Abstract: Alzheimer’s Disease (AD) affects at least 5.7 million Americans, and it is the sixth leading cause of death in the United States. At the onset, patients experience minor memory problems. Next, impairments in speech and motor function manifest as a limitation to well-being and independence. Slowing this pandemic rise is critical, since AD also bears a huge socioeconomical burden. Unfortunately, there is limited prevention and no effective cure has been found, as all clinical trials for promising AD drugs have failed thus far. The pathological hallmarks of AD include amyloid-β plaques (Aβ), neurofibrillary tangles (NFT), and neuroinflammation. Other factors include APOE4 and environmental stressors, such as metal dyshomeostasis, which contribute to AD pathogenesis. Herein, we review major contributing factors involved in AD pathophysiology. Deeper understanding of associated molecular mechanisms underlying AD pathogenesis is critical for developing novel AD theranostics.

Keywords: Amyloid-β; amyloid precursor protein; metals; β-site of APP cleaving enzyme 1 (BACE1 or β-secretase); neurotoxicity; NF-κB; presenilins
INTRODUCTION

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder and is the most represented form of dementia. It is the 3rd most common disease affecting the population, inflicting at least 5.7 million Americans as the trend continues to rise at a pandemic rate (1). AD reduces the quality of a patient’s life, as irreversible cognitive decline becomes apparent due to pathological and morphological changes such as cortical atrophy, neuroinflammation, loss of synaptic connections, and cellular death (2) leaving the individual dependent on significant care, as their memories and motor function deteriorate.

Fortunately, technological advances have afforded researchers the ability to characterize neuronal loss in the hippocampus and cortices (3). Additional work has acknowledged perspectives on multifaceted complexities that have linked risk-associated genes and environmental factors to these differences (4). For example, increased exposure to air pollution, chemicals, and ionizing radiation is harmful (5, 6) and potentially contributes to dementia-related diseases. Unfortunately, AD has no efficacious treatments, and thus, the disease is a critical health concern and has incurred a colossal socioeconomic burden. Recently, the Alzheimer’s Association reported the cost as $236 billion and is projected to rise to $1.1 trillion in 2050 (1). Therefore, identifying an accurate diagnosis and effective treatment is urgent.

The hallmarks of AD are evident, with neuroinflammation and aggregated A\(\beta\) plaques followed by neurofibrillary tangles (NFT). In fact, recent studies observed plaque deposits within cognitively normal individuals up to 20 years before the onset of cognitive decline (7). Why A\(\beta\) fibrils aggregate into plaques has yet to be elucidated; however, there is evidence that its exacerbated presence is toxic to neuronal cells. For example, A\(\beta\) inhibits respiratory function, reduces ATP levels (8), and leads to mitochondrial dysfunction (9). In vitro studies of PC12 cells observed depolarization of the mitochondrial membrane potential and decreased activity of mitochondrial electron transport chain complexes. As A\(\beta\) aggregates, it leads to signaling impairments causing the cells to undergo apoptosis. Anti-A\(\beta\) drugs tested in human clinical trial have failed to produce promising results. As such, the credibility of the amyloid hypothesis has been questioned, and the true role of A\(\beta\) is currently being investigated.

AMYLOID PRECURSOR PROTEIN

Amyloid plaques, or the insoluble A\(\beta\) peptides, in the brain form through the cleavage of amyloid precursor protein (APP) by the b-site of APP cleaving enzyme 1 (BACE1 or \(\beta\)-secretase) and \(\gamma\)-secretase (10, 11). APP is located on chromosome 21, and it is a type I transmembrane protein involved in secretory and endocytic processes (12). It contains a metal-binding domain, heparin, collagen, laminin, and a protease inhibitor domain (13). Although the function of APP is unclear, there is evidence to suggest that the ectodomain of APP may be involved in cell adhesion, trophic support, cell growth, and differentiation of neuronal stem cells (14). Conversely, the intracellular domain may modulate mitochondrial function (15).
APP can be processed through two pathways: the amyloidogenic pathway and the non-amyloidogenic pathway. In the amyloidogenic pathway, β-secretase cleaves APP at amino acid 671 releasing APP β (sAPPβ). Next, the CTF99 embedded in the plasma membrane is cleaved by γ-secretase, made up of 4 subunits (16, 17), including the catalytic domains Presenilin1 gene (PS1) and Presenilin2 (PS2) (18). BACE1 is a rate-limiting step for Aβ production, and knockout studies result in complete inhibition of Aβ generation (19). In the non-amyloidogenic pathway, APP is cleaved by α-secretase at amino acid 687, releasing soluble APPα (sAPPα). The remaining protein, CTF83 is cleaved by γ-secretase releasing a soluble p3 fragment. α-secretase belongs to a family of single-pass transmembrane and secretes zinc-containing endopeptidases that are dominant in neurons (20). Aggregated Aβ function in normal physiology remains to be elucidated (19); however, Aβ disrupts postsynaptic trafficking in glutamate receptors such as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (21) and N-Methyl-D-Aspartate (NMDA) receptors (22). Their actions may be important for learning and memory, and synaptic plasticity (23–25). Aβ has also been shown to modulate the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) through interaction with KCC2 (26). A study by Senechal et al. investigated APP knockout mice and discovered dysregulated long-term potentiation (LTP) and learning deficits (27, 28). Moreover, theta–gamma oscillation phase–amplitude coupling was also diminished in regions of the parietal cortex and hippocampus compared to the wild type (27). As such, the function of APP is complex, and data so far have linked the role of the protein in neurite growth (29–31), axon guidance (32), and neuronal cell adhesion (33).

**β-SITE OF APP CLEAVING ENZYME 1**

β-site of APP cleaving enzyme 1 (BACE1) is a major drug target for therapy (34) because its expression correlates not only with the onset of AD but also with glucose intolerance. Importantly, this downstream effect is a risk factor for diabetes. Studies on mouse models which inhibit BACE1 expression resulted in improvements in glucose homeostasis, lowered leptin levels, and decreased hypothalamic inflammation (35, 36). However, depletion of BACE1 leads to other harmful effects as evidence suggests it important in regulating adult hippocampal neurons responsible for memory (37, 38) and other important neuronal processes. For example, mice models that possess faulty BACE1 expression result in deficits in synaptic transmission and plasticity in the hippocampal region (39). Furthermore, the cell adhesion molecule Neuregulin-1 (Nrg1), which must be cleaved by BACE1, mediates radial migration of glutamatergic and GABAergic neurons. It is also responsible for myelination and synaptic plasticity (40) and is required for the formation of new synapses while strengthening existing ones. Interestingly, BACE1 null mice result in a reduction of Nrg1 cleavage, resulting in characteristics of schizophrenia (41). Similarly, Sez6 is a protein that is concentrated in areas associated with morphological plasticity. This includes areas within the hippocampus and cerebellum in postnatal brains. Sez6 is also cleaved by BACE1 and mediates dendritic arborization of cortical neurons (42) which is critical for neuronal transfer of information. Thus, defective BACE1 leads
to poor motor coordination, weak balance, and cognitive deficits. Lastly, BACE1 deficiency also affects Jagged-1 (Jag1) that regulates astrogenesis/neurogenesis through Notch signaling pathway (43, 44) and contributes to memory formation. Therefore, suppression of this enzyme is a double-edged sword and more research is needed to help in AD patients.

GENETIC RISK FACTORS OF ALZHEIMER DISEASE PATHOLOGY

There are two forms of AD: sporadic and familial. The majority of the cases (approximately 95%) are classified as sporadic late-onset AD (LOAD), while about 5% are classified as familial early-onset AD (EOAD) with an autosomal dominant inheritance pattern. Sporadic AD is influenced by complex genetic variants combined with environmental factors (45). However, there is little evidence to define how this occurs. Early onset is caused by rare mutations in three genes located on chromosome 21 (46, 47) and chromosome 14 (48). The summary of genetic mutations implicated in LOAD is shown in Table 1 and EOAD is given in Table 2 (45).

### TABLE 1 A summary of genetic mutations implicated in LOAD

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Chromosome</th>
<th>Risk change %</th>
<th>Proposed molecular phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>APOE</td>
<td>Apolipoprotein E</td>
<td>19q13</td>
<td>~400–1500%</td>
<td>Clearance of Aβ Lipid metabolism</td>
</tr>
<tr>
<td>ABCA7</td>
<td>ATP-binding cassette subfamily A member 7</td>
<td>19p13.3</td>
<td>~20%</td>
<td>Lipid metabolism Cellular signaling</td>
</tr>
<tr>
<td>BIN1</td>
<td>Bridgin integrator 1</td>
<td>2q14</td>
<td>~15%</td>
<td>Production of Aβ Clearance of Aβ Cellular signaling</td>
</tr>
<tr>
<td>CR1</td>
<td>Complement component (3b/4b) receptor 1</td>
<td>1q32</td>
<td>~15%</td>
<td>Clearance of Aβ Innate immunity</td>
</tr>
<tr>
<td>PICALM</td>
<td>Phosphatidylinositol-binding clathrin assembly molecule</td>
<td>11q14</td>
<td>~15%</td>
<td>Production of Aβ Clearance of Aβ Cellular signaling</td>
</tr>
<tr>
<td>CD2AP</td>
<td>CD2-associated protein</td>
<td>6p12.3</td>
<td>~10%</td>
<td>Cellular Signaling</td>
</tr>
<tr>
<td>CD33</td>
<td>CD33 (Siglec 3)</td>
<td>19q13.3</td>
<td>~10%</td>
<td>Innate immunity Degradation of</td>
</tr>
<tr>
<td>CLU</td>
<td>Clusterin</td>
<td>8p21.1</td>
<td>~10%</td>
<td>Clearance of Aβ Innate immunity</td>
</tr>
<tr>
<td>EPHA1</td>
<td>EPH receptor A1</td>
<td>7q34</td>
<td>~10%</td>
<td>Cellular signaling Innate immunity</td>
</tr>
<tr>
<td>ATXN1</td>
<td>Ataxin 1</td>
<td>6p22.3</td>
<td>NA</td>
<td>Production of Aβ</td>
</tr>
</tbody>
</table>
Mutations in \textit{APP}, \textit{Presenilin1} (PS1), and \textit{Presenilin2} (PS2) genes have been integral in the development of AD as they cause a disruption in the ratio of A\textbeta 42 production (49). In normal physiology, presenilins, needed for the production of A\textbeta peptides via both \textbeta - and \gamma-secretases-mediated cleavage (50, 51), are responsible for autosomal transmission and the promotion of amyloid plaque. \textit{PS1} regulates calcium homeostasis and mediates neurotransmission (52, 53). The largest amount of mutations occurs for \textit{PS1} at an estimate of 200 mutants, whereas \textit{APP} and \textit{PS2} have 10–25 mutants on the AD and frontotemporal dementia mutation database. Meta-analysis revealed at least 15 potential loci where variations may predispose one to developing AD (46). However, a particular gene appears to be the most burdensome, the \textit{ApoE} gene. It has four different isoforms: ApoEe1, ApoEe2, ApoEe3, and ApoEe4. Apolipoprotein E (ApoE) regulates synaptic function, promotes plasticity, increases the number of dendritic spines, and regulates protein trafficking across neurons (54). It is responsible for the regulation of triglyceride and cholesterol metabolism. Binding of lipidated ApoE facilitates A\textbeta uptake in an isoform-dependent manner, and inhibited clearance contributes to A\textbeta accumulation. One variant of \textit{ApoE} gene has been identified as the largest risk factor for late-onset AD through computational analysis (55–57). It is important to note that possessing ApoEe4 over the e3 (common) or e2 (other variant) alleles is not enough to cause AD but it acts as a determinant which increases overtime as the patients ages (45). Analyses reveal that a heterozygous pair increases AD by threefold, whereas a homozygous pair increases the risk by 15-fold (58). It is thought to be the least effective in binding to, and facilitating the uptake of, A\textbeta. Additionally, its strong ties to neurovascular dysfunction further confirm its contribution in AD manifestation (56). The allele can be investigated for potential biomarkers and to unearth new targets for AD drug discovery due to significant clinical and neurobiological correlations. Among them, \textit{ApoE} e4 allele and low CSF level of A\textbeta42 have been reported (59). Patients with the e4 \textit{allele} tend to present with early-onset memory impairment, decrease in global cognitive function, and weak episodic memory (60). Interestingly, the ApoE e2 variant seems to reduce the risk of dementia compared to the common e3 allele, despite its association with an increased amyloid burden (56). Overall, monitoring \textit{ApoE} gene can play an important role in understanding the AD pathophysiology and be used as an assessment tool for at-risk patients.

Other genes that have a strong association with late-onset AD include \textit{SORL1}, which mediates protein trafficking (61), and \textit{ACE}, which regulates blood pressure (62). Furthermore, testing a single nucleotide for any association with disease

**TABLE 2 A summary of genetic mutations implicated in EAOD**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Chromosome</th>
<th>Molecular phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{APP}</td>
<td>Amyloid \textbeta protein precursor</td>
<td>21q21</td>
<td>Increased A\textbeta_{42}/A\textbeta_{40} ratio</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Increased A\textbeta production</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Increased A\textbeta aggregation</td>
</tr>
<tr>
<td>\textit{PSEN1}</td>
<td>Presenilin 1</td>
<td>14q24</td>
<td>Increased A\textbeta_{42}/A\textbeta_{40} ratio</td>
</tr>
<tr>
<td>\textit{PSEN2}</td>
<td>Presenilin 2</td>
<td>1q31</td>
<td>Increased A\textbeta_{42}/A\textbeta_{40} ratio</td>
</tr>
</tbody>
</table>
pathology can be accomplished through GWAS technology. One avenue leads to the discovery of GRB2, which mediates tau phosphorylation and has a high affinity for APP and the presinilins (63, 64). Other findings identified ATXN1, which affects Aβ levels by modulating β-secretase levels and cleavage of APP (65), and BIN1 (66), which is highly expressed in the central nervous system and plays a role in receptor-mediated endocytosis (67). Furthermore, ADAM10 mutations have impaired enzyme activity and lead to the onset of AD in the elderly (67). Lastly, CD33 has been an interesting discovery because it helps strengthen that Aβ acts as an AMP (11, 68–70).

THE NEUROIMMUNE SYSTEM

There are many challenges in understanding the complexity of inflammation in relation to AD in order to develop appropriate therapeutics. Clinical analysis of AD patients exhibited chronic neuroinflammation, insufficient energy metabolism, and redox stress in postmortem brains (71). These observations have been replicated in both animal and cell culture models. Increased inflammatory cascade by microglia has been observed in areas of Aβ deposits and activation of NF-κB (72, 73).

Due to their high affinity for Aβ deposits, understanding the role of microglia may help identify therapeutic targets. In brief, microglia are recognized as the brain macrophage and play an integral role in housekeeping. Upon signal detection, they act to remove debris, toxins, pathogens, and apoptotic neurons (74, 75) by releasing a cascade of inflammatory factors. As such, they release reactive oxygen species and Th1 cytokines including interleukin 1-beta (IL-1β), IL-6, tumor necrosis factor alpha (TNF-α), and interferon-gamma (76) to ramp up the immune system. Furthermore, they are integral in upregulating MCHII complexes, leading to an inflammatory cascade in innate immune response in many disorders such as Parkinson's disease, HIV, and multiple sclerosis (77–80). In AD brain, microglia are constantly aggregated around Aβ plaques (81) to form a barrier between healthy tissue and areas of injured or infected tissue. Since there is no evidence to suggest microglia can degrade Aβ, they undergo a state of compromised phagocytosis, in which the semi-degraded Aβ are ultimately expelled from the microglial cell (82) causing a dysregulation of homeostasis. Extended exposure to Aβ leads to disrupted calcium homeostasis within astrocytic cells, which also leads to degeneration of neurons (83).

NF-κB regulates the expression of more than 400 genes (72) and can be induced by ROS, interleukinIL-1β, TNF-α, bacterial lipopolysaccharides (LPS), isoproterenol, and ionizing radiation (73, 84). Its activation is dependent on growth factors and the neurotransmitter, glutamate (85). Thus, NF-κB plays an important role in DNA transcription and cellular survival. In general, high levels of NF-κB expression are associated with normal aging and upregulate microglial activity (85–87). This overexpression increases the susceptibility for AD through upregulating BACE1 and APP genes (88).

Furthermore, rodent models have demonstrated the outcomes of unregulated NF-κB, resulting in a destructive feedback loop (89, 90). For example, mice that overexpressed NF-κB had clinical signs of increased apoptosis in the hippocampal
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region through triggering TNF-α and iNOS when exposed to neurotoxins (91, 92). Moreover, drosophila studies that overexpressed NF-κB in the hypothalamus-like pars intercerebralis resulted in deficits in learning, inadequate memory consolidation, and increase in mortality rates compared to the controls (93). Upon clinical analysis, imaging studies resulted in severe neurodegeneration (94).

Studies regarding the relationship between AD and lifestyle choices concluded that an increased risk of AD was associated with diabetes, high blood pressure, and smoking (95–97). Type 2 diabetes mellitus (T2DM) increases a patient’s risk of developing AD by over 50% (45), and it affects the increase of Aβ pathology by its ability to upregulate NF-κB and the expression of BACE1 (98, 99). As researchers continue their efforts in drug therapeutic development, alternative approaches have been sought, including cognitive exercises that have improved the production of dopamine and vitamin C (100, 101). Rats that were subjected to pro-inflammatory diets, and adhered to aerobic exercise, resulted in attenuated NF-κB expression in the liver and muscles. Similarly, regular exercise resulted in an increase of endurance, cognition, and performance (102–104). Unfortunately, standard models are not adequate in analyzing the effect of nutrition on the onset of AD, and no study to date can definitively state the relationship (101).

BLOOD–BRAIN BARRIER

The blood–brain barrier (BBB) plays a vital role in the longevity of an individual’s health, and it is responsible for the clearance of Aβ; thus, any insult that compromises the integrity of BBB can cause neuronal cells damage (105–107). New studies have observed the progression of AD along with compromised BBB (108). This negative effect is alarming as any damage to the neurovascular unit (NVU) results in toxic substance leaking into the CNS circulating in the blood. In fact, the mechanism of transporting Aβ out of brain is impaired in AD patients, which contributes substantially to its accumulation (107). One example is the dysfunction of P-glycoprotein (Pgp) (109) resulting in increased deposits and age-associated cognitive impairments. Furthermore, mediating glucose transport for neuronal functionality is integral for astrocytes and neurons, and expression of GLUTs is downregulated in patients. This decreases brain energy supply as confirmed by brain imaging studies (110). Other risks associated with an impaired BBB lead to insufficient nutrient supply and toxin removal, and altered protein expression, all impacting and upregulating the role of neurodegeneration (111, 112). Although it is not elucidated how the mechanism works, therapeutic interventions in alleviating the disease progression are necessary. Recent findings conclude that AD risk factors can be modulated with lifestyle changes in regard to increase in educational levels, exercise, and healthy dietary choices (113–115).

METALS

Strong evidence suggests that biometals in the brains of AD patients are insufficiently maintained, thereby promoting cognitive loss. Due to their structure and function, the proteins that play a role in AD pathophysiology have capabilities
of interacting with metals, especially zinc and copper. Other transitional metals include lead, aluminum, and iron, which may negatively impact human health if the homeostasis is not maintained (116, 117). Neuronal damage can occur due to dysregulation of integral metals needed to maintain brain function. The accumulation of Cu ions has been identified around plaques in postmortem AD brains (118), suggesting the impact of Cu on AD progression. As such, excessive dietary Cu on high-cholesterol diet in rabbits and AD mouse model induces hallmark pathologies. Research has found that chronic exposure to Cu contributes to an increased risk of AD by facilitating Aβ accumulation (118).

Additionally, zinc regulates many proteins such as SNAP25, PSD95, AMPA receptors, and NMDA receptors. ZnT3, a zinc transporter, allows for the release of zinc from neurons into synapses and is involved in cognition and memory. The disruption in mechanism results in cognitive decline (120). Likewise, AD mouse brains have irregular protein levels of CamKII, spinophilin, NMDA receptors, and BDNF (119, 121). Interestingly, AD transgenic mice studies indicated Aβ amyloid aggregated in areas of high Fe, Cu, and Zn levels, indicating accumulation of metals within the brain promotes the aggregation of the Aβ peptides (122, 123). Recent studies have found that APP can regulate iron levels in the brain by removing it from cells, similar to ceruloplasmin. In AD, this activity is decreased by 70% in cortical tissue (122, 123). Tau knockout mice lacked the ability to clear out iron and developed age-dependent neurodegeneration. Rescue studies provided clues that quinoline activity may be a possible therapeutic for AD (124).

PBT2, currently in clinical trials, is a disease modifying drug that does not act like a chelator but as an ionophore (119, 121). Administration of PBT2 for 12 weeks improved mild forms of AD cases through executive function and composite cognitive z-scores and reduced the levels of Aβ in cerebrospinal fluid (125, 126). Other studies also showed increased neurite outgrowth in vitro and decreased tau phosphorylation (121, 127).

Iron is critical for maintaining neuronal tissue and is involved in the synthesis of myelin and neurotransmitters. Conversely, excessive accumulation can enhance Aβ production and tau dysfunction leading to neuronal cell death. Parallel to how iron increases expression of ferritin and ferroportin, iron also increases the processing of APP (128, 129). This causes formation of senile plaques and leads to oxidative stress, resulting in oligomerization and more Aβ generation (130). Iron dysregulation increases NFT (131) creating an iron-rich population within oxidatively stressed environments (132). Quantitative mapping that displays an increase in iron loading shows a strong predictor for cognitive decline. The disruption of iron levels affects neuronal populations within the hippocampus through Fenton and Haber–Weiss reactions (133), producing oxidative lipids that further increase the neurotoxicity and AD pathogenesis (134). As stated, NFT is the integral for trafficking APP to neuronal membrane to facilitate iron efflux from neurons (122, 135), and thus, the loss of tau expression increases the risk for cognitive loss and cortical atrophy in mice (124).

The effects of aluminum on neurodegeneration have attracted attention since it can cause mitochondrial dysfunction and ATP depletion at the cellular level, and decline in memory and cognitive performance on a psychiatric level (136, 137). It can also cause apoptosis in neurons (138). Biopsy studies have confirmed elevated levels of aluminum in LOAD brains, possible source being drinking water (139).
CONCLUSION

AD significantly reduces patients’ quality of life. Therefore, there is an urgent need to develop early detection diagnostics and preventive measures to slow the progress of the onset until the discovery of a cure. Serial failures of clinical trials for AD experimental drugs have led us to reevaluate the pathology of this devastating disease and to embark on further understanding of the underlying AD pathophysiology and associated contributing factors. Agents against targets such as BACE1 and APP amyloidosis have proved to be ineffective against AD progression so far. Therefore, further studies in AD pathogenic mechanisms and future utility of artificial intelligence (AI)-based drug discovery tools may aid in developing novel theranostic agents for AD (140, 141).

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REFERENCES


