Review

Discovery Medicine 2023; 35(178): 757–776
https://doi.org/10.24976/Discov.Med.202335178.71

Etiology of Alzheimer's Disease

Can Zhang[1,2,*]

[1]Genetics and Aging Research Unit, McCance Center for Brain Health, MassGeneral Institute for Neurodegenerative Disease, Department of Neurology, Massachusetts General Hospital, Boston, MA 02129, USA
[2]Harvard Medical School, Boston, MA 02115, USA
*Correspondence: zhang.can@mgh.harvard.edu (Can Zhang)
Published: 1 October 2023

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the primary cause of dementia in the elderly. The research on AD has markedly evolved since discovering the pathological hallmarks in the brain over the past years. However, the etiology of AD has not been completely elucidated, and presently there is no cure for AD. Furthermore, despite the disease hallmarks commonly manifested in all AD cases, considerable evidence suggests complex heterogeneity of AD pathogenesis. Specifically, studies have identified several disease genes that cause or associate with AD and discovered multiple modifiable risk factors of AD, which may further aggregate the complex etiology of AD. Understanding molecular mechanisms of AD genes and modifying risk factors of AD are making it possible to develop effective interventions to prevent or cure the disease, which may in turn help understand the disease etiology. Recently, there has been keen interest in carrying out multidisciplinary investigations on AD, e.g., the studies on the molecular basis in association with neural network abnormalities in AD. Furthermore, the molecular findings on the pathogenetics of AD have provided translational proof-of-concept to intervene in AD, which has inspired clinical trials by reducing disease neuropathology related to AD genes or risk factors of AD. This article's main aim is to highlight recent progress on the mechanisms and approaches with an attempt to gain an integrated perspective of AD etiology. We envision that this article and its referenced reports may provide a valuable point of reflection that may not only acknowledge past discoveries, but also stimulate future studies, advancing the understanding of the complex etiology of AD and the development of effective therapeutics for AD.

Keywords: Alzheimer's disease; etiology; genetics; mechanism; neurology; pathology; translational medicine; therapeutics

Introduction

Alzheimer's disease (AD) is an aging-related and progressive neurodegenerative disorder, accounting for nearly two-thirds of all cases of dementia and up to 20% of individuals older than 80 years of age [1]. As the primary cause of dementia in the elderly and a global health threat, AD currently affects approximately 5.5 million Americans which may triple to 13.8 million by 2050 with increasing lifespans [2]. The State of US Health places AD as having the fastest growing incidence rate among the most burdensome diseases and among the top 5 leading causes of death [3].

Presently, there are no therapies or approaches that can stop disease progression. Several Food and Drug Administration (FDA)-approved pharmacological medications for AD, including acetylcholinesterase inhibitors and *N*-methyl-D-aspartate (NMDA) receptor partial antagonists, are only palliative and may provide temporary and modest symptomatic relief. They have no impact on the underlying pathology of AD, nor can they halt disease progression. There has been recent FDA-approval of aducanumab [4] and lecanemab [5] as two new medications for AD; however, their effects on disease pathology, efficacy on symptom progression, as well as safety properties require further evaluation (addressed in the manuscript [6–8]). As important and worth noting as FDA-approval of new AD drugs, the past few decades have witnessed tremendous global efforts and studies in elucidating disease etiology, as well as generating new evidence of strategies for preventing and treating AD. Despite the mounting evidence, the etiology of AD has still not been completely elucidated. And there has been an urgent medical need to develop therapeutic agents that can modify the pathology or decrease the risk of AD.

This article attempts to collectively analyze and integrate these new findings through multiple aspects toward a better understanding of the etiology of AD. Specifically, this article will discuss AD etiology and pathogenesis, focusing on the following aspects: (1) AD pathology — from molecular abnormality to brain network impairment; (2) aging-related mechanisms of AD; (3) pathogenetics of AD; (4) associations of life exposures with AD; and (5) cellular and molecular mechanisms of AD (Fig. 1).

We would like to note that although this article covered a comprehensive collection of studies that should help

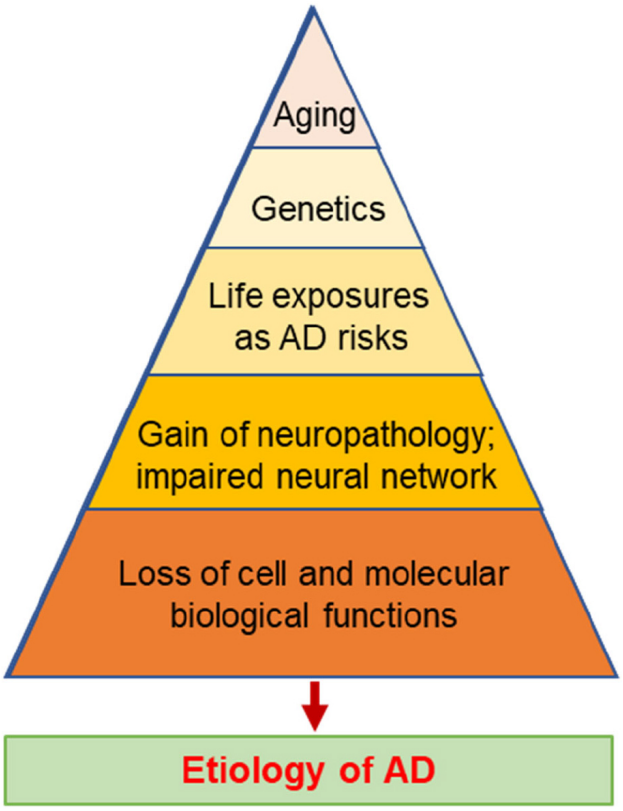

Fig. 1. Multi-level etiology of AD. Alzheimer's disease (AD) is a common neurological disorder and the major cause of dementia. The heterogeneity of AD is characterized by multi-level mechanisms, including: (1) age, (2) genetics, and (3) life exposures; a combined interaction of these mechanisms may lead to the manifestation of (4) gain of neuropathology and neural network abnormality in AD brains; and (5) loss of regular cellular and molecular biological functions. All figures were original and generated using PPT (Microsoft 365 Power-Point Slide Presentation Software).

dissect the nature of this complex disease, the field urgently requires more efforts combining different methodologies to fully capture the underpinnings of AD.

This review article not only discusses knowledge gathered from each mechanism, but also attempts to recognize findings from multidisciplinary studies, for this integrated perspective of AD. This article recognizes the importance of other recently published review articles on AD which may have focused on different aspects of the disease. For example, one article by Tatulian focused on pharmaceutical endeavors for AD and highlighted emerging technologies that may aid the diagnosis and treatment of AD [9]. Moreover, the article by Abeysinghe *et al.* [10], itemized promising therapeutic interventions for AD. Additionally, the article by Ray and Buggia-Prevot [11] highlighted different pathological features in the pathological cascade of AD and further described related therapeutic strategies beyond targeting amyloid pathology. Collectively, although AD brains share substantial manifestations of β-amyloid and tau-related disease neuropathologic hallmarks, they usually appear at the end stage of disease following many years of precipitation of pathogenic events. It is the various mechanisms in association with these pathogenic activities that lead to the heterogeneity of AD. As a complex disease, a future focus using a combination of multidisciplinary approaches on these AD-related pathogenic factors will enable novel and more precise understanding of the etiology and pathogenesis of AD.

AD Neuropathology — From Molecular Abnormality to Brain Network Impairment

Over a century ago, investigation of the neuropathology of postmortem brains by Dr. Alzheimer and others embarked the discovery of "*Alzheimer's disease*". In the current era with contemporary biomedical studies, neuropathological analyses enable comprehensive understanding of AD ranging from molecular abnormality to functional brain network changes [12,13]. The results in this domain are beginning to detect early and precise pathological changes, reflecting complex etiology and neurobehaviors, which may function as biomarkers in AD and can provide great values for disease diagnosis, prediction, and potential intervention.

Specifically, AD brains are characterized by two pathological hallmarks: amyloid plaques comprised primarily of a small peptide named amyloid-β (Aβ), and neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau. Considerable evidence supports "*Aβ hypothesis*", positing that Aβ accumulation and oligomerization is the primary pathological event, occurring initially in specific brain regions, particularly the hippocampus and cortex, which induces phosphorylated tau (p-tau) in neurons and formation of NFTs, as well as neuroinflammation, impaired neural activity, and ultimately dementia [14–20].

On the molecular and cellular basis, studies show wide heterogeneity of Aβ and tau pathology in AD pathogenesis. Aβ heterogeneity is associated with multiple mechanisms, including the proteolytic pathway for generation, accumulation and deposition, clearance and degradation, as well as the interplay with tau pathology and neuroinflammation and neural activity (Fig. 2). Aβ generation occurs through a serial cleavage of the transmembrane amyloid-β protein precursor (APP) by β- and γ-secretase; Aβ generation is precluded by α-secretase cleavage [17]. α-secretase (primarily A disintegrin and metalloproteinase domain-containing protein 10 (ADAM10)) cleavage produces sAPPα and the α-carboxy-terminal fragment (CTFα/C83); β-secretase (or BACE1) cleavage produces sAPPβ and CTFβ/C99. C83 and C99 are further cleaved by γ-secretase to produce P3 and Aβ, respectively [17]. γ-secretase is a heterogeneous protein complex containing four proteins: presenilin (PSEN1/2; PS1/2), presenilin enhancer-2 (PEN2), nicastrin, and anterior pharynx-defective-1 (APH-1) [17]. γ-secretase-processing of APP primarily determines the

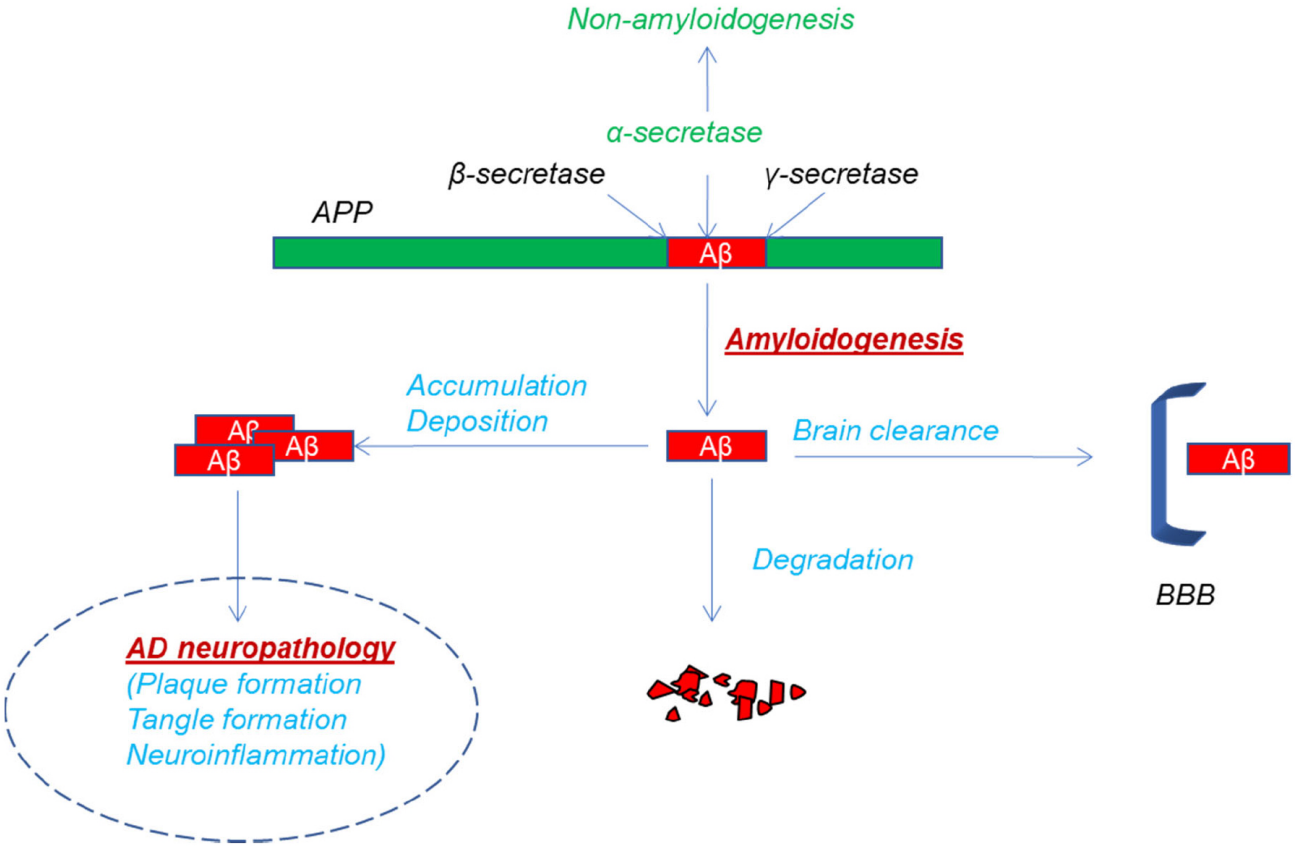

Fig. 2. Amyloid-β (Aβ) metabolism and plaque deposition in the brain are key players of AD neuropathology and underlie AD pathogenesis. Early-onset familial AD (FAD) mutations in amyloid-β protein precursor (*APP*), presenilin (*PSEN1/2*; *PS1/2*) usually increase brain A$\beta_{(42:40)}$ ratios which drives Aβ accumulation and deposition in AD-susceptible brain regions, resulting in subsequent molecular pathological changes, involving tau hyperphosphorylation, tangle formation and neuroinflammation, along with spatiotemporal abnormality and cognitive impairment with disease progression. Understanding molecular mechanisms that regulate AD neuropathology may help understand and potentially treat AD. BBB, brain-blood-barrier.

length of Aβ species. With Aβ_{42} and Aβ_{40} being the two major Aβ species in amyloid plaques, Aβ_{42} is more aggregation-prone and prevalent than Aβ_{40} in AD brains, and readily forms neurotoxic Aβ oligomers [14,16,20]. Additionally, tau heterogeneity has been reported in relationship to post-translational modifications which are linked to AD-related seeding activity, clinical outcomes, and different stages of AD [21,22]. Tau propagation may occur following seeding in AD which can be enhanced by amyloid plaques in AD, by neuronal activity or through communicating neurons [23].

Besides the analysis of amyloid and tau pathology as pathogenic hallmarks of AD, their interplay in AD etiology is recapitulated using a 3D-AD human neural culture model [15,24] and human post-mortem pathological studies [25]. Particularly, mutations in *APP* and presenilin-1 (*PSEN1*; *PS1*) drive β-amyloid which leads to tau pathology; lowering Aβ by β- or γ-secretase inhibitors attenuates β-amyloid and tau pathology; and glycogen synthase kinase 3 (GSK3) regulates Aβ-mediated tau phosphorylation in this 3D-AD human neural culture model. Furthermore, a study shows abundant soluble Aβ oligomers in synapses of early AD cases, but not those of control cases (AD symptom-free and pathology-positive), as well as late stage-dependent increase of synapse-associated p-tau in AD. These results suggest that synaptic soluble oligomers are associated with the onset of disease, which proceeds p-tau accumulation and synaptic spreading, and also suggest that an effective anti-amyloid therapy should be engaged before the development of tau pathology in AD [15,24,25].

Despite manifestation of amyloid plaques and NFTs as hallmarks in classical AD cases, AD clinical neuropathology is complex due to heterogeneous etiology which requires further classification. To better characterize AD-related complex neuropathology and advance drug development, an “ATN” binary (positive or negative) biomarker classification protocol is established and utilized [26]. The ATN system represents three primary pathological biomarkers of AD: including A for Aβ abnormality (by cortical amyloid positron emission tomography (PET) ligand binding or cerebrospinal fluid (CSF) Aβ_{42}); T for tau abnormality (by cortical tau PET ligand binding or CSF

p-tau), and N for neurodegeneration or neuronal injury (by ^{18}F-fluorodeoxyglucose (FDG) PET, brain atrophy on Magnetic resonance imaging (MRI), or elevated CSF total tau). This ATN system not only can detect AD progression or analyze complex neuropathology of AD, but also may differentiate classical AD cases with atypical AD or individuals with primary age-related tauopathy who display only NFTs without amyloid plaques. Recently, there has been interest in using plasma analytes as potential biomarkers for AD, which particularly include Aβ peptides and different forms of p-tau proteins [27–30]. Because of the approachability that is easier than PET and CSF, identification and characterization of plasma and other biofluid analytes may help early screening for AD in cognitively normal individuals and potentially improve early diagnosis of AD.

In addition to amyloid and tau pathology in AD, abnormal neuroinflammatory events play key roles in AD pathogenesis and are considered as a potential hallmark for AD (Fig. 2). Linked to the initiation and progression of amyloid and tau pathology, neuroinflammation occurs throughout the entire course of AD stages and closely interacts with progression of neuropathology and neurobehaviors, which provide potentials to understand and treat AD [19,24,31,32] (more information on this topic appears in recent Reviews [33,34]). It is characterized by impaired microglia/astrocytes and altered proinflammatory cytokines. The mechanisms by which neuroinflammation relates to AD may involve microglia-associated Aβ phagocytosis and clearance [35], tau propagation [36], and the interaction of microglia to the immunological milieux in AD brains [37,38]. Genetic studies have identified and characterized genes that affect microglia-related amyloid pathology, e.g., cluster of differentiation 33 (*CD33*) and triggering receptor expressed on myeloid cells 2 (*TREM2*) [31,39–43]. Interestingly, AD-related CD33 protein interacts with TREM2 and inhibits microglial uptake of Aβ [31,44], highlighting effects of microglia in AD. Furthermore, soluble epoxide hydrolase affects neuroinflammation and its change in AD, inhibitors of which attenuate AD neuropathology [45]. AD-related misfolded proteins can trigger innate immune responses and activate microglia and astroglia to generate and release inflammatory mediators in the brain and contribute to disease progression in AD [46]. Brain immunity may contribute to various nervous system functions, e.g., long-term potentiation (LTP), neural plasticity, and neurogenesis. Furthermore, upregulation of Receptor-interacting serine/threonine protein kinase 1 (RIPK1) in microglia and its related necroptosis may lead to AD which occurs in postmortem human AD brains in positive correlation with the Braak clinical stage; and lowering necroptosis activation by RIPK1 inhibitors reduces AD neuropathology and normalizes microglial inflammation in AD transgenic animals [47]. A recent study discovered a new PET radioligand, [(18)F]CNY-07, which enabled brain imaging of RIPK1 and has provided a valuable tool in studying RIPK1-associated neurological disorders in animals and potentially humans [48]. Additionally, as a different mechanism on neuroinflammation, NAIP, CIITA, HET-E and TP1 (NACHT), leucine-rich repeat (LRR) and pyrin domain (PYD) domains-containing protein 3/nucleotide-binding oligomerization domain-like receptor family pyrin domain containing 3 (NALP3/NLRP3) inflammasome functions in innate immunity and may respond to Aβ and affect microglial activation and subsequently tau pathology [32,49,50]. Collectively, neuroinflammation is key to understanding AD which involves different pathways, and regulating AD-related neuroinflammation has been a promising therapeutic strategy for treating AD [51,52].

In parallel to findings of molecular neuropathology in AD, the synaptic and neural network impairments in AD have been characterized using biochemical, electrophysiological, and functional imaging approaches [53]. The brain forms complex structural and neural networks (aka connectomics) with highly regulated and complex brain circuitries that perform memory and other neurobehaviors [54]. On the synaptic level, loss of presenilin leads to impaired memory functions and synaptic plasticity with age-dependent neurodegeneration. Probing brain network by functional magnetic resonance imaging (fMRI) reveals multiple interacting subsystems, primarily including the medial temporal lobe (MTL) subsystem, which provide experience-based information in the form of memories and associations and function as building blocks of mental simulation, facilitating flexible utilization of associated information. The medial temporal and prefrontal lobe subsystems integrate and form important nodes, including the posterior cingulate cortex subsystem [54]. In AD brains, molecular and functional network studies show that Aβ accumulation and progression usually occur in certain brain networks, particularly the default mode network (DMN), a conserved and large-scale brain circuit of interacting brain regions that highly correlate with one another and control memory network formation and automatic information processing, distinct from other brain circuits [55–58]. As key functional hubs located within DMN, the hippocampal formation plus entorhinal cortex and MTL are often brain regions with early Aβ accumulation in AD [55–57]. Impairment of DMN occurs early in AD and can be a biomarker for AD, consistent with amyloid accumulation in AD brains and supported by neuronal electrophysiology findings of early defects in neural network of AD [53]. Emerging data suggested that DMN connectivity is associated with computerized cognitive training, in subjects at risk for AD [59]. It is anticipated that the impaired network of AD can be normalized by potential AD therapeutics that attenuate AD neuropathology.

Above all, molecular neuropathological and brain network findings have provided insights in understanding AD and facilitated drug development for AD. In future studies, it is imperative to determine whether potential AD thera-

peutics may attenuate AD molecular pathological impairment and normalize altered network abnormality in AD brains.

Aging-Related Mechanisms of AD

Aging is an inevitable and complex physiological process and the biggest risk factor of AD. However, the mechanisms by which age links to AD are still elusive [60]. AD is not simply an advanced or exacerbated state of normal aging [61]. Aging-dependent changes may in part explain neurodegenerative areas of AD brains. In fact, the atrophy rate in specific brain regions of the elderly is higher than middle-aged adults, and the atrophy is not uniform across regions. The brain regions matured during late-stage development (e.g., medial orbitofrontal cortex) tend to display more complex structures than early maturing areas, and display higher vulnerability to age-related degeneration [62]. In addition to aging-related atrophy, AD brains may be further explained by neuroplasticity or neural adaptability which reflects structural and/or functional reorganization under internal and external stimuli. AD risk is precipitated by aging and other non-aging components, e.g., family inheritance, sex, and various life exposures, which collectively determine the status of disease risk. Although aging is the primary risk factor for AD, life exposures, which represent and include a range of different activities across the lifespan (e.g., early education, exercise, sleep, among others) have been increasingly investigated with keen interest in the field. The increased demand for neuroplasticity in cognition-related brain regions may result in increased vulnerability to age-related changes and subsequently trigger AD. Areas with higher plasticity are vulnerable to aging-related pathophysiology, linked to selective vulnerability in AD. Particularly, hippocampal neurogenesis correlates with unparalleled plasticity of the entire hippocampal circuitry and is often affected in AD [63]. The abnormality of dendritic spine plasticity in the prefrontal cortex is associated with age-dependent vulnerability and may affect memory and cognitive processes in AD. Thus, brain atrophy and neuroplasticity are among the mechanisms that account for the association between aging and neuronal vulnerability in AD, particularly during disease early stage in which cognitive decline begins when neurons are unable to compensate for the cumulative insults during aging.

On the cell and molecular levels, aging-related processes usually display multiple mechanisms, which may include epigenetic regulation [64,65], altered autophagy and increased endoplasmic reticulum (ER) stress and reactive oxygen species (ROS), among others. Aging-related mechanisms have been investigated and suggested to function as common mechanisms that may result in the progression of AD in the brain [66]. Furthermore, aging-dependent signaling pathways that link to ER stress and ROS caused by accumulation of misfolded proteins in AD have been reported, including the insulin/insulin-like growth factor 1 (IGF1) and ghrelin signaling. The insulin/IGF1 signaling pathway is responsible for energy homeostasis, stress resistance, longevity, and aging-related neurodegeneration, which is sensitively regulated and usually shows abnormality during early-stage of aging [67]. Ghrelin and ghrelin signaling pathway are associated with longevity, glucose and lipid metabolism, brain functions, e.g., learning, memory, and sleep-wake rhythm [68], as well as neuroprotection against Aβ oligomer-induced neurotoxicity [69]. Additionally, a potential mechanism may involve a transcription factor called RE1 silencing transcription factor (REST) that performs conserved functions of regulation on the excitatory-inhibitory balance of neural circuits in aged brains, which are usually dysregulated in AD brains [70]. Collectively, these cell and molecular changes reduce neuroplasticity, neurogenesis, and neuro-metabolism for orchestrating adaptions of neurons to stressors at both functional and neuroanatomical levels.

Furthermore, epigenetic-related nicotinamide adenine dinucleotide $(NAD)^+$/sirtuin pathways take part in normal aging and protect against aging-related neuronal vulnerability and AD events [71]. The sirtuins (SIRTs; silent information regulator protein types) are NAD^+-dependent deacetylases and can deacetylate N^{ε}-acyl-lysine of histones and other target proteins. SIRTs are a family of intracellular enzymes, containing seven members (SIRT1-7) with distinct intracellular localization: SIRT1/2 residing in nucleus or cytoplasm, SIRT3-5 in mitochondrion, SIRT6 in nucleus, and SIRT7 in nucleolus. In particular, SIRT1 can deacetylate both histones (H3K9 (H3 at lysine 9), H3K14 and H4K16) and non-histones (Peroxisome Proliferator-Activated Receptor-gamma (PPARγ), Nuclear Factor kappa B (NF-kB) and p53) [72] and are involved in the pathophysiology of various aging-related diseases, including cancer, metabolic diseases, and neurodegenerative disorders including AD.

From the therapeutic point of view, reversing epigenetic-related aging-dependent changes may provide regenerative benefits to AD-related neurodegeneration. Both genetic and pharmacological approaches have been research interests [64], evidenced by recent findings showing NAD^+ supplementation-related normalization of AD features in animals [73] and chemical-induced reprogramming to reverse cellular aging [74]. Furthermore, SIRT1 has attracted considerable attention as a therapeutic target for AD and other disorders. SIRT1 activators, e.g., resveratrol (a natural activator) and SRT2104 (a synthesized activator) have been tried for diabetes/obesity [75]. SIRT1 inhibitors have been evaluated for anticancer therapies. And Ex-527 (Selisistat) is a small molecule that is highly potent and selective in inhibiting SIRT1 (IC_{50} = 0.098 μM), which provides a candidate PET ligand for imaging SIRT1 in the brain [76]. Recently, a study reported a new molecular imaging probe for SIRT1 [76], which may enable visualization of

age-related changes in the brain and can be utilized in combination with amyloid and tau and other imaging probes to further elucidate longitudinal and neuroanatomical changes of AD.

Pathogenetics of AD

As a genetically complex disease, the genetic basis of AD has been extensively investigated over the past several decades through the primary unbiased and "top-down" approach. Specifically, the effort of AD genetics with the focus on discovering disease genes and genetic variants of AD has resulted in new insights for understanding AD pathogenesis and advancing drug development of AD (more information on this topic can be found in a recent Review [77]). The influence of genetic factors in combination with aging and other AD risk factors may greatly vary among individuals and collectively lead to the magnitude of AD pathogenesis, being linked to complex neuropathology, cognitive decline, and progression to AD. The genetic heritability and its association with AD are described below.

Early-Onset AD (EOAD)

The EOAD usually occurs in individuals younger than 65 years of age. It consists of familial AD (FAD) (a small percentage of EOAD) and other EOAD with genetic basis remaining to be identified. FAD is characterized by autosomal dominant mutations in *APP*, *PS1*, and *PS2* [14,16,20]. These fully penetrant and pathogenic FAD mutations account for approximately 10% of all the heritability in EOAD, suggesting the involvement of other genetic variants in this AD subtype [78]. The breakthrough of identifying disease genes of FAD leads to the "amyloid cascade hypothesis" of AD [20], which has been developed and extensively tested. Earlier studies are primarily focused on cellular and molecular mechanisms of Aβ and tau metabolism in AD without particularly considering neuroinflammation. Current studies have broadened insights of AD based on prior discoveries and are characterized by integrated analyses on mechanisms and interactions involving Aβ, tau, and neuroinflammation by neurons, microglia, and astrocytes, as well as unraveling AD risk factors and brain biomaterials [79–83].

Because FAD guarantees absolute penetrance of AD onset in individuals expressing these mutations, analysis of these genes has led to mechanistic findings of AD in using preclinical studies of cell and animal models expressing these mutations. For example, various cell models of AD have been extensively developed and reported [15] (more information on this topic appears in a recent Review [84]). Additionally, rodents that express $APP^{Swedish}$ (or Tg2576) [85] or $APP^{Swedish/Florida/London}$ and $PS1^{M146L/L286V}$ (or 5XFAD) [86] have provided useful models to study amyloid neuropathology of these animals. Age-dependent APP upregulation in astrocytes occur in AD transgenic animals [87,88], supporting mechanisms of astrocytes in AD [89]. With AD progression, astrocytes may respond to β-amyloid plaques by changing their morphology to a reactive appearance. Furthermore, late-stage AD-related astrocytes display an impaired ability in Aβ uptake and neuroprotection. While neurons and glia all express APP, APP proteins in neurons seemed to be more likely to be proteolytically cleaved with more Aβ generation than those in glia. In addition to AD, astrocyte expression of APP is upregulated following brain injury. APP is not necessarily related to reactive gliosis because reactive gliosis occurs in APP-deficient mice. Besides APP-related functional changes in astrocytes, a recent study reported that microglial APP may regulate abilities to acquire a proinflammatory phenotype related to AD [90].

In addition to the gain of pathological functions due to FAD mutations, the etiology of AD may associate with a loss of physiological functions or impaired functions in APP and PS1/2 [91]. Their functions may display Aβ-independent mechanisms, e.g., abnormalities in autophagy and proteasome-lysosomal functions, intracellular signaling and synaptic dysfunction, and neurotransmitter release with neurodegeneration. Although independent of Aβ, these mechanisms may indirectly precipitate Aβ-induced neurotoxicity and further aggregate AD progression. Several studies have reported pathophysiological functions of APP and PS1 in AD in addition to Aβ generation. For example, a co-expression of APP and cell death mediator p75 neurotrophin receptor (p75-NTR) triggers cell death and mediates selective neuronal vulnerability in AD. Besides, PS1 regulates the neuronal threshold to excitotoxicity, and deficiency of *PS1* increases calcium-related vulnerability of neurons to oxidative stress.

Late-Onset AD (LOAD)

In contrast to EOAD, LOAD (or sporadic AD; SAD), usually occurs over the age of 65 years and is associated with polymorphism in the apolipoprotein E gene (*APOE*). *APOEε2*, *APOEε3*, and *APOEε4* are the three common alleles in *APOE*, encoding APOE2, APOE3, and APOE4, respectively. The *APOEε2* is related to a reduced AD risk, and the *APOEε4* confers an increased AD risk comparing to the *APOEε3* [92].

Human APOE protein contains 299 amino acids following the cleavage of the 18-amino acid signal peptide. The polymorphism of *APOE* for AD is primarily originated from two single nucleotides causing two amino acid residue differences, which include rs429358 (related to Cys112Arg), and rs7412 (related to Arg176Cys). Correspondingly, APOE protein isoforms differ based on these two amino acid residues (Cys112 and Cys158 for APOE2; Cys112 and Arg158 for APOE3; Arg112 and Arg158 for APOE4). The frequency of *APOEε4* is approximately 40% in LOAD patients, and the presence of *APOEε4* markedly decreases the age of AD onset. *APOEε4* carriers and particularly those of LOAD patients exhibit higher atrophy in

hippocampus and surrounding temporal regions [93]. In LOAD, APOE protein is highly expressed in the brain, primarily in astrocytes. In the central nervous system (CNS), APOE is produced by astrocytes, microglia, choroid plexus cells, and vascular mural cells, and performs multiple biological functions, including lipid transport and cerebrovascular function, glucose metabolism, synaptic integrity, and microglial homeostasis [92].

Regarding mechanisms, *APOEε4* may associate with AD through different pathways, although the complete mechanisms have not been elucidated (more information on *APOE* in AD can be found in a recent Review [94]). APOE4 protein may increase amyloid neuropathology of AD by promoting Aβ aggregation and inhibiting Aβ clearance [95]. Notably, there has been longtime interest in studying the direct binding of APOE and Aβ over the past years focusing on the pathogenesis of AD as well as the potential toward developing therapeutics by disrupting the binding of APOE and Aβ associated with AD (this topic is covered in a recent Review [96]). Particularly, the binding of APOE to Aβ displays isoform-specific properties with highest binding identified in APOE4 among the isoforms. Also, it has been shown that the binding of APOE to Aβ is associated with Aβ conformation. In addition to mechanistic studies, strategies that modulate APOE-Aβ binding for therapeutic purposes have been investigated, which has been tested in both model systems and humans [97]. Interestingly, a recent study showed that PS is essential for APOE secretion and *PS* mutations affect APOE secretion in AD, suggesting a novel mechanism linking PS and APOE in FAD and SAD pathogenesis [98]. The neuronal APOEε4 is associated with increased cell death and phosphorylation-related tau pathology [99]. Both *APOEε4*-genotype and *APOE* deficiency display impaired hippocampal neurogenesis [100]. *APOEε4* is related to dysfunctional γ-aminobutyric acid (GABA)-expressing interneurons in the hippocampus [101]. Because of the diverse physiological functions of APOE, *APOEε4* may lead to LOAD by loss-of-function mechanisms and its interaction with several other life exposures, such as hypertension, diabetes, and depression [102]. Furthermore, *APOEε4* is associated with the vulnerability of cerebral regions beyond the medial temporal areas in EOAD patients, and simultaneously seems to predispose LOAD patients to vulnerability in the MTL region.

In addition to *APOE*, variants in many other genes (e.g., Adenosine triphosphate (ATP)-binding cassette, subfamily A, member 7 (*ABCA7*), Bridging integrator 1 (*BIN1*), CD2-associated protein (*CD2AP*), *CD33*, Clusterin (*CLU*), Complement C3b/C4b Receptor 1 (*CR1*), Erythropoietin-producing hepatoma (EPH) receptor A1 (*EPHA1*), membrane-spanning 4-domains subfamily A (*MS4A*), Phosphatidylinositol binding clathrin assembly protein (*PICALM*), Sortilin-related receptor 1 (*SORL1*), and *TREM2*, among others) have been recently identified and characterized [103–106] in genome-wide association studies (GWAS) or whole-exome and whole-genome sequencing. Functional investigation of these genes shows heterogeneous pathological mechanisms in AD, e.g., neuroinflammation [31], Aβ toxicity and endocytic trafficking, changes of mitochondrial respiration and protein synthesis in amyloid-vulnerable regions, and low expression of genes for neural plasticity in neurodegeneration-vulnerable regions.

Epigenetic Mechanisms of AD

Epigenetic factors regulate gene transcription in response to endogenous and environmental changes with widespread aging-dependent remodeling properties [65, 107] and may provide insights for AD [108–110]. Epigenetic regulation of biology may occur through mechanisms involving DNA methylation and variation, histone modifications and miRNA regulation. DNA methylation in early life can impact the susceptibility to oxidative DNA damage in aged brains. DNA methylation in AD-susceptible genes, e.g., *ABCA7*, *BIN1*, Major histocompatibility complex class II, DR beta 5 (*HLA-DRB5*), *SORL1*, and Solute carrier family 24, member 4 (*SLC24A4*), is associated with neuropathology of disease [111]. A recent study profiled genome-wide methylomic variations leveraging methylomic, epigenomic, transcriptomic, and proteomic data from post-mortem brains, which found that methylation is a key mechanism that regulates both gene and protein networks of AD [110]. Furthermore, microRNA 126 (miRNA-126/miR-126) displays an association of neuronal vulnerability in the presence of neurotoxicity [112]. An overexpression of miR-29a significantly reduces the ischemic injury in the brain, especially in the hippocampal CA1 area. In addition, the proportion of aneuploid neurons and neuronal DNA content variations increase in AD, which may result in neuronal death in selective brain areas [113]. Mitochondrial DNA variations may also occur and enhance neuronal vulnerability in AD [114].

Furthermore, investigators speculated and tested that targeting epigenetic regulation can be a therapeutic approach for AD [107,115]. For example, a recent focus is on the bromodomain containing protein 4 (BRD4), which belongs to the bromodomain and extra-terminal (BET) protein family. BRD4 acts as an epigenetic regulator that regulates gene transcription [116]. JQ1 is a well-studied inhibitor for BRD4, by binding the central benzodiazepine receptor within BRD4, and improves cognitive performance in AD transgenic mouse models. The potential of targeting BRD4 for understanding AD requires future studies [117].

Associations of Life Exposures with AD

Like other common and complex diseases (e.g., cancer, cardiovascular diseases, and stroke) that have a multitude of causes, AD is also regulated by different components in lifespan, which can be evaluated in association with

AD risk and pathological changes in individuals of different populations. Life exposure-related components, both endogenous and exogenous, have been discovered that may increase or reduce the risk of AD. Below we will introduce common and compelling risk factors of AD, and then extend our discussions focusing on microbiome, sleep and peripheral mechanisms because of emerging data in these domains, which we believe should collectively suggest a wide range of the heterogeneity of AD and help elucidate the etiology of AD (Fig. 1).

Risk Factors of AD

In addition to aging and genetics as the major and common risk factors for AD, sex shows significant differences in AD which contributes to neuropathological abnormalities [118,119]. For example, a study was performed using a longitudinal and clinicopathologic cohort and reported sex differences in the clinical manifestations of amyloid and tau pathology in AD brains, with more pathology in women than in men [120]. Additionally, a recent study identified novel loci (Glutamate ionotropic receptor delta type subunit 1 (*GRID1*), RIO kinase 3 (*RIOK3*), Microcephalin 1 (*MCPH1*), Zinc finger and BTB domain containing 7C (*ZBTB7C*)) showing sex-specific association with AD risk through the family-based association analysis of whole-genome sequence data [121]. Because sex is an important factor for AD, more studies are urgently required to characterize sex-specific clinicopathological features of AD which may help advance patient stratification and personalized treatment of AD [119].

Furthermore, several modifiable risk factors of AD have been identified, which consist of sleep [122], diet [123], stress, education, low cognitive activity, and other non-AD conditions and diseases, including orthostatic hypotension, hypertension in midlife, head trauma, hyperhomocysteinemia, high body mass index in late-life, diabetes, and depression [83]. Extensive research supports that diet plays substantial functions to AD-related pathophysiology and may significantly change the risk of AD [124–127]. For example, a recent study investigated fasting-mimicking diet (FMD) cycles, which has been previously shown to reduce several aging-related disease risks, and found that FMD significantly attenuated cognitive decline in Alzheimer's models expressing amyloid and/or tau pathology through mechanisms involving reduced neuroinflammation and/or superoxide production in the brain [126].

Moreover, many studies show that physical and cognitive exercise as well as social activity are also important factors in association with AD which deserve close attention. Physical exercise is a key event that may significantly reduce the neuropathology of AD and improve cognitive functions associated with AD [128]. Exercise-related underlying molecular mechanisms are complex and are beginning to be elucidated, which may involve regulating neurogenesis in the brain as well as modulating brain and peripheral circulating cytokines or neurotrophic factors, e.g., brain-derived neurotrophic factor (BDNF), clusterin, Interleukin-6 (IL-6), IGF-1, and irisin [129–133]. For example, a recent study focused on identifying and characterizing new secreted mediators that may confer exercise-related cognitive benefits in AD model animals [131]. It discovered that irisin is a crucial regulatory molecule offering exercise-related cognitive benefits, which thus can be a potential therapeutic for treating AD and potentially other cognitive disorders [131]. Furthermore, cognitive exercise, e.g., by meditation or computerized cognitive games, has been assessed with beneficial effects in improving cognitive functions [59,134,135]. The underlying mechanism of action has been shown to associate with modulating brain connectivity, as well as plasma Aβ proteins and cytokines [59,134,135].

In addition to physical and cognitive exercise, the association of social activity with AD and molecular phenotypes has been investigated. A study by Wilson *et al.* [136] reported that loneliness is a significant factor of an increased risk of AD. This study, in combination with the other article, suggested that a trial may be designed to reduce loneliness and potentially reduce the risk of AD [137]. Indeed, a mindfulness-based stress reduction training was performed in older adults through a randomized controlled trial (RCT), which reduced loneliness and affected pro-inflammatory gene expression profiles, including NF-kB-related gene expression in circulating leukocytes [138]. Collectively, physical and cognitive exercise as well as social activity are all significant players that regulate brain functions and the risk of AD using inflammatory regulation and several other mechanisms. These findings warrant future studies based on these molecular mechanisms to develop potential therapeutics of AD.

Also, certain medical exposures (non-steroidal anti-inflammatory drugs) and specific dietary exposures (folate, vitamin E and C, and coffee) are found to be protective factors of AD [139]. Recently a systematic and meta-analysis was able to strengthen the association of age-dependent life components and AD risk based on 243 observational prospective studies and 153 randomized controlled trials [83]. The underlying mechanisms of these modifiable exposures require to be further characterized in association with other common AD risk factors, including aging and genetics. Notably, these factors are considered modifiable endogenous or internal exposures which can be managed during different stages in lifespan with a potential to maintain healthy brains and ultimately reduce AD risks or prevent AD [140].

In addition to the aforementioned endogenous factors, exogenous or external factors also exist that can alter AD risks. Air pollution may increase AD risks by inducing neurochemical, pathological, and cognitive changes [141]. In combination with air pollution, other environmental components have been identified which act together as an "en-

vironmental exposome" and affect individuals' AD risks by interacting with aging and other endogenous factors [142]. An animal-based study showed that life exposure is able to reduce AD-related and Aβ oligomer-induced impairment of hippocampal neuroplasticity [143]. Specifically, increased exposure to novelty in animals activates β-adrenergic signaling and enhances hippocampal synaptic plasticity and attenuates synaptotoxicity in the presence of Aβ oligomers [143]. The exposome concept has been conceived to assess lifetime internal and environmental exposures of individuals in relationship to health and complex disease [144]. To study the exposome of AD combining exogenous and environmental exposures, the analysis by Mendelian randomization has been conducted, and validated previously known exposures linked to AD [145]. New exposures that change AD risks are anticipated to be identified in future studies in the presence of new results gathered for analysis. Above all, these results suggest that family inheritance, including early onset and sporadic AD genetic variants, may work together with life exposures in age-dependent manners and determine the ultimate AD risks of different individuals.

Microbiome and AD

Recently, there has been a keen interest on the role of the human microbiome on the pathophysiology of AD. Consisting of the bacterial, viral, and other residents of the human body, the human microbiome is a substantial lifelong player for controlling human pathophysiology. It particularly affects the immune and metabolic system in the gut, as well as the brain, which is referred as the "microbiota-gut-brain axis" for such interacting functions [146].

The discovery of the involvement of the human microbiome in AD began several decades ago with the identification of a number of microbes in AD brains, including *Borrelia spirochetes* [147], *Chlamydia pneumonia* [148], and herpes simplex virus type 1 (HSV-1) [149], among others. The clinical relevance of gut microbiota in AD has recently been analyzed in humans [150]. Increased antibody titers specific for HSV-1 are found present in the serum of early AD cases and positively correlate with brain grey matter volumes, suggesting protective mechanisms of neuroimmune activity [151]. HSV-1 DNA is distributed mainly in the hippocampus and temporal cortex in human postmortem brains [152]. HSV-1 may mediate a transsynaptic and interneuronal cascade for propagating aggregated proteins in AD brains [149]. A study for the bacterial taxonomic composition of fecal samples demonstrates distinct composition and decreased microbial diversity comparing AD patients to age- and sex-matched controls [150]. Particularly, differences in bacterial abundance are represented by decreased *Firmicutes* and *Bifidobacterium* and increased *Bacteroidetes*, and levels of differentially abundant genera are correlated with CSF biomarkers of AD [150].

To further investigate the mechanisms by which microbiome changes the outcome of AD, a series of studies have attempted to manipulate microbiome in animals. First, gut microbiota significantly affects AD neuropathology, in which gut-administrated antibiotics result in attenuated amyloid neuropathology, altered microglial morphology, and reduced plaque-localized glial reactivity in brains of AD transgenic animals, in association with prolonged shifts in gut microbial composition and diversity and altered circulating cytokine levels and chemokine signatures in the blood [153,154]. Next, microbial infection (e.g., herpes viral infection) may promote Aβ amyloidosis in brains of AD transgenic animals [155,156]. Hippocampal and neocortical neurons are highly vulnerable to HSV-1 infection in animals [157]. Furthermore, toll-like receptor-4 (TLR4) and pattern-recognition receptors (PRRs) signalings may sense microorganisms in association with differential neuronal vulnerability in the presence of Aβ-related neurotoxicity and oxidative stress in AD brains [158]. The gut microbiota can synthesize and release lipopolysaccharides (LPS), which may activate inflammatory signaling and trigger pathological changes in AD-susceptible brain regions, particularly the hippocampus [159].

Overall, these findings provide evidence suggesting AD as a disease linked to microbial involvement. Gut microbiota may not only affect innate immunity in the gut, but also regulate the systemic immune and metabolic status through the gut-brain crosstalk and, by extension, neuroinflammation [160]. These microbes may act as exogenous exposures to gain access to human bodies and become endogenous exposures, in early and late stages of life and may lie dormant for many years. They may associate with AD by regulating the host immune system in response to microbes during microbial reactivation, which may lead to neuronal damage in aging-related AD brains or conditions with reduced neural immunity.

Sleep, Circadian Clock, and AD

Sleep is a physiological process required for human health, cognitive performance, and mental health. Although the mechanisms controlling human sleep remain poorly understood, sleep has been repeatedly identified as one of endogenous life exposures in significant association with AD [83,161–163]. Notably, sleep is a key player for memory formation and consolidation [164] which primarily occurs in the hippocampus [165]. Sleep is regulated at neuroanatomical, molecular, genetic, and circadian clock levels. Notably, emerging evidence showed strong contribution of thalamic reticular nucleus (TRN) underlying the etiology of sleep impairment in AD [161]. Specifically, TRN regulates sleep maintenance, initiation of sleep spindles, and slow-wave sleep (SWS) using its GABAergic neurons [166,167]. It contains calbindin ("core") and somatostatin ("shell")-expressing GABAergic neurons, which differ neuroanatomically and differentially affect sleep spin-

dles and bouts [166,167]. While TRN displays reduced activity in AD, restoring TRN activity by DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) improves sleep impairment and ameliorates AD pathology in AD transgenic mice [161,168]. Presently, further mechanistic studies of TRN in sleep impairment-related AD are required.

On the molecular basis, recent studies have linked abnormality of Aβ clearance to sleep in AD [122,169]. As one of the brain's metabolic wastes, Aβ is removed from the brain by the glymphatic system, mostly during sleep [122,170]. Accordingly, insomnia is associated with impairment of glymphatic system-mediated Aβ clearance and hippocampal atrophy [122]. Aβ-related neurotoxicity causes neuronal hyperexcitability that can further drive cellular Aβ release. Thus, abnormality of sleep, such as aging-related reduced sleep quality and sleep deprivation, as well as sleep disorders, may increase AD risk through lowered Aβ clearance and associated neurotoxicity and hyperexcitability in AD brains [162,171].

Sleep is governed by circadian clock, an endogenous and conserved 24-hour cycle timing system that regulates immune system and other functions [172]. Alzheimer's Aβ may result in circadian rhythm disruption in AD by inducing responsible proteins, i.e., Basic helix-loop-helix ARNT like 1 (BMAL1) and CREB-binding protein (CBP) [173]. In addition to aging-related functions aforementioned, SIRT1 is an NAD^+-dependent circadian deacetylase that governs circadian control by regulating transcription of the major circadian regulators, BMAL1 and Clock circadian regulator (CLOCK) [174]. The activity by which SIRT1 regulates circadian control decays with aging [174] and can be reversed by supplementation of NAD^+ [175]. Furthermore, SIRT1 affects neurodegeneration, and hippocampal SIRT1 levels fall in AD transgenic animals [176]. These data support a mechanism by which Aβ drives circadian rhythm protein abnormality and SIRT1-related reduction and circadian rhythm disruption in AD.

Furthermore, sleep genes have been discovered that control sleep/wake duration and human sleep length, which encode, respectively, the transcriptional repressor DEC2 (aka Basic helix-loop-helix family, member E41 (BHLHE41)) [177,178] and the β1-adrenergic receptor (ADRB1) [179]. Humans with mutations in DEC2 or ADRB1 display short sleep phenotype [179], partly due to changing levels of the neuropeptide hormone orexin [178]. Transgenic mice with these human mutations show short sleep behavior [179].

In summary, these results provide evidence that AD is partly a sleep disorder linked to age-dependent impaired circadian control. These genetic and molecular findings on sleep/wake require further studies which may lead to sleep-orientated strategies that can potentially attenuate sleep impairment in AD and enhance brain health and cognitive performance.

Mechanisms of AD from Peripheral Abnormality to Neuropathology

Emerging evidence shows that AD pathology in the brain can be caused by factors from the peripheral systems (systems that are indirectly related to the brain), thus providing pathogenic mechanisms and therapeutic potentials for AD.

Notably and as afore-described in this article, life exposure-related conditions and components may modulate the risks of AD in humans, including cardiovascular systems, hyperhomocysteinemia, high body mass index (BMI) in late-life, diabetes [83,139], and gut microbiota, which interact with aging and affect the neuropathology of AD [153,154]. Inflammation is a mechanism by which the peripheral factors cause AD-related changes, which can lead to both acute and chronic impairment of cognitive functions underlying AD and other neurodegenerative disorders [180–182]. AD-related peripheral factors may lead to changes of peripheral inflammatory events which then engender an inflammatory microenvironment in brain regions responsible for impaired memory and cognitive functions. Once stimulated, peripheral inflammatory cytokines can enter the brain through a defective BBB (brain-blood-barrier) and get recruited to activated microglia and astrocytes [182]. At the neuroanatomical level, the MTL is a susceptible brain area in AD which highly expresses receptors and messenger RNA for pro-inflammatory cytokines with special sensitivity to inflammation [183]. Particularly, the hippocampus is vulnerable to the increase of the IL-1β level, linked to a reduction of adult hippocampal neurogenesis and cognitive impairment [184], as well as age-dependent reduction of LTP in hippocampus and dentate gyrus [183]. In addition to inflammatory molecules, migration of blood immune cells from the peripheral to the brain may occur in AD [37] or peripheral organ inflammation [185], in response to Tumor necrosis factor (TNF)-α signaling [185].

In addition to the mechanism of peripheral inflammation in AD, abnormality of protein clearance is a mechanism by which the peripheral system may contribute to AD. Aβ metabolism has been comprehensively characterized to be regulated by multiple pathways, including generation (β-/γ-secretase-mediated APP processing), degradation (by Insulin-degradation enzyme (IDE)), oligomerization, aggregation, and propagation, as well as clearance. Following generation, clearance of Aβ, tau, and other brain wastes may occur by different mechanisms in the brain. They may enter into peripheral circulation from BBB through various transporters and receptors or get degraded by BBB in vascular smooth muscle and endothelial cells [186]. BBB-related P-glycoprotein affects the elimination rate of neurotoxic proteins, the activity of which is reduced in AD [187]. BBB-associated low-density lipoprotein receptor-related protein1 (LRP1) can also mediate transport of Aβ out of the brain in association with AD [188]. Brain Aβ

clearance can also be through the glymphatic system, which is located in the perivascular space surrounding cerebral blood vessels [186]. Once transported outside of the brain, brain-derived Aβ and potentially other wastes maintain homeostasis by further clearance in the peripheral tissues and organs [189], including the kidney [190]. Pathologically, the kidney function abnormality is associated with AD [190]. Thus, studying the pathophysiological capacity of peripheral tissues and organs in clearing brain-derived Aβ is opening a novel avenue to understanding the etiology of AD [189,191].

Cellular and Molecular Mechanisms of AD

AD-Related Disease Protein Seeding, Aggregation, and Spreading

AD is a disease of protein aggregation and is characterized by cerebral proteinopathy caused by composition, deposition, and distribution of misfolded proteins [192] as well as propagation of amyloid and tau pathology. Aβ generation and metabolism display complex mechanisms and are highly regulated [117,193]. Following generation, Aβ homeostasis is maintained by multiple mechanisms in combination with degradation, which occurs at both intracellular and extracellular levels, before seeding into pathogenic proteins and subsequent aggregation and spreading. Intracellularly, the common degradation mechanisms consist of autophagy, lysosome, and ubiquitin-proteasome system; extracellularly, the degradation pathways involve plasmin, IDE, and neprilysin [186]. Aβ is transported across and/or diffuses into the extracellular space of the brain before aggregation [194]. Cerebral amyloidogenesis of AD may even derive from transmission of peripherally originated Aβ (e.g., Aβ-containing brain extracts) in animal models [195].

Prior to manifestations of clinical neurobehavioral changes, AD-related pathological proteins, in the presence of unbalanced homeostasis, may first seed and aggregate in local regions with cerebral vulnerability and then spread to other brain areas [192]. The spatial and temporal distribution of AD disease proteins manifest heterogeneous and complex mechanisms related to neuroanatomy, BBB and neurovascular system, and neuroimmune system [196]. The initiation of cerebral amyloidogenesis is determined by the agent and the host [197]. Reactive astrocytes may occur in an age-dependent manner with upregulation of astrocytic APP expression and Aβ generation [88], and can mediate the spreading of AD-related Aβ and tau proteins [198]. At the neuroanatomical level, oligomeric and fibrillar Aβ species are associated with brain regional differences [199], and amyloid pathology spreading may occur at the temporobasal and frontomedial areas and the remaining associative neocortex, primary sensory-motor areas, the MTL, and striatum [200].

Furthermore, propagation of AD-related tauopathy has been well-documented, which can first occur in individual neurons of local brain areas and then transmit to other neurons at different brain areas [201]. Pathologic tau transmission may display prion-like properties in animal studies [202] with different biochemical forms (e.g., soluble monomeric or fibrillar tau) [203]. Tau proteinopathy is involved in several clinical conditions other than AD; however, specific clinical features of tauopathies seem to be dependent on disease-specific tau proteins that propagate in these diseases [204]. Different tau fibrillar strains may induce specific pathologic changes in mouse brains, particularly the hippocampus which displays high vulnerability [205]. Enhanced neuronal activity can increase the release of tau proteins *in vitro* and enhance tau propagation *in vivo* [206]. Neuroanatomically, spreading of AD-related tau pathology partially overlaps with amyloid pathology and may occur from medial temporal regions/entorhinal cortex-hippocampal system to the basal and lateral temporal cortices, inferior parietal cortices, posterior cingulate cortices, and other associative cortices [207]. Finally, Aβ can accelerate tau propagation in early stages of AD [208].

Association of γ-Secretase with Amyloid Pathology and Neuroinflammation

Collective findings suggest AD being a protein aggregation and proteinopathy disorder which is driven by age-related upregulation of A$\beta_{(42:40)}$ ratios [14,16,20]. Therefore, understanding mechanisms that increase A$\beta_{(42:40)}$ ratios can help to understand the etiology of AD. Molecules that preferentially decrease Aβ_{42} levels may lower protein aggregation in AD and become promising therapeutics. γ-Secretase in AD controls the length of Aβ proteins and A$\beta_{(42:40)}$ ratios and therefore has been a prime focus for disease understanding and intervention. As a heterogeneous protein complex, γ-secretase contains the presenilin (PS1/2) proteins that harbor the active site enabling proteolytic processing of its substrates, as well as PEN2, nicastrin, and APH-1 [17]. Over 300 FAD mutations in presenilin-encoding genes have been identified [14,16,20]. PS1 holoprotein lacks the proteolytic activity and requires endoproteolysis to generate the active subunits, i.e., PS1-NTF (N-terminal fragment)/CTF (C-terminal fragment), which regulate the intramembrane proteolysis of APP and other substrates, including Notch [17].

Therapeutically, γ-secretase is a prime therapeutic target which has investigated primarily through two strategies, either inhibiting or modulating γ-secretase [209–213]. Small molecule inhibitors of γ-secretase are represented by avagacestat and semagacestat, which reduce Aβ_{42} production, however, have caused serious adverse events such as skin cancer and worsened functional ability in participants of clinical trials in addition to being clinically ineffective in improving cognitive status [214]. These adverse safety issues may be associated with gamma-secretase in-

Table 1. Translational medicine of AD: advancing from etiology to potential therapeutics of AD.

From Etiology to Therapeutics of AD	
Genetics	• FAD genes: *APP*, *PS1*, *PS2* • LOAD risk genes: *APOE* and other candidate genes
Antibodies	• Aducanumab, Lecenumab, and Donanemab
BACE1i	• Verubecestat: Lack of efficacy and adverse safety issues
GSI	• Semagacestat: Lack of efficacy and adverse safety issues
GSMs	• Small molecule GSM776890
Other potential therapeutics (examples)	• New druggable targets (e.g., epigenetic molecule) • New strategies (e.g., neuronal stimulation, repurposing of non-AD therapeutics of AD)

Understanding the molecular pathogenesis of AD has started through discovering disease genes and elucidating mechanisms by which disease genes associate with AD, which has resulted in finding a series of different strategies that may reduce AD neuropathology and cognitive abnormality. While some of these therapies have shown cognitive benefits in clinical trials (e.g., antibodies), some displayed a lack of efficacy and even adverse effects (e.g., small molecule BACE1i (BACE1 inhibitor) and gamma-secretase inhibitor (GSI)), and there are some other new targets and potential therapeutics that have yet to be further tested. GSM, γ-secretase modulator; FAD, familial AD; *PS1*, presenilin 1; *PS2*, presenilin 2; LOAD, Late-onset AD; *APOE*, apolipoprotein E gene.

hibitor (GSI)-related increase of APP-CTFs and impaired γ-secretase cleavage of substrates other than APP [215]. In addition to GSIs, BACE1 inhibitors have also been developed and tested which however displayed a lack of clinical efficacy and adverse safety issues [216,217].

Different from small inhibitors of β- or γ-secretase, γ-secretase modulators (GSMs) are a group of small molecules that specifically modulate γ-secretase cleavage of APP and preferentially lower Aβ_{42} levels and A$\beta_{(42:40)}$ ratios, without affecting γ-secretase cleavage of non-APP substrates or increasing levels of APP-CTF proteins [15, 209,210,212,218–220].

Furthermore, in addition to Aβ generation, γ-secretase-related neuroinflammation has been recently discovered. Using GSM molecules, one study uncovered interferon-induced transmembrane protein 3 (IFITM3) as a γ-secretase modulatory protein [80], which stimulates γ-secretase in the presence of neuroinflammation, resulting in increased Aβ levels and AD risk. The complex heterogeneity of γ-secretase in Aβ generation is reported *in vitro* [221] and *in vivo*, indicated by age-dependent changes in Aβ accumulation in animal brains [79]. These findings add new evidence to the heterogeneity of Aβ, agreeing with previous findings showing γ-secretase changes in sporadic AD dependent on age and sex in mouse brains [222].

The correlation of γ-secretase with both early onset and sporadic AD suggests a potential use of positron emission tomography (PET) to visualize γ-secretase for analyzing and predicting AD in human brains. As a non-invasive imaging method, PET can visualize and analyze biochemistry-based molecular targets in inaccessible tissues, e.g., the brain, and therefore provide distinct advantages for studying AD and other neurodegenerative disorders [108,223]. It is in contrast with *in vitro* techniques that cannot provide analyses of system-level intact organisms over time in endogenous environment. PET imaging has been developed and widely utilized to evaluate AD progression in preclinic and clinic settings, including ligands for Aβ plaques [224] and NFTs [225]. Molecular imaging probes for β- [226] and γ-secretase have been reported in preclinical models [227]. Since γ-secretase has a substantial role in AD, imaging γ-secretase in the brain may improve the drug development and a potential success of AD trials. A recent study discovers an GSM-based γ-secretase probe which enables, for the first time, visualization of γ-secretase in animal brains, with a potential enabling brain imaging in humans [227]. Collectively, the results on γ-secretase provide evidence suggesting that AD is a disease linked to γ-secretase, genetically and biochemically, which also offers therapeutic potentials for targeting γ-secretase.

Mechanism-Based Translational Medicine to Test AD Pathogenesis

A successful treatment may better explain disease etiology, which is considered as a “bottom-up approach”. For example, because the recently FDA-approved medications for AD, including aducanumab [4] and lecanemab [5], as well as the drugs under ongoing trials, e.g., donanemab [228], are designed to reduce amyloid patholgy, their effects will be useful to comprehensively test the association of neuropatholgy with symptom progression of AD (Table 1). More detailed information on ongoing clinical trials can be found in a recent Review [229]. In addition to the efficacy of antibody-based anti-Aβ drugs, the safety should be carefully monitored which may associate with adverse effects caused by amyloid-related imaging abnormalities (ARIAs) and brain atrophy [230]. Additionally, because PS1/γ-secretase-related FAD genes cause complete penetrance of disease, the etiology of AD can be potentially addressed by Clustered regularly interspaced short palindromic repeats (CRISPR)-based genetic correction of disease mutations. This genetic approach is becoming possible with the technology advancement in the field of genetic editing. Alternatively, GSM-based therapeutic ap-

proach for γ-secretase also provides a promising potential for disease treatment in clinic [15,209,212,218–220,227]. Furthermore, there are other approaches that can offer therapeutic potentials for AD, e.g., neuronal stimulation [231], or small molecule pharmacological agents that may work either by epigenetic regulation [108,232,233] or by repurposing non-AD drugs for AD [13,52] (Table 1).

Perspectives of AD Translational Research

Before reaching the end of our article, we would like to particularly describe our perspectives on the translational research of AD, for its current problems and challenges, and will then speculate future directions needed for the successful drug development of AD.

Through our present review article, we may realize that developing useful therapeutics through AD clinical trials may go through a process of trial and error, because of the multi-level heterogeneity of this extremely complex disease. The heterogeneity of AD etiology across the disease continuum may bring the major issues and challenges of current translational studies of AD in the field, which are primarily lacking adequate and suitable tools to precisely stratify patients and analyze AD changes with high spatiotemporal resolutions. Therefore, we speculate that the future of successful AD research and drug development, may require more efforts focused on developing useful tools and methods that may readily enable molecular biomarker discovery from the brain or the biofluid and can allow early and prompt analysis of dynamic changes of AD pathology and brain functions. For example, despite the wide clinical use of PET probes for amyloid and tau pathology which has resulted in recent FDA-approvals of antibody-based AD therapies, it requires to the development of new and specific PET probes that may detect disease-related changes of microglia and enable analysis of spatiotemporal changes of neuroinflammation in AD brains. For future clinical trials, we speculate that the success will be facilitated by multiple approaches, including, e.g., (1) disease mechanism and pathology-based experimental designs based on biomarker discovery and insights gained from previous findings, (2) identification of novel mechanisms or drug targets that safely and effectively attenuate the neuropathology of AD, and (3) regulation of life exposures that may modify AD risks and neuropathology. We speculate that suitable tools and biomarker discovery of AD will accelerate drug development and identify novel drug targets of AD. Once such trials are tested successful in treating AD, the drug targets involved in the trials will prove to be the underpinnings of AD pathogenesis. Collectively, we speculate that the development of adequate tools for the mechanism and biomarker discovery of AD are key areas of attention in future translational research of AD, which may help engage patient stratification and trial selection, better predict clinical outcome, and ultimately provide precise and new insights for the etiology of AD.

Conclusion

In summary, etiologically, AD is an aging-related neurodegenerative disorder that is caused by genetic heterogeneity in relationship to a multitude of life exposures, and is pathologically characterized by gain of cellular and molecular abnormalities and neural network impairment, as well as loss of normal functions in AD brains (Fig. 1). Although AD brains share common manifestation of β-amyloid and tau-related disease neuropathologic hallmarks, the mechanisms causing these changes may significantly differ among patients depending on their genetics and precipitating life exposures. It is the various mechanisms in association with these pathogenic activities that may collectively lead to the heterogeneity of AD.

In conclusion, this article focuses on dissecting the mechanism-based heterogeneity of AD based on previous findings. It is challenging to cover all areas due to the expansive literature in this field. Nevertheless, this article attempts to not only recognize the rich knowledge gleaned from different research methodologies, but also discuss the findings and mechanisms that are identified by multidisciplinary efforts in areas of pathology, genetics, cell and molecular biology, biomedicine, and biochemistry. These efforts have resulted in an integrated perspective for AD etiology. As a complex disease, we speculate that the success of AD's future research will require continued combination of multidisciplinary approaches to attain more precise mechanisms underlying the etiology of AD.

Availability of Data and Materials

All Data and Materials are included in the manuscript.

Author Contributions

CZ originated, revised, and approved the manuscript. The author has participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

The author would like to thank colleagues who have provided helpful suggestions for this article.

Funding

This article was supported by the grants from the National Institutes of Health R01AG055784 (Can Zhang), the Cure Alzheimer's Fund (Can Zhang) and the Massachusetts General Hospital Neuroscience SPARC Award (Can Zhang).

Conflict of Interest

The author declares no conflict of interest.

References

[1] Mazure CM, Swendsen J. Sex differences in Alzheimer's disease and other dementias. The Lancet Neurology. 2016; 15: 451–452.

[2] Tekirian TL. Commentary: Abeta N- Terminal Isoforms: Critical contributors in the course of AD pathophysiology. Journal of Alzheimer's Disease: JAD. 2001; 3: 241–248.

[3] US Burden of Disease Collaborators, Mokdad AH, Ballestros K, Echko M, Glenn S, Olsen HE, *et al*. The State of US Health, 1990-2016: Burden of Diseases, Injuries, and Risk Factors Among US States. JAMA. 2018; 319: 1444–1472.

[4] Sevigny J, Chiao P, Bussière T, Weinreb PH, Williams L, Maier M, *et al*. The antibody aducanumab reduces Aβ plaques in Alzheimer's disease. Nature. 2016; 537: 50–56.

[5] Van Dyck CH, Swanson CJ, Aisen P, Bateman RJ, Chen C, Gee M, *et al*. Lecanemab in Early Alzheimer's Disease. The New England Journal of Medicine. 2023; 388: 9–21.

[6] Hardy J, Mummery C. An anti-amyloid therapy works for Alzheimer's disease: why has it taken so long and what is next? Brain: a Journal of Neurology. 2023; 146: 1240–1242.

[7] Karran E, De Strooper B. The amyloid hypothesis in Alzheimer disease: new insights from new therapeutics. Nature Reviews Drug Discovery. 2022; 21: 306–318, 2022

[8] Tanzi RE. FDA Approval of Aduhelm Paves a New Path for Alzheimer's Disease. ACS Chemical Neuroscience. 2021; 12: 2714–2715.

[9] Tatulian SA. Challenges and hopes for Alzheimer's disease. Drug Discovery Today. 2022; 27: 1027–1043.

[10] Abeysinghe AADT, Deshapriya RDUS, Udawatte C. Alzheimer's disease; a review of the pathophysiological basis and therapeutic interventions. Life Sciences. 2020; 256: 117996.

[11] Ray WJ, Buggia-Prevot V. Novel Targets for Alzheimer's Disease: A View Beyond Amyloid. Annual Review of Medicine. 2021; 72: 15–28.

[12] Miller SJ, Wray S, Sattler R, Zhang C. Editorial: Mechanisms of Action in Neurodegenerative Proteinopathies. Frontiers in Neuroscience. 2022; 16: 968994.

[13] Zhang C, Fang EF, Wang C. Editorial: Progress of Translational Medicine in Alzheimer's Disease. Frontiers in Aging Neuroscience. 2022; 14: 970044.

[14] Bertram L, Tanzi RE. Thirty years of Alzheimer's disease genetics: the implications of systematic meta-analyses. Nature Reviews Neuroscience. 2008; 9: 768–778.

[15] Choi SH, Kim YH, Hebisch M, Sliwinski C, Lee S, D'Avanzo C, *et al*. A three-dimensional human neural cell culture model of Alzheimer's disease. Nature. 2014; 515: 274–278.

[16] Gandy S. The role of cerebral amyloid beta accumulation in common forms of Alzheimer disease. The Journal of Clinical Investigation. 2005; 115: 1121–1129.

[17] Sisodia SS, St George-Hyslop PH. gamma-Secretase, Notch, Abeta and Alzheimer's disease: where do the presenilins fit in? Nature Reviews. Neuroscience. 2002; 3: 281–290.

[18] Wang Z, Ye J, Zhang K, Ding L, Granzier-Nakajima T, Ranasinghe JC, *et al*. Rapid Biomarker Screening of Alzheimer's Disease by Interpretable Machine Learning and Graphene-Assisted Raman Spectroscopy. ACS Nano. 2022; 16: 6426–6436.

[19] Yang HS, Zhang C, Carlyle BC, Zhen SY, Trombetta BA, Schultz AP, *et al*. Plasma IL-12/IFN-gamma axis predicts cognitive trajectories in cognitively unimpaired older adults. Alzheimer's & dementia: the journal of the Alzheimer's Association. 2022; 18: 645–653.

[20] Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science (New York, N.Y.). 2002; 297: 353–356.

[21] Dujardin S, Commins C, Lathuiliere A, Beerepoot P, Fernandes AR, Kamath TV, *et al*. Tau molecular diversity contributes to clinical heterogeneity in Alzheimer's disease. Nature Medicine. 2020; 26: 1256–1263.

[22] Wesseling H, Mair W, Kumar M, Schlaffner CN, Tang S, Beerepoot P, *et al*. Tau PTM Profiles Identify Patient Heterogeneity and Stages of Alzheimer's Disease. Cell. 2020; 183: 1699–1713.e13.

[23] Vogel JW, Iturria-Medina Y, Strandberg OT, Smith R, Levitis E, Evans AC, *et al*. Spread of pathological tau proteins through communicating neurons in human Alzheimer's disease. Nature Communications. 2020; 11: 2612.

[24] Park J, Wetzel I, Marriott I, Dreau D, D'avanzo C, Kim DY, *et al*. A 3D human triculture system modeling neurodegeneration and neuroinflammation in Alzheimer's disease. Nature Neuroscience. 2018; 21: 941–951.

[25] Bilousova T, Miller CA, Poon WW, Vinters HV, Corrada M, Kawas C, *et al*. Synaptic Amyloid-β Oligomers Precede p-Tau and Differentiate High Pathology Control Cases. The American Journal of Pathology. 2016; 186: 185–198.

[26] Jack CR, Jr, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, *et al*. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. Alzheimer's & Dementia: the Journal of the Alzheimer's Association. 2018; 14: 535–562.

[27] Ashton NJ, Pascoal TA, Karikari TK, Benedet AL, Lantero-Rodriguez J, Brinkmalm G, *et al*. Plasma p-tau231: a new biomarker for incipient Alzheimer's disease pathology. Acta Neuropathologica. 2021; 141: 709–724.

[28] Karikari TK, Pascoal TA, Ashton NJ, Janelidze S, Benedet AL, Rodriguez JL, *et al*. Blood phosphorylated tau 181 as a biomarker for Alzheimer's disease: a diagnostic performance and prediction modelling study using data from four prospective cohorts. The Lancet. Neurology. 2020; 19: 422–433.

[29] Palmqvist S, Janelidze S, Stomrud E, Zetterberg H, Karl J, Zink K, *et al*. Performance of Fully Automated Plasma Assays as Screening Tests for Alzheimer Disease-Related β-Amyloid Status. JAMA Neurology. 2019; 76: 1060–1069.

[30] Verberk IMW, Slot RE, Verfaillie SCJ, Heijst H, Prins ND, van Berckel BNM, *et al*. Plasma Amyloid as Prescreener for the Earliest Alzheimer Pathological Changes. Annals of Neurology. 2018; 84: 648–658.

[31] Griciuc A, Patel S, Federico AN, Choi SH, Innes BJ, Oram MK, *et al*. TREM2 Acts Downstream of CD33 in Modulating Microglial Pathology in Alzheimer's Disease. Neuron. 2019; 103: 820–835.e7.

[32] Ising C, Venegas C, Zhang S, Scheiblich H, Schmidt SV, Vieira-Saecker A, *et al*. NLRP3 inflammasome activation drives tau pathology. Nature. 2019; 575: 669–673.

[33] Jorfi M, Maaser-Hecker A, Tanzi RE. The neuroimmune axis of Alzheimer's disease. Genome Medicine. 2023; 15: 6.

[34] Lewcock JW, Schlepckow K, Di Paolo G, Tahirovic S, Monroe KM, Haass C. Emerging Microglia Biology Defines Novel Therapeutic Approaches for Alzheimer's Disease. Neuron. 2020; 108: 801–821.

[35] Ennerfelt H, Frost EL, Shapiro DA, Holliday C, Zengeler KE, Voithofer G, *et al*. SYK coordinates neuroprotective microglial responses in neurodegenerative disease. Cell. 2022; 185: 4135–4152.e22.

[36] Pascoal TA, Benedet AL, Ashton NJ, Kang MS, Therriault J, Chamoun M, *et al*. Microglial activation and tau propagate jointly across Braak stages. Nature Medicine. 2021; 27: 1592–1599.

[37] Chen X, Firulyova M, Manis M, Herz J, Smirnov I, Aladyeva E, *et al*. Microglia-mediated T cell infiltration drives neurodegeneration in tauopathy. Nature. 2023; 615: 668–677.
[38] Jorfi M, Park J, Hall CK, Lin CCJ, Chen M, von Maydell D, *et al*. Infiltrating CD8$^+$ T cells exacerbate Alzheimer's disease pathology in a 3D human neuroimmune axis model. Nature Neuroscience. 2023; 26: 1489–1504.
[39] Deming Y, Filipello F, Cignarella F, Cantoni C, Hsu S, Mikesell R, *et al*. The *MS4A* gene cluster is a key modulator of soluble TREM2 and Alzheimer's disease risk. Science Translational Medicine. 2019; 11: eaau2291.
[40] Falcon C, Monté-Rubio GC, Grau-Rivera O, Suárez-Calvet M, Sánchez-Valle R, Rami L, *et al*. CSF glial biomarkers YKL40 and sTREM2 are associated with longitudinal volume and diffusivity changes in cognitively unimpaired individuals. NeuroImage. Clinical. 2019; 23: 101801.
[41] Gratuze M, Chen Y, Parhizkar S, Jain N, Strickland MR, Serrano JR, *et al*. Activated microglia mitigate Aβ-associated tau seeding and spreading. The Journal of Experimental Medicine. 2021; 218: e20210542.
[42] Jaitin DA, Adlung L, Thaiss CA, Weiner A, Li B, Descamps H, *et al*. Lipid-Associated Macrophages Control Metabolic Homeostasis in a Trem2-Dependent Manner. Cell. 2019; 178: 686–698.e14.
[43] Kosoy R, Fullard JF, Zeng B, Bendl J, Dong P, Rahman S, *et al*. Genetics of the human microglia regulome refines Alzheimer's disease risk loci. Nature Genetics. 2022; 54: 1145–1154.
[44] Griciuc A, Serrano-Pozo A, Parrado AR, Lesinski AN, Asselin CN, Mullin K, *et al*. Alzheimer's disease risk gene CD33 inhibits microglial uptake of amyloid beta. Neuron. 2013; 78: 631–643.
[45] Lee HT, Lee KI, Chen CH, Lee TS. Genetic deletion of soluble epoxide hydrolase delays the progression of Alzheimer's disease. Journal of Neuroinflammation. 2019; 16: 267.
[46] Pereira CF, Santos AE, Moreira PI, Pereira AC, Sousa FJ, Cardoso SM, *et al*. Is Alzheimer's disease an inflammasomopathy? Ageing Research Reviews. 2019; 56: 100966.
[47] Ofengeim D, Mazzitelli S, Ito Y, DeWitt JP, Mifflin L, Zou C, *et al*. RIPK1 mediates a disease-associated microglial response in Alzheimer's disease. Proceedings of the National Academy of Sciences of the United States of America. 2017; 114: E8788–E8797.
[48] Lan Y, Bai P, Liu Y, Afshar S, Striar R, Rattray AK, *et al*. Visualization of Receptor-Interacting Protein Kinase 1 (RIPK1) by Brain Imaging with Positron Emission Tomography. Journal of Medicinal Chemistry. 2021; 64: 15420–15428.
[49] Li S, Fang Y, Zhang Y, Song M, Zhang X, Ding X, *et al*. Microglial NLRP3 inflammasome activates neurotoxic astrocytes in depression-like mice. Cell Reports. 2022; 41: 111532.
[50] Van Zeller M, Dias D, Sebastião AM, Valente CA. NLRP3 Inflammasome: A Starring Role in Amyloid-β- and Tau-Driven Pathological Events in Alzheimer's Disease. Journal of Alzheimer's Disease: JAD. 2021; 83: 939–961.
[51] Liang Y, Lee DYW, Zhen S, Sun H, Zhu B, Liu J, *et al*. Natural medicine HLXL targets multiple pathways of amyloid-mediated neuroinflammation and immune response in treating alzheimer's disease. Phytomedicine: International Journal of Phytotherapy and Phytopharmacology. 2022; 104: 154158.
[52] Zhang C, Griciuc A, Hudry E, Wan Y, Quinti L, Ward J, *et al*. Cromolyn Reduces Levels of the Alzheimer's Disease-Associated Amyloid β-Protein by Promoting Microglial Phagocytosis. Scientific Reports. 2018; 8: 1144.
[53] Palop JJ, Chin J, Roberson ED, Wang J, Thwin MT, Bien-Ly N, *et al*. Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. Neuron. 2007; 55: 697–711.
[54] Vetere G, Kenney JW, Tran LM, Xia F, Steadman PE, Parkinson J, *et al*. Chemogenetic Interrogation of a Brain-wide Fear Memory Network in Mice. Neuron. 2017; 94: 363–374.e4.
[55] [53] Berron D, Van Westen D, Ossenkoppele R, Strandberg O, Hansson O Medial temporal lobe connectivity and its associations with cognition in early Alzheimer's disease. Brain: a journal of neurology. 2020; 143: 1233–1248.
[56] Gatchel JR, Rabin JS, Buckley RF, Locascio JJ, Quiroz YT, Yang HS, *et al*. Longitudinal Association of Depression Symptoms With Cognition and Cortical Amyloid Among Community-Dwelling Older Adults. JAMA Network Open. 2019; 2: e198964.
[57] Jacobs HIL, Hedden T, Schultz AP, Sepulcre J, Perea RD, Amariglio RE, *et al*. Structural tract alterations predict downstream tau accumulation in amyloid-positive older individuals. Nature Neuroscience. 2018; 21: 424–431.
[58] Smith V, Mitchell DJ, Duncan J. Role of the Default Mode Network in Cognitive Transitions. Cerebral Cortex (New York, N.Y.: 1991). 2018; 28: 3685–3696.
[59] Petrella JR, Michael AM, Qian M, Nwosu A, Sneed J, Goldberg TE, *et al*. Impact of Computerized Cognitive Training on Default Mode Network Connectivity in Subjects at Risk for Alzheimer's Disease: A 78-week Randomized Controlled Trial. Journal of Alzheimer's Disease: JAD. 2023; 91: 483–494.
[60] Hou Y, Dan X, Babbar M, Wei Y, Hasselbalch SG, Croteau DL, *et al*. Ageing as a risk factor for neurodegenerative disease. Nature Reviews. Neurology. 2019; 15: 565–581.
[61] Fyfe I. Alzheimer disease: Epigenetics links ageing with Alzheimer disease. Nature Reviews. Neurology. 2018; 14: 254.
[62] Tamnes CK, Walhovd KB, Dale AM, Østby Y, Grydeland H, Richardson G, *et al*. Brain development and aging: overlapping and unique patterns of change. NeuroImage. 2013; 68: 63–74.
[63] Moreno-Jiménez EP, Flor-García M, Terreros-Roncal J, Rábano A, Cafini F, Pallas-Bazarra N, *et al*. Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer's disease. Nature Medicine. 2019; 25: 554–560.
[64] Lu Y, Brommer B, Tian X, Krishnan A, Meer M, Wang C, *et al*. Reprogramming to recover youthful epigenetic information and restore vision. Nature. 2020; 588: 124–129.
[65] Yang JH, Hayano M, Griffin PT, Amorim JA, Bonkowski MS, Apostolides JK, *et al*. Loss of epigenetic information as a cause of mammalian aging. Cell. 2023; 186: 305–326.e327.
[66] Choi I, Wang M, Yoo S, Xu P, Seegobin SP, Li X, *et al*. Autophagy enables microglia to engage amyloid plaques and prevents microglial senescence. Nature Cell Biology. 2023; 25: 963–974.
[67] Sandhof CA, Hoppe SO, Druffel-Augustin S, Gallrein C, Kirstein J, Voisine C, *et al*. Reducing INS-IGF1 signaling protects against non-cell autonomous vesicle rupture caused by SNCA spreading. Autophagy. 2020; 16: 878–899.
[68] Stoyanova II. Ghrelin: a link between ageing, metabolism and neurodegenerative disorders. Neurobiology of Disease. 2014; 72 Pt A: 72–83.
[69] Jeon SG, Hong SB, Nam Y, Tae J, Yoo A, Song EJ, *et al*. Ghrelin in Alzheimer's disease: Pathologic roles and therapeutic implications. Ageing Research Reviews. 2019; 55: 100945.
[70] Zullo JM, Drake D, Aron L, O'Hern P, Dhamne SC, Davidsohn N, *et al*. Regulation of lifespan by neural excitation and REST. Nature. 2019; 574: 359–364.
[71] Lautrup S, Sinclair DA, Mattson MP, Fang EF. NAD$^+$ in Brain Aging and Neurodegenerative Disorders. Cell Metabolism. 2019; 30: 630–655.
[72] Bheda P, Jing H, Wolberger C, Lin H. The Substrate Specificity of Sirtuins. Annual Review of Biochemistry. 2016; 85: 405–429.
[73] Hou Y, Lautrup S, Cordonnier S, Wang Y, Croteau DL, Zavala E, *et al*. NAD$^+$ supplementation normalizes key Alzheimer's

features and DNA damage responses in a new AD mouse model with introduced DNA repair deficiency. Proceedings of the National Academy of Sciences of the United States of America. 2018; 115: E1876–E1885.

[74] Yang JH, Petty CA, Dixon-McDougall T, Lopez MV, Tyshkovskiy A, Maybury-Lewis S, *et al*. Chemically induced reprogramming to reverse cellular aging. Aging. 2023; 15: 5966–5989.

[75] Pulla VK, Battu MB, Alvala M, Sriram D, Yogeeswari P. Can targeting SIRT-1 to treat type 2 diabetes be a good strategy? A review. Expert Opinion on Therapeutic Targets. 2012; 16: 819–832.

[76] Xu Y, Chen Z, Wey HY, Liang Y, Tanzi RE, Zhang C, *et al*. Molecular imaging of NAD^+ -dependent deacetylase SIRT1 in the brain. Alzheimer's & Dementia: the Journal of the Alzheimer's Association. 2021; 17: 1988–1997.

[77] Sierksma A, Escott-Price V, De Strooper B. Translating genetic risk of Alzheimer's disease into mechanistic insight and drug targets. Science (New York, N.Y.). 2020; 370: 61–66.

[78] Cacace R, Sleegers K, Van Broeckhoven C. Molecular genetics of early-onset Alzheimer's disease revisited. Alzheimer's & Dementia: the Journal of the Alzheimer's Association. 2016; 12: 733–748.

[79] Chen WT, Lu A, Craessaerts K, Pavie B, Sala Frigerio C, Corthout N, *et al*. Spatial Transcriptomics and In Situ Sequencing to Study Alzheimer's Disease. Cell. 2020; 182: 976–991.e19.

[80] Hur JY, Frost GR, Wu X, Crump C, Pan SJ, Wong E, *et al*. The innate immunity protein IFITM3 modulates γ-secretase in Alzheimer's disease. Nature. 2020; 586: 735–740.

[81] Ma C, Su J, Sun Y, Feng Y, Shen N, Li B, *et al*. Significant Up-regulation of Alzheimer's β-Amyloid Levels in a Living System Induced by Extracellular Elastin Polypeptides. Angewandte Chemie (International Ed. in English). 2019; 58: 18703–18709.

[82] Walsh DM, Selkoe DJ. Amyloid β-protein and beyond: the path forward in Alzheimer's disease. Current Opinion in Neurobiology. 2020; 61: 116–124.

[83] Yu JT, Xu W, Tan CC, Andrieu S, Suckling J, Evangelou E, *et al*. Evidence-based prevention of Alzheimer's disease: systematic review and meta-analysis of 243 observational prospective studies and 153 randomised controlled trials. Journal of Neurology, Neurosurgery, and Psychiatry. 2020; 91: 1201–1209.

[84] Blanchard JW, Victor MB, Tsai LH. Dissecting the complexities of Alzheimer disease with in vitro models of the human brain. Nature Reviews. Neurology. 2022; 18: 25–39.

[85] Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, *et al*. Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. Science (New York, N.Y.). 1996; 274: 99–102.

[86] Oakley H, Cole SL, Logan S, Maus E, Shao P, Craft J, *et al*. Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation. The Journal of Neuroscience: the Official Journal of the Society for Neuroscience. 2006; 26: 10129–10140.

[87] Heiland T, Zeitschel U, Puchades MA, Kuhn PH, Lichtenthaler SF, Bjaalie JG, *et al*. Defined astrocytic expression of human amyloid precursor protein in Tg2576 mouse brain. Glia. 2019; 67: 393–403.

[88] Liang Y, Raven F, Ward JF, Zhen S, Zhang S, Sun H, *et al*. Up-regulation of Alzheimer's Disease Amyloid-β Protein Precursor in Astrocytes Both in vitro and in vivo. Journal of Alzheimer's Disease: JAD. 2020; 76: 1071–1082.

[89] Wang W, Hou TT, Jia LF, Wu QQ, Quan MN, Jia JP. Toxic amyloid-β oligomers induced self-replication in astrocytes triggering neuronal injury. EBioMedicine. 2019; 42: 174–187.

[90] [89] Manocha GD, Floden AM, Rausch K, Kulas JA, Mcgregor BA, Rojanathammanee L, Puig KR, Puig KL, Karki S, Nichols MR, Darland DC, Porter JE, Combs CK APP Regulates Microglial Phenotype in a Mouse Model of Alzheimer's Disease. The Journal of Neuroscience: the official journal of the Society for Neuroscience. 2016; 36: 8471–8486.

[91] Zhang C, Inamdar SM, Swaminathan S, Marenda DR, Saunders AJ. Association of the Protein-Quality-Control Protein Ubiquilin-1 With Alzheimer's Disease Both *in vitro* and *in vivo*. Frontiers in Neuroscience. 2022; 16: 821059.

[92] Yamazaki Y, Zhao N, Caulfield TR, Liu CC, Bu G. Apolipoprotein E and Alzheimer disease: pathobiology and targeting strategies. Nature Reviews. Neurology. 2019; 15: 501–518.

[93] Saeed U, Mirza SS, MacIntosh BJ, Herrmann N, Keith J, Ramirez J, *et al*. APOE-ε4 associates with hippocampal volume, learning, and memory across the spectrum of Alzheimer's disease and dementia with Lewy bodies. Alzheimer's & Dementia: the Journal of the Alzheimer's Association. 2018; 14: 1137–1147.

[94] Martens YA, Zhao N, Liu CC, Kanekiyo T, Yang AJ, Goate AM, *et al*. ApoE Cascade Hypothesis in the pathogenesis of Alzheimer's disease and related dementias. Neuron. 2022; 110: 1304–1317.

[95] Serrano-Pozo A, Das S, Hyman BT. APOE and Alzheimer's disease: advances in genetics, pathophysiology, and therapeutic approaches. The Lancet. Neurology. 2021; 20: 68–80.

[96] Wisniewski T, Drummond E. APOE-amyloid interaction: Therapeutic targets. Neurobiology of Disease. 2020; 138: 104784.

[97] Johnson NR, Wang ACJ, Coughlan C, Sillau S, Lucero E, Viltz L, *et al*. Imipramine and olanzapine block apoE4-catalyzed polymerization of Aβ and show evidence of improving Alzheimer's disease cognition. Alzheimer's Research & Therapy. 2022; 14: 88.

[98] Islam S, Sun Y, Gao Y, Nakamura T, Noorani AA, Li T, *et al*. Presenilin Is Essential for ApoE Secretion, a Novel Role of Presenilin Involved in Alzheimer's Disease Pathogenesis. The Journal of Neuroscience: the Official Journal of the Society for Neuroscience. 2022; 42: 1574–1586.

[99] Wadhwani AR, Affaneh A, Van Gulden S, Kessler JA. Neuronal apolipoprotein E4 increases cell death and phosphorylated tau release in alzheimer disease. Annals of Neurology. 2019; 85: 726–739.

[100] Tensaouti Y, Stephanz EP, Yu TS, Kernie SG. ApoE Regulates the Development of Adult Newborn Hippocampal Neurons. eNeuro. 2018; 5: ENEURO.0155–18.2018.

[101] Najm R, Jones EA, Huang Y. Apolipoprotein E4, inhibitory network dysfunction, and Alzheimer's disease. Molecular Neurodegeneration. 2019; 14: 24.

[102] Rajan KB, Barnes LL, Wilson RS, Weuve J, McAninch EA, Evans DA. Blood pressure and risk of incident Alzheimer's disease dementia by antihypertensive medications and APOE ε4 allele. Annals of Neurology. 2018; 83: 935–944.

[103] Bellenguez C, Kucukali F, Jansen IE, Kleineidam L, Moreno-Grau S, Amin N, *et al*. New insights into the genetic etiology of Alzheimer's disease and related dementias. Nature Genetics. 2022; 54: 412–436.

[104] [Bis JC, Jian X, Kunkle BW, Chen Y, Hamilton-Nelson KL, Bush WS, *et al*. Whole exome sequencing study identifies novel rare and common Alzheimer's-Associated variants involved in immune response and transcriptional regulation. Molecular Psychiatry. 2020: 25: 1859–1875.

[105] Kunkle BW, Grenier-Boley B, Sims R, Bis JC, Damotte V, Naj AC, *et al*. Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Abeta, tau, immunity and lipid processing. Nature Genetics. 2019; 51: 414–430.

[106] Yuan P, Zhang M, Tong L, Morse TM, McDougal RA, Ding

H, *et al*. PLD3 affects axonal spheroids and network defects in Alzheimer's disease. Nature. 2022; 612: 328–337.

[107] Li P, Marshall L, Oh G, Jakubowski JL, Groot D, He Y, *et al*. Epigenetic dysregulation of enhancers in neurons is associated with Alzheimer's disease pathology and cognitive symptoms. Nature Communications. 2019; 10: 2246.

[108] Bai P, Mondal P, Bagdasarian FA, Rani N, Liu Y, Gomm A, *et al*. Development of a potential PET probe for HDAC6 imaging in Alzheimer's disease. Acta Pharmaceutica Sinica. B. 2022; 12: 3891–3904.

[109] Nativio R, Lan Y, Donahue G, Sidoli S, Berson A, Srinivasan AR, *et al.* An integrated multi-omics approach identifies epigenetic alterations associated with Alzheimer's disease. Nature Genetics. 2020; 52: 1024–1035.

[110] Wang E, Wang M, Guo L, Fullard JF, Micallef C, Bendl J, *et al*. Genome-wide methylomic regulation of multiscale gene networks in Alzheimer's disease. Alzheimer's & Dementia: the Journal of the Alzheimer's Association. 2023; 19: 3472–3495.

[111] Yu L, Chibnik LB, Srivastava GP, Pochet N, Yang J, Xu J, *et al*. Association of Brain DNA methylation in SORL1, ABCA7, HLA-DRB5, SLC24A4, and BIN1 with pathological diagnosis of Alzheimer disease. JAMA Neurology. 2015; 72: 15–24.

[112] Kim W, Noh H, Lee Y, Jeon J, Shanmugavadivu A, McPhie DL, *et al*. MiR-126 Regulates Growth Factor Activities and Vulnerability to Toxic Insult in Neurons. Molecular Neurobiology. 2016; 53: 95–108.

[113] Arendt T, Brückner MK, Lösche A. Regional mosaic genomic heterogeneity in the elderly and in Alzheimer's disease as a correlate of neuronal vulnerability. Acta Neuropathologica. 2015; 130: 501–510.

[114] Phillips NR, Simpkins JW, Roby RK. Mitochondrial DNA deletions in Alzheimer's brains: a review. Alzheimer's & Dementia: the Journal of the Alzheimer's Association. 2014; 10: 393–400.

[115] De Simone A, Milelli A. Histone Deacetylase Inhibitors as Multitarget Ligands: New Players in Alzheimer's Disease Drug Discovery? ChemMedChem. 2019; 14: 1067–1073.

[116] Kulikowski E, Rakai BD, Wong NCW. Inhibitors of bromodomain and extra-terminal proteins for treating multiple human diseases. Medicinal Research Reviews. 2021; 41: 223–245.

[117] Zhang S, Bai P, Lei D, Liang Y, Zhen S, Bakiasi G, *et al*. Degradation and inhibition of epigenetic regulatory protein BRD4 exacerbate Alzheimer's disease-related neuropathology in cell models. The Journal of Biological Chemistry. 2022; 298: 101794.

[118] Deming Y, Dumitrescu L, Barnes LL, Thambisetty M, Kunkle B, Gifford KA, *et al*. Sex-specific genetic predictors of Alzheimer's disease biomarkers. Acta Neuropathologica. 2018; 136: 857–872.

[119] Ferretti MT, Iulita MF, Cavedo E, Chiesa PA, Schumacher Dimech A, Santuccione Chadha A, *et al*. Sex differences in Alzheimer disease - the gateway to precision medicine. Nature Reviews. Neurology. 2018; 14: 457–469.

[120] Barnes LL, Wilson RS, Bienias JL, Schneider JA, Evans DA, Bennett DA. Sex differences in the clinical manifestations of Alzheimer disease pathology. Archives of General Psychiatry. 2005; 62: 685–691.

[121] Prokopenko D, Hecker J, Kirchner R, Chapman BA, Hoffman O, Mullin K, *et al*. Identification of Novel Alzheimer's Disease Loci Using Sex-Specific Family-Based Association Analysis of Whole-Genome Sequence Data. Scientific Reports. 2020; 10: 5029.

[122] Xie L, Kang H, Xu Q, Chen MJ, Liao Y, Thiyagarajan M, O'donnell J, Christensen DJ, Nicholson C, Iliff JJ, Takano T, Deane R, Nedergaard M Sleep drives metabolite clearance from the adult brain. Science. 2013; 342: 373–377.

[123] Shakersain B, Santoni G, Larsson SC, Faxén-Irving G, Fastbom J, Fratiglioni L, *et al*. Prudent diet may attenuate the adverse effects of Western diet on cognitive decline. Alzheimer's & Dementia: the Journal of the Alzheimer's Association. 2016; 12: 100–109.

[124] Lien EC, Westermark AM, Zhang Y, Yuan C, Li Z, Lau AN, *et al*. Low glycaemic diets alter lipid metabolism to influence tumour growth. Nature. 2021; 599: 302–307.

[125] Mitchell SJ, Bernier M, Aon MA, Cortassa S, Kim EY, Fang EF, *et al*. Nicotinamide Improves Aspects of Healthspan, but Not Lifespan, in Mice. Cell Metabolism. 2018; 27: 667–676.e4.

[126] Rangan P, Lobo F, Parrella E, Rochette N, Morselli M, Stephen TL, *et al*. Fasting-mimicking diet cycles reduce neuroinflammation to attenuate cognitive decline in Alzheimer's models. Cell Reports. 2022; 40: 111417.

[127] Sun Y, Liu B, Snetselaar LG, Wallace RB, Shadyab AH, Kroenke CH, *et al*. Association of Major Dietary Protein Sources With All-Cause and Cause-Specific Mortality: Prospective Cohort Study. Journal of the American Heart Association. 2021; 10: e015553.

[128] Wu W, Ding D, Zhao Q, Xiao Z, Luo J, Ganguli M, *et al*. Dose-response relationship between late-life physical activity and incident dementia: A pooled analysis of 10 cohort studies of memory in an international consortium. Alzheimer's & Dementia: the Journal of the Alzheimer's Association. 2023; 19: 107–122.

[129] Choi SH, Bylykbashi E, Chatila ZK, Lee SW, Pulli B, Clemenson GD, *et al*. Combined adult neurogenesis and BDNF mimic exercise effects on cognition in an Alzheimer's mouse model. Science (New York, N.Y.). 2018; 361: eaan8821.

[130] De Miguel Z, Khoury N, Betley MJ, Lehallier B, Willoughby D, Olsson N, *et al*. Exercise plasma boosts memory and dampens brain inflammation via clusterin. Nature. 2021; 600: 494–499.

[131] Islam MR, Valaris S, Young MF, Haley EB, Luo R, Bond SF, *et al.* Exercise hormone irisin is a critical regulator of cognitive function. Nature Metabolism. 2021; 3: 1058–1070.

[132] Tari AR, Norevik CS, Scrimgeour NR, Kobro-Flatmoen A, Storm-Mathisen J, Bergersen LH, *et al.* Are the neuroprotective effects of exercise training systemically mediated? Progress in Cardiovascular Diseases. 2019; 62: 94–101.

[133] Voss MW, Soto C, Yoo S, Sodoma M, Vivar C, van Praag H. Exercise and Hippocampal Memory Systems. Trends in Cognitive Sciences. 2019; 23: 318–333.

[134] Sevinc G, Rusche J, Wong B, Datta T, Kaufman R, Gutz SE, *et al*. Mindfulness Training Improves Cognition and Strengthens Intrinsic Connectivity Between the Hippocampus and Posteromedial Cortex in Healthy Older Adults. Frontiers in Aging Neuroscience. 2021; 13: 702796.

[135] Sheng C, Yang K, Wang X, Li H, Li T, Lin L, *et al*. Advances in Non-Pharmacological Interventions for Subjective Cognitive Decline: A Systematic Review and Meta-Analysis. Journal of Alzheimer's Disease: JAD. 2020; 77: 903–920.

[136] Wilson RS, Krueger KR, Arnold SE, Schneider JA, Kelly JF, Barnes LL, *et al*. Loneliness and risk of Alzheimer disease. Archives of General Psychiatry. 2007; 64: 234–240.

[137] Hsiao YH, Chang CH, Gean PW. Impact of social relationships on Alzheimer's memory impairment: mechanistic studies. Journal of Biomedical Science. 2018; 25: 3.

[138] Creswell JD, Irwin MR, Burklund LJ, Lieberman MD, Arevalo JMG, Ma J, *et al*. Mindfulness-Based Stress Reduction training reduces loneliness and pro-inflammatory gene expression in older adults: a small randomized controlled trial. Brain, Behavior, and Immunity. 2012; 26: 1095–1101.

[139] Xu W, Tan L, Wang HF, Jiang T, Tan MS, Zhao QF, *et al.* Meta-analysis of modifiable risk factors for Alzheimer's disease. Journal of Neurology, Neurosurgery, and Psychiatry. 2015; 86: 1299–1306.

[140] Jia J, Zhao T, Liu Z, Liang Y, Li F, Li Y, *et al*. Association between healthy lifestyle and memory decline in older adults: 10 year, population based, prospective cohort study. BMJ (Clinical Research Ed.). 2023; 380: e072691.

[141] Chen H, Kwong JC, Copes R, Hystad P, van Donkelaar A, Tu K, *et al*. Exposure to ambient air pollution and the incidence of dementia: A population-based cohort study. Environment International. 2017; 108: 271–277.

[142] Finch CE, Kulminski AM. The Alzheimer's Disease Exposome. Alzheimer's & Dementia: the Journal of the Alzheimer's Association. 2019; 15: 1123–1132.

[143] Li S, Jin M, Zhang D, Yang T, Koeglsperger T, Fu H, *et al*. Environmental novelty activates β2-adrenergic signaling to prevent the impairment of hippocampal LTP by Aβ oligomers. Neuron. 2013; 77: 929–941.

[144] Wild CP. Complementing the genome with an "exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology. Cancer Epidemiology, Biomarkers & Prevention: a Publication of the American Association for Cancer Research, Cosponsored by the American Society of Preventive Oncology. 2005; 14: 1847–1850.

[145] Andrews SJ, Goate A, Anstey KJ. Association between alcohol consumption and Alzheimer's disease: A Mendelian randomization study. Alzheimer's & Dementia: the Journal of the Alzheimer's Association. 2020; 16: 345–353.

[146] Cox LM, Yamanishi S, Sohn J, Alekseyenko AV, Leung JM, Cho I, *et al.* Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. Cell. 2014; 158: 705–721.

[147] MacDonald AB, Miranda JM. Concurrent neocortical borreliosis and Alzheimer's disease. Human Pathology. 1987; 18: 759–761.

[148] Balin BJ, Gérard HC, Arking EJ, Appelt DM, Branigan PJ, Abrams JT, *et al*. Identification and localization of Chlamydia pneumoniae in the Alzheimer's brain. Medical Microbiology and Immunology. 1998; 187: 23–42.

[149] Ball MJ, Lukiw WJ, Kammerman EM, Hill JM. Intracerebral propagation of Alzheimer's disease: strengthening evidence of a herpes simplex virus etiology. Alzheimer's & Dementia: the Journal of the Alzheimer's Association. 2013; 9: 169–175.

[150] Vogt NM, Kerby RL, Dill-McFarland KA, Harding SJ, Merluzzi AP, Johnson SC, *et al*. Gut microbiome alterations in Alzheimer's disease. Scientific Reports. 2017; 7: 13537.

[151] Agostini S, Mancuso R, Baglio F, Cabinio M, Hernis A, Costa AS, *et al*. High avidity HSV-1 antibodies correlate with absence of amnestic Mild Cognitive Impairment conversion to Alzheimer's disease. Brain, Behavior, and Immunity. 2016; 58: 254–260.

[152] Jamieson GA, Maitland NJ, Wilcock GK, Yates CM, Itzhaki RF. Herpes simplex virus type 1 DNA is present in specific regions of brain from aged people with and without senile dementia of the Alzheimer type. The Journal of Pathology. 1992; 167: 365–368.

[153] Minter MR, Hinterleitner R, Meisel M, Zhang C, Leone V, Zhang X, *et al*. Antibiotic-induced perturbations in microbial diversity during post-natal development alters amyloid pathology in an aged APPSWE/PS1DeltaE9 murine model of Alzheimer's disease. Scientific Reports. 2017; 7: 10411.

[154] Minter MR, Zhang C, Leone V, Ringus DL, Zhang X, Oyler-Castrillo P, *et al*. Antibiotic-induced perturbations in gut microbial diversity influences neuro-inflammation and amyloidosis in a murine model of Alzheimer's disease. Scientific Reports. 2016; 6: 30028.

[155] Eimer WA, Vijaya Kumar DK, Navalpur Shanmugam NK, Rodriguez AS, Mitchell T, Washicosky KJ, *et al*. Alzheimer's Disease-Associated beta-Amyloid Is Rapidly Seeded by Herpesviridae to Protect against Brain Infection. Neuron. 2018; 99: 56–63.e53.

[156] Kumar DKV, Choi SH, Washicosky KJ, Eimer WA, Tucker S, Ghofrani J, *et al*. Amyloid-β peptide protects against microbial infection in mouse and worm models of Alzheimer's disease. Science Translational Medicine. 2016; 8: 340ra72.

[157] De Chiara G, Piacentini R, Fabiani M, Mastrodonato A, Marcocci ME, Limongi D, *et al*. Recurrent herpes simplex virus-1 infection induces hallmarks of neurodegeneration and cognitive deficits in mice. PLoS Pathogens. 2019; 15: e1007617.

[158] Tang SC, Lathia JD, Selvaraj PK, Jo DG, Mughal MR, Cheng A, *et al*. Toll-like receptor-4 mediates neuronal apoptosis induced by amyloid beta-peptide and the membrane lipid peroxidation product 4-hydroxynonenal. Experimental Neurology. 2008; 213: 114–121.

[159] de J R De-Paula V, Forlenza AS, Forlenza OV. Relevance of gutmicrobiota in cognition, behaviour and Alzheimer's disease. Pharmacological Research. 2018; 136: 29–34.

[160] Rothhammer V, Borucki DM, Tjon EC, Takenaka MC, Chao CC, Ardura-Fabregat A, *et al*. Microglial control of astrocytes in response to microbial metabolites. Nature. 2018; 557: 724–728.

[161] Jagirdar R, Fu CH, Park J, Corbett BF, Seibt FM, Beierlein M, *et al*. Restoring activity in the thalamic reticular nucleus improves sleep architecture and reduces Aβ accumulation in mice. Science Translational Medicine. 2021; 13: eabh4284.

[162] Mander BA, Dave A, Lui KK, Sprecher KE, Berisha D, Chappel-Farley MG, *et al*. Inflammation, tau pathology, and synaptic integrity associated with sleep spindles and memory prior to β-amyloid positivity. Sleep. 2022; 45: zsac135.

[163] Wu R, Tripathy S, Menon V, Yu L, Buchman AS, Bennett DA, *et al*. Fragmentation of rest periods, astrocyte activation, and cognitive decline in older adults with and without Alzheimer's disease. Alzheimer's & Dementia: the Journal of the Alzheimer's Association. 2023; 19: 1888–1900.

[164] Stickgold R, Walker MP. Memory consolidation and reconsolidation: what is the role of sleep? Trends in Neurosciences. 2005; 28: 408–415.

[165] Ramirez-Villegas JF, Besserve M, Murayama Y, Evrard HC, Oeltermann A, Logothetis NK. Coupling of hippocampal theta and ripples with pontogeniculooccipital waves. Nature. 2021; 589: 96–102.

[166] Li Y, Lopez-Huerta VG, Adiconis X, Levandowski K, Choi S, Simmons SK, *et al*. Distinct subnetworks of the thalamic reticular nucleus. Nature. 2020; 583: 819–824.

[167] Martinez-Garcia RI, Voelcker B, Zaltsman JB, Patrick SL, Stevens TR, Connors BW, *et al*. Two dynamically distinct circuits drive inhibition in the sensory thalamus. Nature. 2020; 583: 813–818.

[168] Jagirdar R, Chin J. Corticothalamic network dysfunction and Alzheimer's disease. Brain Research. 2019; 1702: 38–45.

[169] Holth JK, Fritschi SK, Wang C, Pedersen NP, Cirrito JR, Mahan TE, *et al*. The sleep-wake cycle regulates brain interstitial fluid tau in mice and CSF tau in humans. Science (New York, N.Y.). 2019; 363: 880–884.

[170] Kang JE, Lim MM, Bateman RJ, Lee JJ, Smyth LP, Cirrito JR, *et al*. Amyloid-beta dynamics are regulated by orexin and the sleep-wake cycle. Science (New York, N.Y.). 2009; 326: 1005–1007.

[171] Shokri-Kojori E, Wang GJ, Wiers CE, Demiral SB, Guo M, Kim SW, *et al*. β-Amyloid accumulation in the human brain after one night of sleep deprivation. Proceedings of the National Academy of Sciences of the United States of America. 2018; 115: 4483–4488.

[172] Keller M, Mazuch J, Abraham U, Eom GD, Herzog ED, Volk HD, *et al*. A circadian clock in macrophages controls inflamma-

tory immune responses. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106: 21407–21412.

[173] Song H, Moon M, Choe HK, Han DH, Jang C, Kim A, *et al*. Aβ-induced degradation of BMAL1 and CBP leads to circadian rhythm disruption in Alzheimer's disease. Molecular Neurodegeneration. 2015; 10: 13.

[174] Chang HC, Guarente L. SIRT1 mediates central circadian control in the SCN by a mechanism that decays with aging. Cell. 2013; 153: 1448–1460.

[175] Schibler U. Senescence of Timing Reverted: NAD^+ Rejuvenates the Circadian Clock. Molecular Cell. 2020; 78: 805–807.

[176] Zhang QS, Liu W, Lu GX. miR-200a-3p promotes b-Amyloid-induced neuronal apoptosis through down-regulation of SIRT1 in Alzheimer's disease. Journal of Biosciences. 2017; 42: 397–404.

[177] He Y, Jones CR, Fujiki N, Xu Y, Guo B, Holder JL, Jr, *et al*. The transcriptional repressor DEC2 regulates sleep length in mammals. Science (New York, N.Y.). 2009; 325: 866–870.

[178] Hirano A, Hsu PK, Zhang L, Xing L, McMahon T, Yamazaki M, *et al*. DEC2 modulates orexin expression and regulates sleep. Proceedings of the National Academy of Sciences of the United States of America. 2018; 115: 3434–3439.

[179] Shi G, Xing L, Wu D, Bhattacharyya BJ, Jones CR, Mcmahon T, *et al*. A Rare Mutation of beta1-Adrenergic Receptor Affects Sleep/Wake Behaviors. Neuron. 2019; 103: 1044–1055.e1047.

[180] Lehrer S, Rheinstein PH. Alzheimer's Disease and Parkinson's Disease May Result from Reactivation of Embryologic Pathways Silenced at Birth. Discovery Medicine. 2021; 31: 89–94.

[181] Lehrer S, Rheinstein PH. Vaccination Reduces Risk of Alzheimer's Disease, Parkinson's Disease and Other Neurodegenerative Disorders. Discovery Medicine. 2022; 34: 97–101.

[182] Wendeln AC, Degenhardt K, Kaurani L, Gertig M, Ulas T, Jain G, *et al*. Innate immune memory in the brain shapes neurological disease hallmarks. Nature. 2018; 556: 332–338.

[183] Harrison NA, Doeller CF, Voon V, Burgess N, Critchley HD. Peripheral inflammation acutely impairs human spatial memory via actions on medial temporal lobe glucose metabolism. Biological Psychiatry. 2014; 76: 585–593.

[184] Hueston CM, O'Leary JD, Hoban AE, Kozareva DA, Pawley LC, O'Leary OF, *et al*. Chronic interleukin-1β in the dorsal hippocampus impairs behavioural pattern separation. Brain, Behavior, and Immunity. 2018; 74: 252–264.

[185] D'Mello C, Le T, Swain MG. Cerebral microglia recruit monocytes into the brain in response to tumor necrosis factoralpha signaling during peripheral organ inflammation. The Journal of Neuroscience: the Official Journal of the Society for Neuroscience. 2009; 29: 2089–2102.

[186] Boland B, Yu WH, Corti O, Mollereau B, Henriques A, Bezard E, *et al*. Promoting the clearance of neurotoxic proteins in neurodegenerative disorders of ageing. Nature Reviews. Drug Discovery. 2018; 17: 660–688.

[187] Deo AK, Borson S, Link JM, Domino K, Eary JF, Ke B, *et al*. Activity of P-Glycoprotein, a β-Amyloid Transporter at the Blood-Brain Barrier, Is Compromised in Patients with Mild Alzheimer Disease. Journal of Nuclear Medicine: Official Publication, Society of Nuclear Medicine. 2014; 55: 1106–1111.

[188] Donahue JE, Flaherty SL, Johanson CE, Duncan JA, 3rd, Silverberg GD, Miller MC, *et al*. RAGE, LRP-1, and amyloid-beta protein in Alzheimer's disease. Acta Neuropathologica. 2006; 112: 405–415.

[189] Xiang Y, Bu XL, Liu YH, Zhu C, Shen LL, Jiao SS, *et al*. Physiological amyloid-beta clearance in the periphery and its therapeutic potential for Alzheimer's disease. Acta Neuropathologica. 2015; 130: 487–499.

[190] Shi Y, Liu Z, Shen Y, Zhu H. A Novel Perspective Linkage Between Kidney Function and Alzheimer's Disease. Frontiers in Cellular Neuroscience. 2018; 12: 384.

[191] Yu JT, Zhang C. Pathogenesis and Therapeutic Strategies in Alzheimer's Disease: From Brain to Periphery. Neurotoxicity Research. 2016; 29: 197–200.

[192] [191] Jucker M, Walker LC Propagation and spread of pathogenic protein assemblies in neurodegenerative diseases. Nature Neuroscience. 2018; 21: 1341–1349.

[193] Bhattacharyya R, Black SE, Lotlikar MS, Fenn RH, Jorfi M, Kovacs DM, *et al*. Axonal generation of amyloid-β from palmitoylated APP in mitochondria-associated endoplasmic reticulum membranes. Cell Reports. 2021; 35: 109134.

[194] Meyer-Luehmann M, Stalder M, Herzig MC, Kaeser SA, Kohler E, Pfeifer M, *et al*. Extracellular amyloid formation and associated pathology in neural grafts. Nature Neuroscience. 2003; 6: 370–377.

[195] Eisele YS, Obermüller U, Heilbronner G, Baumann F, Kaeser SA, Wolburg H, *et al*. Peripherally applied Abeta-containing inoculates induce cerebral beta-amyloidosis. Science (New York, N.Y.). 2010; 330: 980–982.

[196] Hasegawa M, Nonaka T, Masuda-Suzukake M. Prion-like mechanisms and potential therapeutic targets in neurodegenerative disorders. Pharmacology & Therapeutics. 2017; 172: 22–33.

[197] Meyer-Luehmann M, Coomaraswamy J, Bolmont T, Kaeser S, Schaefer C, Kilger E, *et al*. Exogenous induction of cerebral beta-amyloidogenesis is governed by agent and host. Science (New York, N.Y.). 2006; 313: 1781–1784.

[198] Venegas C, Kumar S, Franklin BS, Dierkes T, Brinkschulte R, Tejera D, *et al*. Microglia-derived ASC specks cross-seed amyloid-β in Alzheimer's disease. Nature. 2017; 552: 355–361.

[199] Grothe MJ, Sepulcre J, Gonzalez-Escamilla G, Jelistratova I, Schöll M, Hansson O, *et al*. Molecular properties underlying regional vulnerability to Alzheimer's disease pathology. Brain: a Journal of Neurology. 2018; 141: 2755–2771.

[200] Grothe MJ, Barthel H, Sepulcre J, Dyrba M, Sabri O, Teipel SJ, *et al*. In vivo staging of regional amyloid deposition. Neurology. 2017; 89: 2031–2038.

[201] De Calignon A, Polydoro M, Suarez-Calvet M, William C, Adamowicz DH, Kopeikina KJ, *et al.* Propagation of tau pathology in a model of early Alzheimer's disease. Neuron. 2012; 73: 685–697.

[202] Tardivel M, Bégard S, Bousset L, Dujardin S, Coens A, Melki R, *et al*. Tunneling nanotube (TNT)-mediated neuron-to neuron transfer of pathological Tau protein assemblies. Acta Neuropathologica Communications. 2016; 4: 117.

[203] Yamada K. Extracellular Tau and Its Potential Role in the Propagation of Tau Pathology. Frontiers in Neuroscience. 2017; 11: 667.

[204] Ayers JI, Giasson BI, Borchelt DR. Prion-like Spreading in Tauopathies. Biological Psychiatry. 2018; 83: 337–346.

[205] Kaufman SK, Sanders DW, Thomas TL, Ruchinskas AJ, Vaquer-Alicea J, Sharma AM, *et al*. Tau Prion Strains Dictate Patterns of Cell Pathology, Progression Rate, and Regional Vulnerability In Vivo. Neuron. 2016; 92: 796–812.

[206] Wu JW, Hussaini SA, Bastille IM, Rodriguez GA, Mrejeru A, Rilett K, *et al*. Neuronal activity enhances tau propagation and tau pathology in vivo. Nature Neuroscience. 2016; 19: 1085–1092.

[207] Cho H, Choi JY, Hwang MS, Kim YJ, Lee HM, Lee HS, *et al*. In vivo cortical spreading pattern of tau and amyloid in the Alzheimer disease spectrum. Annals of Neurology. 2016; 80: 247–258.

[208] Gomes LA, Hipp SA, Rijal Upadhaya A, Balakrishnan K, Ospitalieri S, Koper MJ, *et al*. Aβ-induced acceleration of Alzheimer-related τ-pathology spreading and its association

with prion protein. Acta Neuropathologica. 2019; 138: 913–941.
[209] Raven F, Ward JF, Zoltowska KM, Wan Y, Bylykbashi E, Miller SJ, *et al*. Soluble Gamma-secretase Modulators Attenuate Alzheimer's β-amyloid Pathology and Induce Conformational Changes in Presenilin 1. EBioMedicine. 2017; 24: 93–101.
[210] Rynearson KD, Ponnusamy M, Prikhodko O, Xie Y, Zhang C, Nguyen P, *et al*. Preclinical validation of a potent γ-secretase modulator for Alzheimer's disease prevention. The Journal of Experimental Medicine. 2021; 218: e20202560.
[211] Tagami S, Yanagida K, Kodama TS, Takami M, Mizuta N, Oyama H, *et al*. Semagacestat Is a Pseudo-Inhibitor of γ-Secretase. Cell Reports. 2017; 21: 259–273.
[212] Wagner SL, Rynearson KD, Duddy SK, Zhang C, Nguyen PD, Becker A, *et al*. Pharmacological and Toxicological Properties of the Potent Oral γ-Secretase Modulator BPN-15606. The Journal of Pharmacology and Experimental Therapeutics. 2017; 362: 31–44.
[213] Yang G, Zhou R, Guo X, Yan C, Lei J, Shi Y. Structural basis of γ-secretase inhibition and modulation by small molecule drugs. Cell. 2021; 184: 521–533.e14.
[214] Doody RS, Raman R, Farlow M, Iwatsubo T, Vellas B, Joffe S, *et al*. A phase 3 trial of semagacestat for treatment of Alzheimer's disease. The New England Journal of Medicine. 2013; 369: 341–350.
[215] Mitani Y, Yarimizu J, Saita K, Uchino H, Akashiba H, Shitaka Y, *et al*. Differential effects between γ-secretase inhibitors and modulators on cognitive function in amyloid precursor protein-transgenic and nontransgenic mice. The Journal of Neuroscience: the Official Journal of the Society for Neuroscience. 2012; 32: 2037–2050.
[216] Cai J, Qi X, Kociok N, Skosyrski S, Emilio A, Ruan Q, *et al*. β-Secretase (BACE1) inhibition causes retinal pathology by vascular dysregulation and accumulation of age pigment. EMBO Molecular Medicine. 2012; 4: 980–991.
[217] Sperling R, Henley D, Aisen PS, Raman R, Donohue MC, Ernstrom K, *et al*. Findings of Efficacy, Safety, and Biomarker Outcomes of Atabecestat in Preclinical Alzheimer Disease: A Truncated Randomized Phase 2b/3 Clinical Trial. JAMA Neurology. 2021; 78: 293–301.
[218] Kounnas MZ, Danks AM, Cheng S, Tyree C, Ackerman E, Zhang X, *et al*. Modulation of gamma-secretase reduces beta-amyloid deposition in a transgenic mouse model of Alzheimer's disease. Neuron. 2010; 67: 769–780.
[219] Rynearson KD, Buckle RN, Barnes KD, Herr RJ, Mayhew NJ, Paquette WD, *et al*. Design and synthesis of aminothiazole modulators of the gamma-secretase enzyme. Bioorganic & Medicinal Chemistry Letters. 2016; 26: 3928–3937.
[220] Wagner SL, Zhang C, Cheng S, Nguyen P, Zhang X, Rynearson KD, *et al*. Soluble γ-secretase modulators selectively inhibit the production of the 42-amino acid amyloid β peptide variant and augment the production of multiple carboxy-truncated amyloid β species. Biochemistry. 2014; 53: 702–713.
[221] Maesako M, Sekula NM, Aristarkhova A, Feschenko P, Anderson LC, Berezovska O. Visualization of PS/γ-Secretase Activity in Living Cells. iScience. 2020; 23: 101139.
[222] Placanica L, Zhu L, Li YM. Gender- and age-dependent gamma-secretase activity in mouse brain and its implication in sporadic Alzheimer disease. PloS One. 2009; 4: e5088.
[223] Xiang J, Tao Y, Xia Y, Luo S, Zhao Q, Li B, *et al*. Development of an alpha-synuclein positron emission tomography tracer for imaging synucleinopathies. Cell. 2023; 186: 3350–3367.e3319.
[224] Landau SM, Thomas BA, Thurfjell L, Schmidt M, Margolin R, Mintun M, *et al*. Amyloid PET imaging in Alzheimer's disease: a comparison of three radiotracers. European Journal of Nuclear Medicine and Molecular Imaging. 2014; 41: 1398–1407.
[225] James OG, Doraiswamy PM, Borges-Neto S. PET Imaging of Tau Pathology in Alzheimer's Disease and Tauopathies. Frontiers in Neurology. 2015; 6: 38.
[226] Zhang L, Chen L, Dutra JK, Beck EM, Nag S, Takano A, *et al*. Identification of a Novel Positron Emission Tomography (PET) Ligand for Imaging β-Site Amyloid Precursor Protein Cleaving Enzyme 1 (BACE-1) in Brain. Journal of Medicinal Chemistry. 2018; 61: 3296–3308.
[227] Xu Y, Wang C, Wey HY, Liang Y, Chen Z, Choi SH, *et al*. Molecular imaging of Alzheimer's disease-related gamma-secretase in mice and nonhuman primates. The Journal of Experimental Medicine. 2020; 217: e20182266.
[228] Pontecorvo MJ, Lu M, Burnham SC, Schade AE, Dage JL, Shcherbinin S, *et al*. Association of Donanemab Treatment With Exploratory Plasma Biomarkers in Early Symptomatic Alzheimer Disease: A Secondary Analysis of the TRAILBLAZER-ALZ Randomized Clinical Trial. JAMA Neurology. 2022; 79: 1250–1259.
[229] Cummings J, Lee G, Nahed P, Kambar MEZN, Zhong K, Fonseca J, *et al*. Alzheimer's disease drug development pipeline: 2022. Alzheimer's & Dementia (New York, N. Y.). 2022; 8: e12295.
[230] Alves F, Kalinowski P, Ayton S. Accelerated Brain Volume Loss Caused by Anti-β-Amyloid Drugs: A Systematic Review and Meta-analysis. Neurology. 2023; 100: e2114–e2124.
[231] Martorell AJ, Paulson AL, Suk HJ, Abdurrob F, Drummond GT, Guan W, *et al*. Multi-sensory Gamma Stimulation Ameliorates Alzheimer's-Associated Pathology and Improves Cognition. Cell. 2019; 177: 256–271.e22.
[232] Liu G, Mondal P, Sang N, Li Z, Ding W, Yang L, *et al*. Design, synthesis, and anti-inflammatory activity characterization of novel brain-permeable HDAC6 inhibitors. European Journal of Medicinal Chemistry. 2023; 254: 115327.
[233] Yan S, Wei X, Jian W, Qin Y, Liu J, Zhu S, *et al*. Pharmacological Inhibition of HDAC6 Attenuates NLRP3 Inflammatory Response and Protects Dopaminergic Neurons in Experimental Models of Parkinson's Disease. Frontiers in Aging Neuroscience. 2020; 12: 78.