 2; HLA-DRB5, human leucocyte antigen DRB5; MEF2C, myocyte enhancer factor 2C; RIN3, Ras and Rab interactor 3; TREM2, triggering receptor expressed on myeloid cells 2; CLU, clusterin; NYAP1, neuronal tyrosine phosphorylated phosphoinositide-3-kinase adaptor 1; EPHA1, EPH receptor A1; PLD3, phospholipase D family member 3; PTK2B, protein tyrosine kinase 2 β ; PICALM, phosphatidylinositol binding clathrin assembly protein; CR1, complement component 3b/4b receptor 1; ZCWPW1, zinc finger CW-type and PWWP domain containing 1; SLC24A4, solute carrier family 24 member 4; CELF1, CUGBP Elav-like family member 1; BIN1, bridging integrator 1; ADAM10, a disintegrin and metalloproteinase domain-containing protein 10; AAP, amyloid precursor protein; DGS2, DiGeorge syndrome/velocardiofacial syndrome complex 2.

Serotonin receptors and AD. An increased serotonin (5-hydroxytryptamine) concentration in the synaptic cleft has been reported to be a potential therapeutic strategy to slow the progression of AD (48,49). Serotonin targets specific receptors at postsynaptic neurons and mediates downstream signaling pathways that control cognition. It has been reported that ≥ 16 different types of serotonin receptors exist, which are categorized into 7 subfamilies (5-HT1-5-HT7) (50). All serotonin receptors are G-protein-coupled receptors (GPCRs), excluding the 5-HT3 receptor (50). The activation of these receptors stimulates downstream signal transduction pathways that govern certain intracellular responses. The protein kinase A (PKA) signaling cascade is responsible for the inhibition and stimulation of phospholipase C/protein kinase C, which regulates the ERK/MAPK pathways (51,52). Subsequently, these activations affect cognitive impairment in neurodegenerative diseases.

Results from animal and clinical experiments have also demonstrated the importance of 5-HT in cognitive dysfunction and memory deficits (53). Increases in the expression of 5-HT1A receptors were reported to be associated with cognitive impairment, and these receptors are therefore considered to be potential targets for the treatment of AD (54). Furthermore, Garcia-Alloza *et al* (55) demonstrated that

5-HT1B/1D receptors were associated with cognitive dysfunction in AD. It was also observed that the density of the 5-HT2A receptor was significantly reduced in the frontal and temporal cortical neurons in patients with AD compared with healthy participants. Furthermore, various studies have reported an important association between 5-HT2 receptors and cognitive decline in AD (56-58).

The serotonin receptor 5-HT6 has an important role in various mechanistic pathways within the brain (59). It is primarily expressed in the striatal, hippocampal and cortical areas (60). Notably, it was previously reported that inhibition of the 5-HT6 receptor improved learning and memory (61,62). Another animal study also demonstrated the importance of 5-HT6, as the agonist SB-271046 improved age-associated deficits and spatial recognition memory in aged mice (63). It was also reported that, another agonist, WAY-181187, may also be used to modulate synaptic plasticity via attenuation of LTP (64).

Serotonin receptors and downstream signaling pathways in AD. 5-HT receptor-mediated signaling pathways are associated with improvements in cognitive deficits (65). The 5-HT6 receptor stimulates G-proteins, which results in cAMP production via adenylyl cyclase activation (66,67). cAMP subsequently triggers PKA, which, via phosphorylation, activates CREB (67). A number of studies have indicated that the 5-HT6 receptor modulates various neurotransmitters, including glutamate and Ach, to aid memory processes (Fig. 2C) (68,69).

Adrenergic receptors and AD. Adrenergic receptors are metabotropic GPCRs, which are divided into two major groups, α and β . Adrenergic receptors are typically sensitized for norepinephrine and epinephrine neurotransmitters. A number of studies have reported that adrenergic receptors (α and β) are closely associated with cognitive decline in AD (70,71). An expression study by Kalaria and Harik (72) demonstrated that $\beta 2$ levels were increased in the cortex and hippocampus of patients with AD. In addition, a behavioral study reported that certain structural changes in adrenergic receptors were associated with the presence or absence of aggressive behavior in AD patients (73).

Dopamine receptors and AD. Dopamine receptors exhibit important roles in various human functions, including cognition and learning (50). Dopamine receptors are divided into two different classes, D1- and D2-like receptors, which consist of five subtypes. D1-type receptors include D1 and D5 receptors, whereas D2-type receptors include D2, D3 and D4 receptors (74). Functionally, D1- and D2-type receptors function in synaptic plasticity and cognition by stimulating the protein signaling cascade of cAMP/PKA and CREB modulation (43,44). However, another study demonstrated that dopamine receptors were directly associated with AD and Parkinson's disease (50).

N-methyl-D-aspartate (NMDA) receptors and its pathway in AD. NMDA/glutamate receptors have been extensively studied, and are abundantly expressed in the cerebral cortex, hippocampus, nucleus accumbens and striatum (75,76).

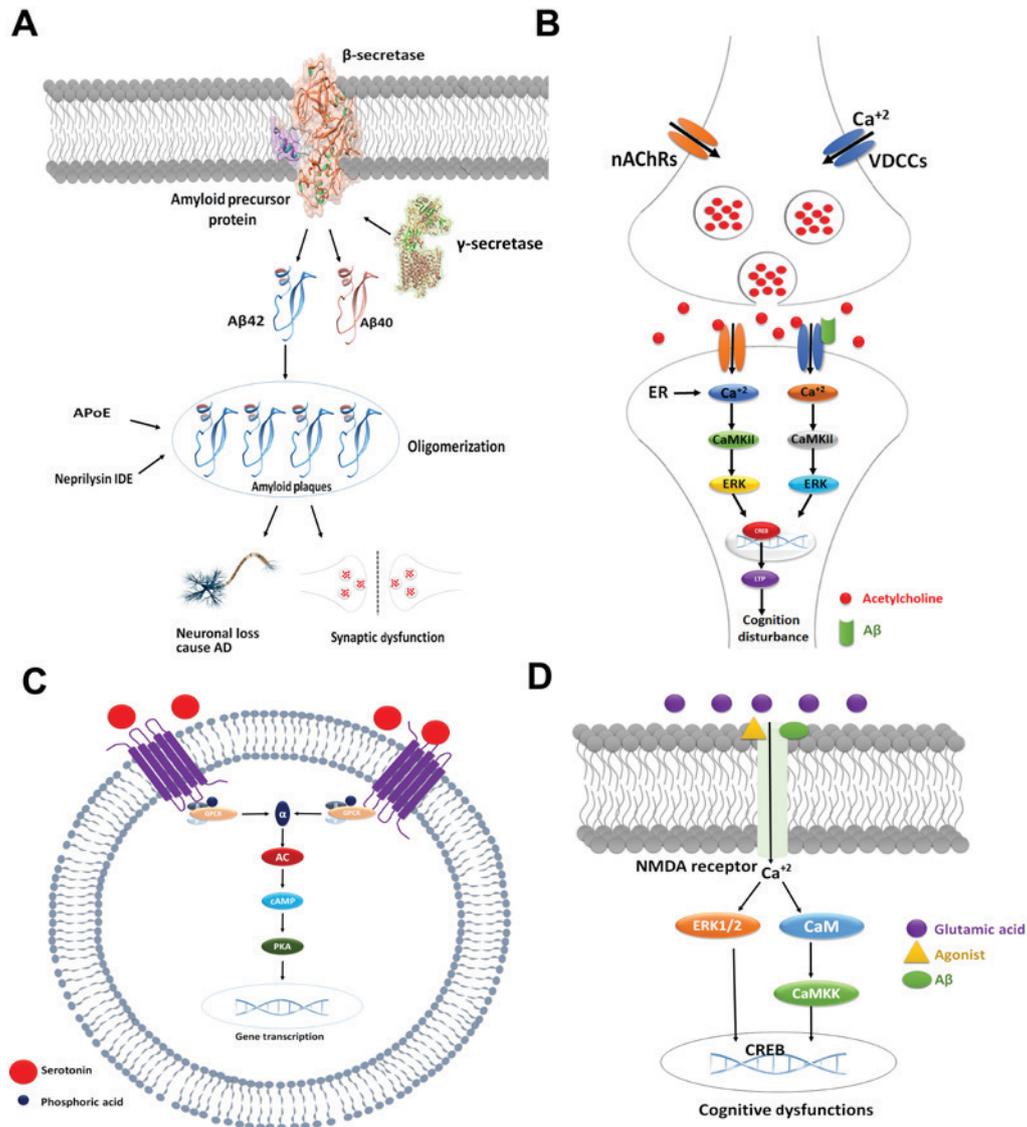


Figure 2. Mechanistic overview of AD with neuronal signaling pathways. (A) General mechanism of tau-mediated AD is presented. A double membrane is highlighted in silver containing an embedded complex of β -secretase and APP (80). β -secretase and APP protein are indicated by maroon and purple colors, respectively. The γ -secretase enzyme is acting to cleave APP into A β 40 and A β 42 subunits. The clump of A β 40, termed amyloid plaques, are generated by a process termed oligomerization and interactions with other two enzymes, APoE and neprilysin IDE. The aggregated plaques lead to neuronal loss and synaptic dysfunction, which ultimately results in cognition deficits. (B) In the acetylcholine signaling pathway, acetylcholine stimulates calcium influx after interacting with its respective receptor at the synaptic complex. This calcium flux activates a series of signaling proteins, including CaMKII/IV, ERK/MAPK and CREB. As a result, the activated enzymatic cascade leads to altered gene expression and may govern cognition symptoms via LTP (40-43). (C) In the serotonin signaling pathway, activation of the 5-HT₆ receptor stimulates G-proteins, which results in increased cAMP production via AC activation. This cAMP triggers PKA activation, which phosphorylates and regulates the CREB transcription factor, which subsequently leads to cognition dysfunction (65). (D) In the glutamic acid signaling pathway, activation of the NMDA receptor by glutamic acid mediates calcium signaling from presynaptic to postsynaptic neurons. CaM and ERK1/2 protein cascades are activated, which ultimately leads to CREB activation and cognition dysfunction (77-79). AD, Alzheimer's disease; APP, amyloid precursor protein; A β , amyloid β ; APoE, apolipoprotein E; IDE, insulin-degrading enzyme; CaMK, calcium/calmodulin-dependent protein kinase; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; CREB, cAMP response element-binding protein; LTP, long-term potentiation; AC, adenylyl cyclase; PKA, protein kinase A; NMDA, N-methyl-D-aspartate; nAChRs, nicotinic acetylcholine receptors; VDCCs, voltage-dependent calcium channels; ER, endoplasmic reticulum; CaM, calmodulin; CaMKK; calcium/calmodulin-dependent protein kinase kinase; GPCR, G-protein coupled receptor.

Variations in glutamatergic receptors are implicated in the pathogenesis of neurodegenerative diseases, such as AD, as they are associated with neuronal death (77). A reduced expression study on NMDA, NMDA receptor subunit 1 and subunit 2B proteins in rat models reported that there is a close association between NMDA receptors and cognitive deficits (78). Neuronal loss induced by amyloid plaques are a consequence of NMDA receptor modulation. Amyloid plaques activate NMDA receptors, which results in higher calcium

influx into neurons, ERK1/2 activation and mediation of respective downstream enzymes (79-82). Therefore, NMDA signaling pathways have a potential role in the pathogenesis of cognitive dysfunctions (Fig. 2D).

Acetylcholinesterase (AChE) as an AD drug target. AChE is a type of hydrolase, and exhibits key functions in cholinergic neurotransmission in the autonomic and somatic nervous systems (83). AChE interacts with Ach, converting it into

choline and acetic acid (82). Expression studies have demonstrated that AChE is frequently present in motor neurons and certain other types of conducting tissue, including nerve and muscle, motor and sensory fibers, and cholinergic and non-cholinergic fibers (84,85). In AD, cholinergic neurons mediate memory deficits and cognitive decline by reducing the level of ACh (86,87). Therefore, AChE may be considered as a novel target to reverse AD symptoms. Furthermore, butyrylcholinesterase (BChE) is also considered to be a minor player in the regulation of synaptic ACh levels (88). Therefore, inhibition of BChE may also be considered a valid approach to restore cholinergic function in AD (89,90).

4. The use of receptor-based pharmaceutical agents to treat AD

AChE-based inhibitors. The majority of neuromuscular problems are treated by AChE inhibitors, which are also considered to be first-generation drugs for the treatment of AD. There are four established inhibitors (donepezil, galantamine, rivastigmine and tacrine) that are commonly used to improve cognition (91). However, tacrine is not as reputable due to poor tolerability (92,93). Donepezil demonstrated its neuroprotective effects by diminishing the excitotoxicity of glutamate by reducing A β load and cell toxicity, as well as increasing cell life span (94,95). Rivastigmine is a cholinergic agent that targets AChE and BChE. Clinical trials have indicated that rivastigmine improves cognition, with few side effects in patients with AD (96). Tacrine is another inhibitor that increases ACh levels from cholinergic nerve endings. Tacrine inhibits the activity of certain enzymes, including monoamine oxidase, and suppresses γ -aminobutyric acid (GABA) signaling, which results in the release of dopamine, noradrenaline and serotonin from nerve endings, and improves memory in patients with AD (97).

Recently, novel inhibitors have been synthesized from natural and synthetic sources for patients with AD. Huperzine A (Hup A) is an AChE inhibitor that is primarily used in the treatment of memory disorders. Hup A is highly potent and has a higher bioavailability compared with donepezil and tacrine, but is less effective compared with BChE inhibitors for treating AD symptoms (98). Recent attempts have demonstrated that derivatives of Hup A, with aromatic rings, exhibit potential therapeutic effects for AD symptoms (99). However, further studies are required to assess the potential benefits of Hup A for treating AD (100). Camps *et al* (101) synthesized hybrids of innovative tacrine and Hup A as a cholinesterase agonists to treat AD. This designed agonist exhibits different functional moieties at basic nuclei of chemical compounds, and provided good results at various positions. The halogen moiety had a higher activity and increased therapeutic effectiveness of treating AD compared with tacrine. However, it also exhibits limited inhibition of BChE. Furthermore, the agonist designed by Camps *et al* (101) also has the propensity to cross the blood-brain barrier.

Huperzine B (Hup B) is also considered to be an AChE inhibitor with reversible and effective properties. Hup B is less potent compared with Hup A, and is also used as a template structure to synthesize novel compounds that inhibit AChE (102). Another potent derivative is bis-Hup B, which

consists of two Hup B molecules connected to a carbon-nitrogen chain by an amine group. The bis-Hup B compound has exhibited higher inhibitory potential against AChE compared with against BChE (103).

Berberine is another candidate compound with multiple biological activities, including the potential to cross the blood brain barrier and target the central nervous system (CNS). Berberine acts as an inhibitor of AChE (104,105) and also performs a neuroprotective function by reducing NMDA-induced excitotoxicity (105).

β -secretase (BACE) as a therapeutic target for AD. A number of BACE1 inhibitors are being synthesized for the treatment of AD (105). OM99-2 is a peptide inhibitor of BACE1 that exhibits strong hydrogen binding within the active binding region of target proteins (106-108). KMI-429 is also considered to be an effective BACE1 inhibitor, with a 50% inhibition concentration (IC₅₀) of 3.9 nM. In a mouse study, Asai *et al* (109) demonstrated that A β production was reduced in APP transgenic and normal mice following KMI-429 treatment. Another mouse study was performed by Luo and Yan (110) using the GSK188909 agonist (non-peptide) against BACE1. The results indicated potential therapeutic effects of GSK188909-induced inhibition of BACE1, via reduction of A β levels in the brain. These results demonstrate that GSK188909 may be considered a beneficial inhibitor in the treatment of AD (110,111). In addition, another orally administered inhibitor, 4-phenoxyprolidine, is also considered to be a potent agent against BACE1, and it has similar functions and pharmacokinetic/pharmacodynamic properties to GSK188909 (110,112). Furthermore, GRL-8234 is also a potent inhibitor of BACE, with an inhibitory constant (K_i) value of 1.8 (107). Chang *et al* (113), in a study on transgenic mice, demonstrated the prominent effects of GRL-8234 on cerebrospinal fluid (CSF) and A β production. CTS-21166 has also been reported to reduce A β levels in the brain by >35-40%, and was the only inhibitor to pass phase I clinical trials (114).

γ - and α -secretase-based drugs for AD. The designing of novel γ -secretase agonists remains a challenging approach due to its non-amyloid behavior and interaction with metabolic processes. Various undesired effects are also generated, including gastrointestinal lethality, hematological toxicity and skin reactions (114).

The first compound that was synthesized as a γ -secretase agonist was DAPT (115,116). A pharmacokinetic evaluation study demonstrated that DAPT overdose is required to inhibit A β production in the neuronal cells of APP transgenic mice (117). LY-450139 (semagacestat) is also an inhibitor of APP cleavage (118). However, certain side effects are associated with LY-450139, including thymus atrophy and a reduction in the number of lymphocytes (118). BMS-708163 is another inhibitor that is reported to reduce A β 40 levels in CSF without causing adverse effects (119). PF-3084014, a non-competitive compound, has been investigated in mice and humans (118). Begacestat (GSI-953) is also an effective agonist against γ -secretase, which controls A β production. Furthermore, in Tg2576 transgenic mice studies, high doses of GSI-953 reduced A β 41 levels in the brain (120,121). A clinical study was performed using GSI-953 for AD treatment (119,122), and the results demonstrated that

GSI-953 does not exhibit a positive effect on the reduction of A β 40 levels in the CSF of patients with AD (120).

In addition, a number of candidate molecules have been synthesized by considering α -secretase as a target molecule (123). Of these, etazolate (EHT-0202) activates neuronal α -secretase and, as a result, enhances soluble APP production (124). Etazolate was investigated in a phase II study in patients with mild-to-moderate AD. Results revealed that etazolate exhibited good clinical efficacy in patients with AD (124). Bryostatins and exebryl-1 are potent inhibitors of α -secretase, which significantly affect A β production and improve memory (125).

GSK-3 inhibitors in the treatment of AD. Hu *et al* (126) demonstrated the importance of GSK-3 as a receptor molecule in the prevalence of AD. GSK-3 agonists may have positive therapeutic effects on patients with AD. The investigated compound SB216763-a (a GSK-3 inhibitor) was synthesized for potential use in the treatment of AD. Functionally, SB216763 reduced phospho-glycogen synthase by 39% and increased glycogen levels by 44%, which demonstrates its potent inhibition of receptor activity (126).

It is difficult to fully understand all of the receptor-based mechanistic signaling pathways and the interactions of neurotransmitters with drugs by experimentation. Therefore, computational modeling and simulation approaches are considered to be important for targeting and investigating the neurodegeneration disorders. The present review will highlight a number of computational modeling attempts and biomarker interpretations to improve the understanding of the pathogenesis and symptoms of AD.

5. Computational modeling and simulations of AD

To interpret the basic mechanism of AD, computational models have been designed on the basis of amyloid plaques, NFTs and hippocampus functions. Furthermore, additional models are based on neuronal functionality and the synaptic transmission of neurotransmitters.

Biochemical and morphological modeling. A β is considered to be a major hallmark and pathological feature of AD (127). Based on A β aggregation factors, kinetics, mechanistic pathways and its morphological appearance, A β is considered to be a key feature in the design of computational models for AD. Experimental and theoretical investigations on A β have investigated the kinetics, mechanistic pathways and fibrillogenesis (128-138). Various computational models have been proposed on the basis of A β kinetics, which include fibril elongation and A β self-association (130,137). Through computational modeling, the monomers of the oligomers of β -peptides in elongated fibrils were arranged into compact aggregations of complexes of pro-peptides in irregular symmetry (132,133,136). The key factors regarding oligomeric β -peptides in previous models include the exclusion of filaments and fibril discrimination, and the use of non-physiological (pH ~1) experimental conditions (139).

Pallitto and Murphy (140) designed a mathematical model on the basis of the kinetics of A β aggregation. The core feature of this model was identifying that A β is partitioned between

two pathways. The first pathway produces a stable structure of monomers and dimers, and the second pathway produces an unstable β -sheet, containing intermediate-aggregated oligomers (141). A model by Kim *et al* (131) further explained the involvement of A β oligomers in the fibrillogenic pathway by evaluating the effect of urea on aggregation kinetics, size distribution and aggregate morphology. An enhanced urea concentration has a direct effect on β -sheet contents, including a decrease in the aggregation size and changes in the morphology of aggregates. The computational model results supported the hypothesis that the amyloidogenesis pathway and the globular aggregates were involved as intermediates rather than an alternative aggregated species.

Plaque-based computational modeling. Amyloid plaque formation is also considered a key biochemical concept to design models (142,143). In addition, the kinetics of APP processing and downstream intracellular interactions of calcium and A β were observed in the AD brain (144-146). The secretases (α , β and γ) function as cleaving agents of APP. It has been observed that secretase agonists target APP and minimize the A β production, and may slow the progression of AD (147). Based on intracellular calcium and A β interactions, a computational model was built to account for established characteristics of AD, which include its irreversibility, acute to chronic pathology and inherent random characteristics of sporadic AD.

Anastasio (148) developed a computational model of AD on the basis of an amyloid hypothesis. The regulatory pathway in the model was justified by interrelated equations. Furthermore, the molecular conditions were symbolized by arbitrary integer values in the equations, and a set of rules were employed to justify the changes in model elements, which change the levels of other elements. The model explained the disruption of A β regulation through the interconnection of various diseases and pathological processes, including cerebrovascular disease (CVD), inflammation and oxidative stress. More precisely, it was reported that CVD contributes to the progression of AD. Additionally, multiple target therapies were more effective compared with single target treatments. In addition, Anastasio (149) designed an additional model based on the knowledge of previous model evaluations and incorporated factors such as the role of estrogen in the regulation of A β to predict effective AD therapy. The predicted model results demonstrated that, by administering non-steroidal anti-inflammatory drug therapy, estrogen levels decrease, which leads to marked reductions in A β . Furthermore, Anastasio (150) extended this work to further understand the synaptic plasticity dysregulation of A β . The predicted results indicated a normalization of nAChRs. Neuronal proteins responded to the neurotransmitter ACh, which addresses the effects of A β on synaptic plasticity. These results contributed substantially to the understanding of how combinations of drugs may be used in the pathogenesis of synaptic diseases (150).

Craft *et al* (151) investigated the association between AD treatment effects and A β levels in different parts of the body. The study investigated fluctuations in A β levels in the brain, CSF and plasma prior to and following medication states. This was achieved by employing an infinite set of nonlinear differential equations. Based on the polymerization ratio calculation,

results indicated that, when values were >1 , A β amalgamation increased indefinitely. Whereas the A β levels in the CSF and plasma remained in a steady state. However, polymerization ratio calculations <1 demonstrated a steady-state of A β levels throughout the body.

GSK-3b, p53, A β and tau-based modeling. In intracellular signaling, multiple proteins are interconnected through specific receptor-mediated pathways. GSK-3b, p53, A β and tau proteins are observed in computational modeling to investigate the mechanistic pathways that mediate AD (152). A multi-compartment model for GSK-3b, p53, A β and tau proteins was designed to determine the associations among these proteins (152). The predicted results demonstrated that abrupt changes in DNA damage the p53 and Mdm2 complex. As a result, GSK-3b/p53 complexes are formed, which enhance the transcriptional activity of p53 and GSK-3b. Consequently, there are increases in the production of A β , Mdm2, mRNA and tau phosphorylation. Computational model results indicate that, in normal states, A β is degraded in cells and, upon dephosphorylation, degradation become optimized within cells. However, under conditions of stress, A β production and tau phosphorylation increase. Therefore, adjusting the DNA damage parameter may clear A β and stop the phosphorylation of the tau protein. Additionally, plaque and tangle formation were independent, even with GSK-3b overactivity (152).

In another computational model based on A β functionality, which was developed by Diem *et al* (153), the results indicated that the deposition of A β in human artery walls reflect the lymphatic drainage pathway with the progression of AD. Initially, the diffusion of A β occurs from the brain to basement membranes in capillaries and arteries via extracellular spaces of gray matter in the brain. However, the exact mechanism of perivascular elimination of A β remains under consideration. Based on this mechanistic approach, a computational model was designed to explain the process of periarterial drainage with regards to diffusion in the brain, and demonstrated that periarterial drainage along basement membranes is rapid compared with diffusion. The predicted results indicated that failure of periarterial drainage is a mechanism underlying the pathogenesis of AD, in addition to complications associated with its immunotherapy (153).

Immunity-based modeling. Proctor *et al* (154) investigated the passive and active immunization effects against A β , plaques, phosphorylated-tau and tangles. In their extended model, A β clearance was elicited by the administration of antibodies, which were modeled by the addition of species termed 'anti-A β ' and 'Glial' (with an additional species to represent microglia). Both additions (antibodies and microglia) were run by predetermined time points during simulation. The predicted model results demonstrated that immunization helped to clear the plaques. However, immunization only exhibited limited effects on soluble A β , tau or tangles. The results of this model demonstrated that immunotherapy against A β is more effective during early stages of AD.

An additional network interaction model of A β , neuroinflammation, mitochondrial dysfunction and lipid metabolism dysregulation was designed by Kyrtos and Baras (155). The basic purpose of this computational model was to investigate

the short and moderate level effects of inflammation, and mutational effects on the ApoE allele. Their model was based on cellular and molecular levels. In cellular levels, four different types of cells, which included neurons, astrocytes, microglia and brain endothelial cells, were used to interact with each other. While at the molecular level, each cellular downstream metabolic network was addressed to mediate the metabolic responses of particular cell types. Modeling of chemical species for each cell type was performed by average distribution. The simulation results indicated that the ApoE4 allele ultimately led to an increase in A β . This increase causes ATP to collapse and an elevation of glutamate levels, which is the major cause of neuronal loss in a local region. The computational model results demonstrated that inflammation may be considered as a key component in the pathogenesis of AD. Furthermore, inflammation strength and duration are also important factors in AD progression (155).

Single cell-based models for AD. To interpret the A β functionality more adequately against AD, single-cell-based models were employed. The different cell-based-models act as a single framework, which investigates the different properties of individual cells. Chen (156) revealed that a higher expression of A β in cells leads to intrinsic disruption of electrical properties in the dendrites of the hippocampus. In the dendrites of pyramidal cells, A β blocks the A-type potassium channels, which results in enhanced membrane excitability and calcium influx (156). Hyperexcitability of dendrites gradually leads to degenerative changes or neuronal cell death (157). The effect of A β was modeled by decreasing the maximal conductance in transient A-type potassium channels. The simulation results for this experimental study demonstrated that, when A β affected the potassium current, there was increased invasion of back-propagated action potentials (bAPs) from the cell body into the apical dendritic trunk of CA1 pyramidal neurons. In another study by Hoffman *et al* (158), similar results were observed following the administration of pharmacological agents that blocked the A-type potassium current (158). The simulation results indicated that the disturbance of normal dendritic electrical activity caused by an intra-articular blockade produces significant differences between the depolarizations of A β and normal cases in the distal oblique branches compared with the dendritic trunk. Furthermore, a number of studies have reported that modified synaptic membrane properties disturb the firing properties of CA1 pyramidal neurons under current and voltage clamp conditions by the amalgamation of A β (159,160).

In addition, Abramov *et al* (161) identified that increases in endogenous A β enhances the initial release probability (p_0) at the CA3-CA1 synapses of the hippocampus, without altering the intrinsic neuronal excitability and postsynaptic function. The increased level of p_0 is also directly involved in the destruction of vesicles by increasing A β production (162). Furthermore, a hippocampal CA1 pyramidal neuron model demonstrated that the enrichment in p_0 affects the synaptic short-term plasticity of the synapse and the firing probability of the CA1 output neuron (163).

Neural network and drug-based models for AD. A neural network model of corticohippocampus formation was

designed to investigate the effects of scopolamine, a drug that blocks cellular effects of ACh, on the encoding and retrieval of memories in paired associate tasks (163). Four modules were present in this model by Hasselmo and Wyble, which included the entorhinal cortex (EC), dentate gyrus (DG), region CA3 and region CA1 (164). In each module, 'memory' was represented as a pattern of neural activation. The information following the patterns among the four modules were represented as EC to DG to CA3 to CA1. The represented items, such as individual words, in CA3 neurons exhibited weaker recurrent connections compared with contextual information. Their detailed model simulation demonstrated that scopolamine impaired the encoding of new input patterns, but had no effect on previously learnt recalled patterns. Results indicated that impairment is selective in free recall, upon the recognition of items that are not already encoded. This model was the first attempt to simulate the effects of a drug on human memory. The experiment investigated and quantified the physiological effects at a cellular level. To design novel drugs against neurodegenerative diseases, modeling attempts to interlink the behavior, physiology and molecular biological aspects in a single constrained model for human memory functions.

An additional computational modeling approach was established to investigate the modulation and control storage, and AD dynamics within the hippocampal CA3 network on the basis of subcortical cholinergic and GABAergic inputs (165). To build upon Meschnik and Finkel's model (165), Buzsaki developed a 'two-stage' memory model and highlighted the importance of interneurons, basket cells and chandelier cells in memory (166-168). Furthermore, Lisman *et al* (168) designed a computational model on the basis of embedded γ cycles within the θ cycles. Their results demonstrated that attractor-based auto-associative memory may be implemented by the synchronization of γ -frequency ranges. Each newly arrived input pattern at the commencement of θ cycle with embedded 5-10 γ -cycles generated a network activity to congregate various γ -cycles as a steady attractor, which characterizes the stored memory. Their predicted results support the hypothesis that CA3 pyramidal cells generate distinct behavioral functions by bursting and spiking patterns. In addition, the change between behavioral states associated with the online processing and recall of information is regulated by cholinergic input in the hippocampus. A deficiency of cholinergic neurons is associated with a reduction of γ frequency. The reduction of γ -cycles within the θ rhythm results in memory loss and cognition, which is associated with AD (169).

Roberts *et al* (170) created a cortical circuitry computational model using preclinical data available on pharmacological receptors for cholinergic and catecholamine neurotransmitters (170). Working memory was identified as a measure of cognitive functions. The pathology of AD includes neuronal and synaptic loss, and decreases in cholinergic tone. The model explains the differential effects of an NMDA agonist, memantine, in EOAD and LOAD conditions. In addition, the model also explains the inhibition of the NMDA receptor NR2C/NR2D subunits, which are present on inhibitory interneurons; the NMDA receptor is inhibited to compensate for the higher excitatory decay detected with pathology.

Bianchi *et al* (171) developed a memory encoding and retrieval model in the brain based on a previous computational

study by Cutsuridis *et al* (172). Their model explains that by enhancing CREB activity, hippocampal CA1 pyramidal neuron properties change, which may contribute to improvements in memory storage. The CREB effects were modeled with a decrease in the conductance peaks of medium after-hyperpolarization (52%) and small after-hyperpolarization (64%), and increased conductance peak of AMPA (266%) currents. The results demonstrated that, by improving CREB function in AD-like conditions, the stored pattern in network recall quality increased significantly. The results confirmed that CREB-based agonists may be used as a novel approach for the treatment of AD.

The synaptic deletion and compensation model. The initiation of synaptic deletion and compensation model was initially proposed by Horn *et al* (173) and further developed by Rupp and Reggia (174). The artificial neural progression model for AD (173) deviates from the excitotoxicity, which does not account for cognitive impairment. It has been observed that a 50% loss of synaptic connections is considered a primary factor for cognitive deficits. In earlier stages of AD, the loss of connections is compensated by strengthening the remaining connections. Horn's model demonstrated that synaptic connections are associated with memory loss and disturbances in learning patterns. The rate of memory deterioration may be minimized by enhancing the remaining connection weight of a constant multiplication factor (173).

The synaptic runaway model was based on associative memory and memory storage as a pattern of neuronal spatio-temporal activation (175,176). Memory storage activates different analog patterns that interact with previous associations. For example, if there is an overlap between patterns or if the memory capacity is exhausted. The results demonstrated that the significant increase in the number of associations stored by the network may govern pathological increases in the strength of synaptic connections. As a result, such synaptic connections give rise to an increase in neuronal activity, high metabolic demand and may eventually cause excitotoxicity. The synaptic runaway model investigated two basic mechanistic approaches to memory, encoding and retrieval, to attempt to reduce neuronal cell death (176). In normal conditions, neuromodulation is satisfactory to preclude the variations in runaway synaptic modification (RSM). Whereas, in manifestations of disease conditions such as AD, the RSM neuromodulation is inevitable. However, the threshold levels for RSM in AD are lower compared with controls (177).

Bhattacharya *et al* (178) designed a computational model to determine the association between active synapses and α -band frequency amongst individuals that are healthy, exhibit mild cognitive impairments and patients with AD. An additional aim was to duplicate the dysfunctional electroencephalogram experimental data in patients with AD. Their model simulated neurological regions composed of various multilayer neurons, three for the thalamus and four for the cortex module. However, this model was limited as it did not simulate the association between α -band frequencies and ACh.

Neurocomputational model at system level. Computational exploration of AD has reported changes in hippocampal functionality and behavioral performance (178,179). For example,

the ability to learn and adapt learning to novel situations is impaired in AD. Moustafa *et al.* (180) also identified that simulated learning occurred through an interaction between the hippocampal region and basal ganglia (180). Hebbian learning and temporal difference algorithms were used to train the model, and the results indicated that hippocampal damage leads to impaired learning performance.

Additionally, a computational model by McAuley *et al.* (181) investigated the functional association between cortisol and the hippocampus in aged individuals and patients with AD (180). The results of this model demonstrated the existence of an antagonistic association; as cortisol levels increase, there is a decline in hippocampal functions and cognitive performance. An estimation approach of this model depicts that, in 90-year-old individuals, increases in cortisol levels lead to a 30% reduction in hippocampal functions. A limitation of this model was that it only considered the effect of cortisol receptors in the hippocampus; the effects of cortisol on other brain regions were not considered.

6. AD Biomarkers

Neurological biomarkers. Generally, a biomarker is a parameter of physiological, biochemical or anatomical domains that indicates normal biological and pathological processes or reactions to a therapeutic intervention (182). Biomarkers are currently considered to be important factors in the diagnosis of neurodegeneration (183). AD biomarkers, which include A β plaques, and tau-associated and fluid biomarkers, have been validated in clinical trials (183), and are currently being used within therapeutic trials (184). There are two major categories of AD markers, which are A β plaques and tau-associated neurodegeneration. Furthermore, certain types of AD models based on imaging measurements and CSF analytes exist (185-189). Various targeted proteins and receptors may also be used as markers by inhibiting their downstream signaling pathways by using antagonists. A β and tau proteins are employed as early markers in the treatment of certain cognitive disorders, including LOAD, lewy body dementia, mild cognitive impairment, vascular dementia and frontotemporal lobar degeneration (190,191).

Neuronal death occurs due to a loss of neuronal synapses (192,193), which results in structural and functional changes (which may be used as markers) in brain regions associated with memory, which include frontal, temporal and parietal lobes (194). The strength of these markers is dependent upon the time scale of disease, whether it is early or late-stage AD. The disturbance of a single neuron or neurotransmitter could not be considered as a sole factor for the prevalence of neurodegenerative diseases; the risk of developing neurodegenerative diseases may be due to the disruption of interconnected signaling pathways across multiple neurological regions (195). For example, damage to neuronal cells or neurotransmitters have been reported to govern atrophy of structures in frontal, temporal and parietal lobes (196-200).

Studies concerning AD have demonstrated that damage may occur at various regions of the brain, including the neocortex, EC, hippocampus, amygdala, nucleus basalis, anterior thalamus and the corpus callosum within these lobes (201-204). Neuronal damage results in atrophy of

Table II. Biomarkers based on clinical and exploratory research.

AD biomarkers	Clinical research	Exploratory research
Neurological	-	Yes
BACE1	Yes	-
Amyloid plaque-based	Yes	-
Glucose metabolism	Yes	-
LOAD	Yes	-
Blood-based	Preclinical	

'-' indicates that no data is available at present. AD, Alzheimer's disease; BACE, β -secretase; LOAD, late-onset AD.

structures in the frontal, temporal and parietal lobes. Consistent with the heterogeneous symptomatology of AD, damage may be localized to numerous sites within these lobes. An abnormal paleness of the ceruleus locus, which contains neuromelanin neurons, is also considered to be a key feature of AD (205). The neuropathological structures, NFTs and senile plaques, within affected brain regions of AD are also considered to be markers. The accumulation of NFTs in the affected regions following neuronal death causes abnormalities in structure of the cytoskeleton, which is important for preserving the cell structure as well as for transportation (206). In addition, the hyperphosphorylation of tau interrupts axonal transport, which leads to disturbances in various molecular movements and results in neuron death (202,207).

BACE1 and amyloid plaque-based biomarkers. The secretases (β - and γ -secretase) cleave APP into various types of A β protein. It has been reported that an elevated level of BACE1 activity may contribute to the amyloidogenic process in AD (207,208). Therefore, BACE1 is considered to be a biomarker for monitoring amyloidogenic APP metabolism in the CNS (209).

A β is the fundamental element of senile plaques, which is considered to be a common biomarker for AD (210). Depending on the structure of senile plaques, they are classified as either neuritic or diffuse plaques (201). Neuritic plaques have spherical morphology with a periphery of neurites, which may include axons, astrocytes and microglia, with neighboring dense amyloid proteins (206). Diffuse plaques have an amorphous morphological appearance without neurites. Diffuse plaques may be present in normal aging brain tissue (211). However, a number of studies have also reported that diffuse plaques may or may not be ancestors of neuritic plaques (201,212). Amyloid angiopathy is a generic term for blood vessel (arteries, veins and capillaries) disease. Amyloid angiopathy is also considered to be a marker for AD, as it involves the accumulation of amyloid protein in the cerebral blood vessels of patients with AD (213-215).

Glucose metabolism and oxidative free radicals as biomarkers. Additional reported biomarkers for the pathogenesis of neurodegenerative diseases include glucose metabolism, oxidative

free radical damage to mitochondrial DNA, neuroreceptors and neurotransmitter functional activity (216,217). It has been reported that decreases in glucose metabolism (218,219) and augmented oxidative free radical damage (220-222) are responsible for neuronal death in the temporal and temporoparietal regions of the brain. Furthermore, changes in the neurotransmitter activity may govern abnormal types of neuroreceptor responses. Data mining revealed that a reduced density of nAChR, and serotonin and α 2-epinephrine receptors (217), reduces the binding of neurotransmitters and may disturb synaptic efficiency. Any modulator of neurotransmitters, including ACh, serotonin and GABA, may be considered beneficial in the improvement of cognition in AD. In addition, other neurochemical markers, including N-acetylaspartate and myoinositol, have also been reported as potential treatments for AD (223).

LOAD biomarkers. Modeling AD biomarkers becomes more important in the elderly state, due to the neurodegenerative nature of AD. A previous study using autopsy demonstrated that the medial temporal tauopathy may be decreased by two-thirds after the age of 50, and is present in the majority of individuals >70 years of age (224). Furthermore, a number of studies have reported that tauopathy precedes LOAD (224-226). In A β deposition, CSF levels of A β 42 and amyloid PET scans are highly effective parameters for biomarkers to accurately identify EOAD (227).

It has been observed that magnetic resonance imaging (MRI) studies may be considered as quantitative biomarker measures for AD, on the basis of calculating the values of AD-signature regions (228). The summation calculation is primarily performed by an anatomic atlas that is spatially registered to the subject's imaging study (228). Additional potential AD biomarkers that may be employed to investigate the pathology of AD include visinin-like protein 1, a CSF analyte (229), diffusion and perfusion MRI (230) and agonist of tau PET imaging (231). These biomarkers are reported as novel suggestions and limited experimental data exists currently.

Blood-based biomarkers. Certain blood-based proteomic biomarkers are also being used for AD treatment (232). However, there are disadvantages associated with the complex nature of blood-based biomarkers. One of the most prominent hindrances is the presence of multiple dynamic ranges of proteins in the blood (233). The blood-brain barrier is interrupted in aging patients with AD. This results in enhanced permeability, which is considered to be the first indicator of cognitive impairment in AD (234). The association between the blood and the brain is strengthened by blood-brain barrier disruption. This association may aid with the detection of protein-based biomarkers during the earlier stages of AD (235). However, blood-based biomarker associations with AD are lower compared with the CSF, due to the reduced peptide (A β) concentration in the CSF (235). AD biomarkers at preliminary exploratory stages, and biomarkers that are currently being tested in clinical studies are presented in Table II.

7. Conclusions and future directions

AD is a slow neurodegenerative disorder in which pathophysiological irregularities lead to obvious symptoms such

as severe memory loss. This review has demonstrated that mechanistic gene/receptor-mediated signaling pathways may be used as novel therapeutic targets to treat cognitive symptoms. To interpret such receptors and their effects on A β , various computational modeling and simulation approaches have been employed to identify novel targets for AD. Furthermore, identification of potential biomarkers may also be considered an important approach prior to the implementation of *in vitro* and *in vivo* experiments. Therefore, the design of interventional approaches (modeling and simulations) that target the appropriate molecular pathways in developmental stages of AD depends upon specific AD biomarkers. This may improve treatment by allowing individual patients to receive the most appropriate drug for them in the shortest amount of time (236-240). However, current AD models have limitations, which include not explaining the effects of mechanistic pathways and cytotoxicity. Furthermore, there is no comprehensive explanation of the ACh neuronal transmission that leads to AD and other neurodegenerative diseases. Future models should aim to investigate and explain the molecular mechanisms underlying the implication of ACh in the development of AD in the human hippocampus. In addition, drug simulations should also be addressed to determine their effects on other brain compartments. Notably a model has already been suggested to explain the dysfunction of ACh in AD (164). Finally, drug models may be more helpful if they considered key knowledge regarding dosage form, targeted receptors and their associated downstream signaling pathways. Detailed computational modeling and simulation approaches are essential to understanding what chemical compounds may be synthesized in order to treat or cure AD.

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