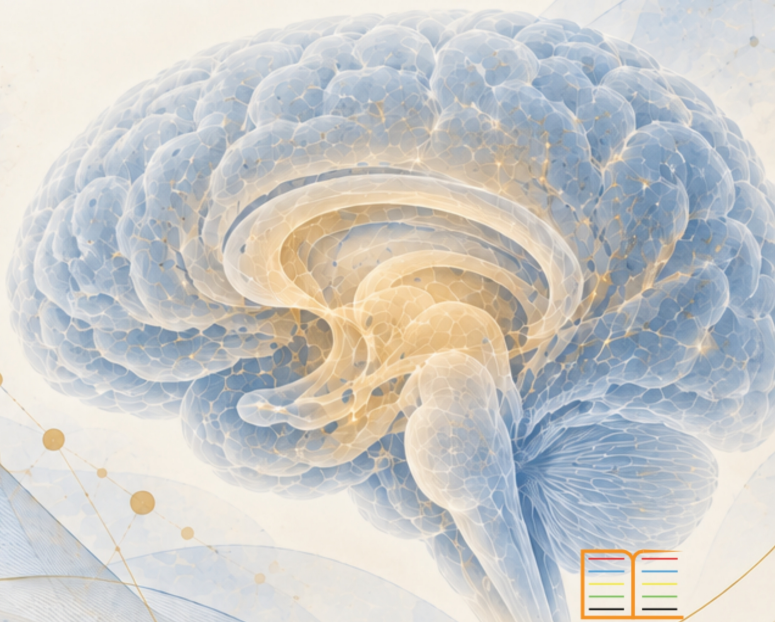
ALZHEIMER'S DISEASE:
MOLECULAR MECHANISMS AND THERAPEUTIC APPROACHES

EDITORS:
ASSOC. PROF. DR. AHMET GÖKHAN AĞGÜL &
ASST. PROF. DR. EMRAH DİKİCİ



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Alzheimer’s Disease: Molecular Mechanisms and Therapeutic Approaches

Editor: Assoc. Prof. Dr. Ahmet Gökhan AĞGÜL & Asst. Prof. Emrah DİKİCİ

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CHAPTER 1

AN OVERVIEW OF ALZHEIMER'S DISEASE

ERGİN TAŞKIN[1]
İREM ARSLANTÜRK[2]

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the leading cause of dementia worldwide, clinically defined by an insidious decline in memory and other cognitive domains that ultimately erodes a person's capacity for independent living. First described by the German psychiatrist and neuropathologist Alois Alzheimer in 1906 in a 51-year-old woman presenting with progressive memory loss, disorientation, and psychosocial impairment, the condition was for decades regarded as a rare presenile dementia before being recognized, over the course of the twentieth century, as the dominant form of dementia in older adults (Baviskar & Mahajan, 2026). More than a century after its first description, AD has evolved from a clinical curiosity into one of the defining public health challenges of the twenty first century,

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characterized by a long asymptomatic phase, a heterogeneous genetic and environmental risk architecture, and a pathophysiology that intersects protein misfolding, synaptic failure, vascular compromise, and chronic neuroinflammation (Safiri et al., 2024). This chapter provides an orienting overview of AD intended to frame the more detailed molecular, biochemical, and therapeutic discussions that follow in this volume. It surveys the historical background of the disease, its global epidemiological burden, the clinical continuum from preclinical changes to overt dementia, the classification of its major subtypes, its principal nonmodifiable and modifiable risk factors, a brief synthesis of the leading pathophysiological hypotheses, the general framework used for clinical diagnosis, and the socioeconomic burden the disease imposes on patients, families, and health systems. Rather than duplicating the molecular and pharmacological depth provided by later chapters on amyloid-β and tau biology, oxidative stress, the gut–brain axis, biochemical biomarkers, cholinergic and sigma-1 receptor signaling, and natural or synthetic therapeutic strategies, this chapter is deliberately structured as a conceptual map of the disease, situating those specialized discussions within the broader clinical and epidemiological landscape of AD.

2. Historical Perspective

The disease that today bears his name was first documented by Alois Alzheimer, who in 1906 presented the clinicopathological case of Auguste Deter, describing profound memory impairment, paranoid ideation, and progressive cognitive deterioration alongside the neuropathological findings of extracellular plaques and intracellular neurofibrillary changes on postmortem examination (Baviskar & Mahajan, 2026). For much of the twentieth century, this presentation was considered a distinct and uncommon presenile disorder, clinically separated from the far more common age related cognitive decline described under the umbrella of senile dementia. It

was only in the latter decades of the century, as neuropathological studies demonstrated that the amyloid plaques and neurofibrillary tangles described by Alzheimer were essentially identical in presenile and senile cases, that AD came to be understood as a single, continuous disease process spanning both early and late life (Baviskar & Mahajan, 2026). This reconceptualization, together with the subsequent identification of amyloid-β and tau as the principal constituents of these lesions, catalyzed the modern era of AD research and set the stage for the amyloid cascade, tauopathy, cholinergic, and neuroinflammatory hypotheses that continue to shape investigation into the disease's biological mechanisms (Safiri et al., 2024). Over the past two decades, this trajectory has continued: diagnostic practice has progressively shifted from reliance on clinical presentation and exclusion of other causes toward a biologically anchored framework incorporating cerebrospinal fluid and, increasingly, blood based biomarkers, while therapeutic development has moved from purely symptomatic agents toward disease modifying monoclonal antibodies targeting amyloid-β (Baviskar & Mahajan, 2026).

3. Epidemiology and Global Burden of Disease

AD and related dementias represent one of the fastest growing sources of morbidity and mortality among older adults globally. Global Burden of Disease modeling indicates that the age standardized prevalence of AD and other dementias among adults aged 65 years and older rose by approximately 160% between 1991 and 2021, from an estimated 18.7 million to 49 million affected individuals, with dementia attributable mortality in this age group more than doubling over the same interval (Zhu et al., 2025). Consistent with these trends, worldwide dementia prevalence is estimated to have surged by roughly 148% between 1990 and 2019, currently affecting approximately 55 million people, with projections indicating that more than 150 million individuals will be

living with the disease by 2050 as global populations continue to age (Twiss et al., 2025). More recent Global Burden of Disease projections extend this trajectory further, forecasting a 50.1% increase in age standardized prevalence by 2050, corresponding to approximately 191 million cases and 6.8 million deaths annually (Liu et al., 2026), while separate age period cohort modeling projects that annual incident AD cases will reach 19.12 million by 2036 (Xu et al., 2025). Epidemiological trends are not uniform across regions: incidence and prevalence rates are consistently higher in high income countries such as Japan, the United States, and members of the European Union, yet the sharpest relative increases in burden are increasingly observed in low and middle income countries, where population aging is occurring rapidly against a backdrop of limited diagnostic and care infrastructure (Baviskar & Mahajan, 2026). Women bear a disproportionate share of this burden, both because of their greater longevity and because several studies report higher age specific incidence rates among women than men across most world regions (Zhu et al., 2025).

4. The Clinical Continuum: From Preclinical Changes to Dementia

AD is now understood not as a discrete clinical event but as a continuum that unfolds over one to three decades, beginning with asymptomatic biological changes and culminating in progressive functional dependence. The National Institute on Aging Alzheimer's Association (NIA–AA) framework, first articulated in 2011, formalized this continuum into three broad stages: a preclinical stage, in which individuals harbor biomarker evidence of amyloid and, later, tau pathology without measurable cognitive or functional impairment; mild cognitive impairment (MCI) due to AD, characterized by measurable decline in memory or other cognitive domains that does not yet compromise independence in daily activities; and dementia due to AD, in which cognitive and

functional impairment become severe enough to interfere with everyday life (Sperling et al., 2011). The preclinical phase can begin twentyfive to thirty years before the emergence of overt symptoms, reflecting the slow, self-amplifying accumulation of amyloid-β and, subsequently, hyperphosphorylated tau (whose molecular mechanisms are examined in detail in the following chapter of this volume). Progression through the continuum is neither uniform nor guaranteed: only a fraction of individuals with MCI, on the order of 10–12% annually, go on to convert to dementia, while others remain clinically stable or, less commonly, revert to normal cognition (Safiri et al., 2024). Clinically, the dementia stage itself is typically subdivided into mild, moderate, and severe categories, reflecting an initial predominance of episodic memory loss and word finding difficulty that gradually extends to impairment of executive function, visuospatial skills, and language, and is eventually accompanied by behavioral and psychiatric symptoms, loss of activities of Daily living independence, and, in advanced stages, motor and swallowing dysfunction (Baviskar & Mahajan, 2026).

4.1. Classification: Early onset and late onset, familial and sporadic disease

AD is clinically and genetically heterogeneous and is conventionally classified according to age of onset. Early onset AD (EOAD), defined by symptom onset before the age of 65, accounts for a minority of cases, on the order of 5–10%, and is disproportionately associated with autosomal dominant mutations in the amyloid precursor protein (APP), presenilin-1 (PSEN1), or presenilin-2 (PSEN2) genes, which together account for a substantial fraction of familial, early onset disease (Dato et al., 2023). Late onset AD (LOAD), by contrast, accounts for the great majority of cases, typically manifests after age 65, and is best understood as a complex, polygenic, and largely sporadic disorder shaped by the cumulative interaction of numerous common genetic variants with age related,

vascular, metabolic, and lifestyle factors (Dato et al., 2023). This distinction is clinically consequential: EOAD more frequently presents with atypical, nonamnestic phenotypes involving language, visuospatial, or executive dysfunction, and its strong genetic determinism has made it the primary focus of biomarker confirmatory genetic testing and of dominantly inherited AD research cohorts, whereas LOAD, as the population level driver of the global dementia burden, is the principal target of the risk factor and prevention framework discussed in Section 5.

5. Risk Factors

5.1. Non modifiable risk factors: age, sex, and genetics

Advancing age remains, by a wide margin, the single strongest risk factor for AD, with incidence rising markedly across each successive decade after age 65 (Safiri et al., 2024). Female sex is independently associated with higher lifetime prevalence, reflecting both greater longevity and evidence of biological differences in disease susceptibility and progression (Zhu et al., 2025). Among genetic determinants, the ε4 allele of the apolipoprotein E (APOE) gene is the most robust and consistently replicated genetic risk factor for late onset AD, acting in a dose dependent manner such that ε3/ε4 heterozygosity confers an approximately three-fold increase in risk and ε4/ε4 homozygosity an even greater increase, through mechanisms that include impaired amyloid-β clearance, altered lipid transport, and heightened neuroinflammatory responsiveness (Dato et al., 2023). Genome wide association studies conducted over the past decade have identified dozens of additional common risk loci, including genes involved in innate immunity and microglial function, endocytosis, and lipid metabolism, that collectively refine polygenic risk beyond APOE alone, while rare, highly penetrant mutations in APP, PSEN1, and PSEN2 remain the defining genetic cause of autosomal dominant, early onset disease (Dato et al., 2023).

5.2. Modifiable risk factors

A substantial proportion of dementia risk is, encouragingly, attributable to factors that are at least partially modifiable across the life course. The 2024 report of the Lancet Standing Commission on dementia prevention, intervention, and care identifies fourteen such factors distributed across early life (less education), midlife (hearing loss, hypertension, obesity, excessive alcohol consumption, traumatic brain injury, and, newly added in the 2024 update, high LDL cholesterol), and later life (smoking, depression, physical inactivity, social isolation, diabetes, air pollution, and untreated vision loss, the latter also newly added in 2024), estimating that eliminating all fourteen factors could theoretically prevent or delay approximately 45% of dementia cases worldwide (Livingston et al., 2024). These risk factors do not act independently of genetic susceptibility: several cohort analyses indicate that the relationship between individual modifiable factors, such as diabetes or hypertension, and dementia risk is itself modulated by APOE ε4 carrier status, underscoring the gene–environment interactions that shape individual trajectories toward or away from clinical disease (Livingston et al., 2024). From a public health perspective, this evidence reframes AD not as an inevitable consequence of aging but as a condition whose population level incidence can be meaningfully influenced through cardiovascular risk management, sensory rehabilitation, cognitive and social engagement, and injury and pollution mitigation, even as effective disease modifying pharmacotherapy remains limited.

6. An Overview of Pathophysiological Hypotheses

AD pathophysiology is now understood as multifactorial rather than attributable to a single causal cascade, and several complementary hypotheses converge to explain its clinical and neuropathological features. The amyloid cascade hypothesis holds that the abnormal

accumulation of misfolded amyloid-β, generated through sequential cleavage of the amyloid precursor protein, initiates a chain of downstream neuronal dysfunction, while the tauopathy hypothesis emphasizes hyperphosphorylated tau and neurofibrillary tangle formation, which correlate more closely with the severity of cognitive decline than amyloid burden alone; the detailed molecular biology of both processes, including secretase activity and the kinases that drive tau hyperphosphorylation, is examined in the following chapter of this volume. The cholinergic hypothesis attributes much of the memory impairment characteristic of AD to degeneration of basal forebrain cholinergic neurons and the consequent loss of acetylcholine mediated signaling, a mechanism and its therapeutic implications that are addressed in depth later in this book, while oxidative stress is increasingly recognized as an early, self amplifying contributor that both promotes tau hyperphosphorylation and is aggravated by amyloid-β induced mitochondrial toxicity, a topic explored in its own dedicated chapter. Finally, systems level views converge on chronic neuroinflammation, driven substantially by microglial activation and the gut brain axis, as a hub that links amyloid, tau, vascular, and metabolic dysfunction into a single, self reinforcing pathological network (Safiri et al., 2024). Rather than competing explanations, these hypotheses are best understood as complementary and interacting facets of a single, multifactorial disease process, together constituting the principal molecular targets explored throughout the remainder of this volume.

7. The Diagnostic Approach

Clinical diagnosis of AD has traditionally rested on a structured history of progressive cognitive decline, standardized cognitive screening instruments such as the Mini Mental State Examination or Montreal Cognitive Assessment, informant based functional assessment, and the exclusion of alternative or reversible causes of

cognitive impairment through laboratory testing and structural neuroimaging (Safiri et al., 2024). Over the past decade, however, diagnostic practice has shifted toward an increasingly biologically anchored framework, formalized by the NIA–AA research criteria, which characterize disease status along the amyloid, tau, and neurodegeneration (ATN) axes using cerebrospinal fluid analysis and positron emission tomography, allowing preclinical and prodromal disease to be identified in vivo, years before the onset of measurable cognitive symptoms (Sperling et al., 2011). More recently, the emergence of validated blood based biomarkers, most notably plasma phosphorylated tau species, has begun to make biologically confirmed diagnosis feasible outside specialized tertiary centers, a development with substantial implications for earlier and more equitable detection (Baviskar & Mahajan, 2026). The full biochemical and molecular basis of these biomarkers, together with their diagnostic performance and clinical implementation, is treated as a dedicated subject later in this volume; here it suffices to note that contemporary diagnosis increasingly integrates clinical, cognitive, and biological evidence rather than relying on clinical impression alone.

8. Socioeconomic and Caregiving Burden

Beyond its epidemiological scale, AD imposes a profound socioeconomic burden that extends well beyond direct medical expenditure. Global spending attributable to dementia was estimated at approximately $263 billion in 2019 and is projected to rise to $1.6 trillion by 2050 under baseline assumptions, representing an increasing share of overall global health expenditure as populations age (Pedroza Velandia et al., 2022). These figures substantially understate the true societal cost of the disease, since the majority of day to day care is provided informally by family members, whose lost wages, reduced working hours, and diminished quality of life are frequently omitted from formal healthcare spending estimates yet

constitute the dominant component of the overall economic burden in most regions of the world (Twiss et al., 2025). The burden of informal caregiving also carries a substantial toll on caregivers themselves, who report elevated rates of physical and psychological strain as the disease progresses through its later, more dependent stages. Taken together with the direct costs of medical care, long term residential care, and lost productivity among both patients and caregivers, the socioeconomic burden of AD underscores the urgency of the search for accurate early biomarkers, effective disease modifying therapies, and scalable models of care that are examined throughout the remainder of this book (Safiri et al., 2024).

9. The Current Therapeutic Landscape: A Brief Overview

Pharmacological management of AD has, until recently, remained largely symptomatic. Acetylcholinesterase inhibitors and the N-methyl-D-aspartate receptor antagonist memantine, whose mechanisms are discussed in greater detail elsewhere in this volume, offer modest and time limited symptomatic benefit without altering the underlying disease trajectory (Baviskar & Mahajan, 2026). The recent regulatory approval of anti amyloid monoclonal antibodies, including lecanemab and donanemab, marks the first shift toward disease modifying therapy, producing statistically significant, though clinically modest, slowing of cognitive decline; these agents nonetheless carry substantial safety considerations, most notably amyloid related imaging abnormalities requiring ongoing magnetic resonance imaging monitoring, and their overall benefit–risk balance continues to be debated among regulators and clinicians alike (Baviskar & Mahajan, 2026). This combination of modest efficacy, safety burden, and cost has intensified interest in complementary and adjunctive strategies, including cholinesterase- and sigma-1 receptor targeted approaches and naturally derived multitarget compounds such as phenolic acids and flavonoids, whose distinct mechanisms and translational evidence are each addressed in dedicated chapters

later in this volume. A detailed treatment of current pharmacological and emerging therapeutic strategies is reserved for the closing chapter of this book.

10. Conclusion

Alzheimer's disease sits at the intersection of molecular neurobiology, clinical medicine, and public health, and no single chapter can capture its full complexity. This overview has traced the disease from its historical origins through its present day epidemiological scale, its clinical continuum from silent preclinical changes to overt dementia, its principal genetic and modifiable risk factors, a high level synthesis of its interacting pathophysiological mechanisms, the evolving framework used for its diagnosis, and the socioeconomic burden it imposes on individuals, families, and health systems worldwide. Each of these threads is taken up in far greater depth in the chapters that follow: the molecular mechanisms of amyloid-β and tau pathology, the role of oxidative stress, the emerging significance of the gut brain axis, the biochemical biomarkers that increasingly anchor diagnosis, the pathophysiological and therapeutic relevance of acetylcholinesterase and the sigma-1 receptor, the neuroprotective potential of phenolic compounds and flavonoids, the broader therapeutic promise of natural compounds, and, finally, the current and emerging pharmacological strategies being developed to change the trajectory of this disease. Together, these chapters aim to provide a comprehensive, mechanistically grounded, and clinically relevant account of Alzheimer's disease as it is understood at the present moment, while acknowledging that the scale of the epidemiological challenge described in this chapter continues to demand faster translation from laboratory discovery to effective clinical care.

References

Baviskar, P. S., & Mahajan, H. S. (2026). Unveiling Alzheimer's disease (1901–2025): Historical insights, global burden, biological mechanisms, diagnostics, and therapeutic strategies. *Ageing Research Reviews, 114*, 102990. https://doi.org/10.1016/j.arr.2025.102990

Dato, S., De Rango, F., Crocco, P., Pallotti, S., Belloy, M. E., Le Guen, Y., Greicius, M. D., Passarino, G., Rose, G., & Napolioni, V. (2023). Sex- and APOE-specific genetic risk factors for late-onset Alzheimer's disease: Evidence from gene–gene interaction of longevity-related loci. *Aging Cell, 22*(9), e13938. https://doi.org/10.1111/acel.13938

Liu, S., Wu, G., Ma, X., Xu, K., Lan, X., Wang, L., Li, H., Gu, D., Wang, M., & Liu, J. (2026). Trends and cross-country inequalities in the global burden of Alzheimer's disease and other dementias among adults aged 65+ years, 1990–2021: A population-based study with projections into 2050. *Frontiers in Aging Neuroscience, 17*, 1683158. https://doi.org/10.3389/fnagi.2025.1683158

Livingston, G., Huntley, J., Liu, K. Y., Costafreda, S. G., Selbæk, G., Alladi, S., Ames, D., Banerjee, S., Burns, A., Brayne, C., Fox, N. C., Ferri, C. P., Gitlin, L. N., Howard, R., Kales, H. C., Kivimäki, M., Larson, E. B., Nakasujja, N., Rockwood, K., . . . Mukadam, N. (2024). Dementia prevention, intervention, and care: 2024 report of the Lancet standing Commission. *The Lancet, 404*(10452), 572–628. https://doi.org/10.1016/S0140-6736(24)01296-0

Pedroza Velandia, P., Miller-Petrie, M. K., Chen, C., Chakrabarti, S., Chapin, A., Hay, S., Tsakalos, G., Wimo, A., & Dieleman, J. L. (2022). Global and regional spending on dementia care from 2000–2019 and expected future health spending scenarios

from 2020–2050: An economic modelling exercise. *eClinicalMedicine, 45*, 101337. https://doi.org/10.1016/j.eclinm.2022.101337

Safiri, S., Ghaffari Jolfayi, A., Fazlollahi, A., Morsali, S., Sarkesh, A., Daei Sorkhabi, A., Golabi, B., Aletaha, R., Motlagh Asghari, K., Hamidi, S., Mousavi, S. E., Jamalkhani, S., Karamzad, N., Shamekh, A., Mohammadinasab, R., Sullman, M. J. M., Şahin, F., & Kolahi, A.-A. (2024). Alzheimer's disease: A comprehensive review of epidemiology, risk factors, symptoms diagnosis, management, caregiving, advanced treatments and associated challenges. *Frontiers in Medicine, 11*, 1474043. https://doi.org/10.3389/fmed.2024.1474043

Sperling, R. A., Aisen, P. S., Beckett, L. A., Bennett, D. A., Craft, S., Fagan, A. M., Iwatsubo, T., Jack, C. R., Kaye, J., Montine, T. J., Park, D. C., Reiman, E. M., Rowe, C. C., Siemers, E., Stern, Y., Yaffe, K., Carrillo, M. C., Thies, B., Morrison-Bogorad, M., . . . Phelps, C. H. (2011). Toward defining the preclinical stages of Alzheimer's disease: Recommendations from the National Institute on Aging–Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & Dementia, 7*(3), 280–292. https://doi.org/10.1016/j.jalz.2011.03.003

Twiss, E., McPherson, C., & Weaver, D. F. (2025). Global diseases deserve global solutions: Alzheimer's disease. *Neurology International, 17*(6), 92. https://doi.org/10.3390/neurolint17060092

Xu, L., Wang, Z., Li, M., & Li, Q. (2025). Global incidence trends and projections of Alzheimer disease and other dementias: An age-period-cohort analysis 2021. *Journal of Global Health, 15*, 04156. https://doi.org/10.7189/jogh.15.04156

Zhu, X., Yu, J., Lai, X., Wang, X., Deng, J., Long, Y., & Li, B. (2025). Global burden of Alzheimer's disease and other dementias in adults aged 65 years and older, 1991–2021: Population-based

study. *Frontiers in Public Health, 13*, 1585711. https://doi.org/10.3389/fpubh.2025.1585711

CHAPTER 2

AMYLOID-β AND TAU: MOLECULAR MECHANISMS IN ALZHEIMER'S DISEASE

EMRAH DİKİCİ[1]

Alzheimer's disease is a progressive and multilayered neurodegenerative process characterized by the coexistence of senile plaques associated with extracellular amyloid-β (Aβ) accumulation and neurofibrillary tangles composed of intracellular hyperphosphorylated tau aggregates (Paula et al., 2009; Ashford, 2019; Roda et al., 2022; Abyadeh et al., 2024; Abdulkhaliq et al., 2026). However, the current literature has moved beyond the classical approach that reduces the biopathological framework of the disease solely to the presence of "plaques" and "tangles"; it has demonstrated that the mutually reinforcing interaction between Aβ and tau, together with synaptic failure, network-level disruption, glial response, mitochondrial stress, and neuronal loss, constitutes the principal destructive axis of the disease (Busche & Hyman, 2020; Zhang et al., 2021; Garbuz et al., 2021; Aranda-Abreu et al., 2026; Abdulkhaliq et al., 2026). From both clinical and neuropathological

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perspectives, it has been reported that Aβ accumulation often begins years before the onset of disease symptoms, whereas the relationship between the regional spread of tau pathology and cognitive impairment as well as neuronal damage is stronger (Ashford, 2019; Roda et al., 2022; van der Kant et al., 2020; Busche & Hyman, 2020; Capilla-López et al., 2025). For this reason, the current perspective considers Aβ not merely as an initiating trigger and tau not merely as an end product, but rather regards both proteins as dynamic determinants that shape one another throughout the temporal and spatial evolution of the disease (Zhang et al., 2021; Busche & Hyman, 2020; Zhuang et al., 2024; Abdulkhaliq et al., 2026).

The biochemical basis of Aβ accumulation relies on the non-physiological processing of amyloid precursor protein (APP) (Paula et al., 2009; Kikuchi et al., 2017; Roda et al., 2022; Azargoonjahromi, 2024; Abdulkhaliq et al., 2026). In the non-amyloidogenic pathway, when APP is cleaved by α-secretase within the Aβ sequence, sAPPα and C83 are generated; subsequent γ-secretase activity then produces the non-toxic P3 fragment (Paula et al., 2009; Roda et al., 2022; Azargoonjahromi, 2024; Abdulkhaliq et al., 2026). In contrast, in the amyloidogenic pathway, β-secretase/BACE1 converts APP into the C99 fragment, and γ-secretase releases Aβ peptides from this fragment (Kikuchi et al., 2017; Roda et al., 2022; Abyadeh et al., 2024; Azargoonjahromi, 2024; Abdulkhaliq et al., 2026). The resulting peptides are predominantly in the forms of Aβ40 and Aβ42; although Aβ40 is produced in greater abundance, Aβ42 is recognized as being more hydrophobic, more prone to aggregation, and more susceptible to plaque nucleation (Paula et al., 2009; Roda et al., 2022; Abyadeh et al., 2024; Azargoonjahromi, 2024; Abdulkhaliq et al., 2026). Therefore, the problem in Alzheimer’s disease is not merely the presence of Aβ production, but rather the shift of APP metabolism from the non-amyloidogenic pathway to the amyloidogenic pathway

and the chronic disruption of this balance, particularly in favor of Aβ42 (Kikuchi et al., 2017; Polanco et al., 2018; Garbuz et al., 2021; Azargoonjahromi, 2024).

Aβ peptides are not uniform molecules; rather, they exhibit multiple interconvertible structural states, including monomers, oligomers, protofibrils, fibrils, and plaques (Polanco et al., 2018; Roda et al., 2022; Azargoonjahromi, 2024; Abdulkhaliq et al., 2026). Within this transformation continuum, current evidence indicates that the principal neurotoxic species are, in many cases, soluble Aβ oligomers rather than large mature plaques (Roda et al., 2022; Guntupalli et al., 2016; Behroozi et al., 2026; Abdulkhaliq et al., 2026; Garbuz et al., 2021). Oligomers can target the synaptic membrane, disrupt glutamatergic transmission, perturb calcium homeostasis, alter receptor trafficking, and suppress synaptic plasticity, thereby weakening the cellular basis of learning and memory processes (Guntupalli et al., 2016; Behroozi et al., 2026; Santos et al., 2024; Capilla-López et al., 2025). It is emphasized that Aβ can contribute pathologically not only as an extracellular plaque component but also in the form of intraneuronal oligomeric species; moreover, it accumulates especially around synapses in the early stages, thereby initiating communication defects (Roda et al., 2022; Abdulkhaliq et al., 2026; Behroozi et al., 2026). Nevertheless, it has also been reported that, at physiological levels, Aβ may assume certain regulatory roles in processes such as synaptic plasticity, neuronal survival, and even antioxidant defense, but that these physiological functions are transformed into a harmful biochemical burden under conditions of pathological overaccumulation and misfolding (Roda et al., 2022; Azargoonjahromi, 2024; Garbuz et al., 2021).

It has become increasingly accepted in recent years that Aβ accumulation cannot be explained solely by increased production and that, particularly in sporadic Alzheimer’s disease, impaired

clearance is a decisive factor (Kikuchi et al., 2017; Xin et al., 2018; Roda et al., 2022; van der Kant et al., 2020). The removal of Aβ from the brain occurs through multiple mechanisms, including proteases, the ubiquitin–proteasome system, the autophagy–lysosomal pathway, microglial phagocytosis, transport across the blood–brain barrier, perivascular drainage, glymphatic flow, and meningeal lymphatic drainage (Xin et al., 2018; Kikuchi et al., 2017; Reddy & Oliver, 2019). Age-related weakening of these mechanisms, APOE4-associated transport defects, disruption of vascular integrity, and collapse of proteostasis accelerate Aβ accumulation and contribute to the progressive increase in amyloid burden (Kikuchi et al., 2017; Xin et al., 2018; van der Kant et al., 2020; Aranda-Abreu et al., 2026). Indeed, when the production–clearance balance, defined as Aβ economy, is disrupted, a neurotoxic environment is created through the combined effects of a peptide composition shifted particularly in favor of Aβ42 and an insufficient removal capacity (Kikuchi et al., 2017; Azargoonjahromi, 2024; Abdulkhaliq et al., 2026). In this context, Aβ accumulation should be interpreted not only as an increase in protein levels, but also as a systemic failure of the brain's intrinsic clearance networks (Xin et al., 2018; Garbuz et al., 2021; Aranda-Abreu et al., 2026).

Under physiological conditions, tau protein is a microtubule-associated protein that is highly abundant in neuronal axons, supports microtubule stabilization, and regulates axonal transport (Paula et al., 2009; Abyadeh et al., 2024; Zhang et al., 2021; Bloom & Norambuena, 2024; Abdulkhaliq et al., 2026). The six major isoforms generated through alternative splicing of the MAPT gene contain either three or four microtubule-binding repeats, and the binding strength and intracellular behavior of tau vary according to this isoform composition and post-translational modifications (Abyadeh et al., 2024; Polanco et al., 2018). The normal function of tau is to support tubulin polymerization, maintain cytoskeletal

organization, and preserve neuronal integrity; however, modifications such as phosphorylation, acetylation, truncation, nitration, and ubiquitination may transform this physiological role into a pathological form (Abyadeh et al., 2024; Polanco et al., 2018; Zhang et al., 2021). Hyperphosphorylation in particular reduces the affinity of tau for microtubules, leads to its mislocalization from the axonal compartment to the soma and dendrites, and facilitates the emergence of soluble toxic tau species (Zhang et al., 2021; Rosa et al., 2016; Bloom & Norambuena, 2024). This process is important because it demonstrates that tau may be biologically harmful not only as a final tangle-forming product, but also before tangle formation occurs (Rosa et al., 2016; Busche & Hyman, 2020; Abdulkhaliq et al., 2026).

One of the defining events in tau dysregulation is abnormal phosphorylation occurring at multiple serine/threonine sites (Zhang et al., 2021; Lloret et al., 2015; Abdulkhaliq et al., 2026). GSK-3β, CDK5, MAPK/ERK, p38 MAPK, and other related kinase networks facilitate tau hyperphosphorylation, whereas impairments in phosphatase function further intensify this process (Lloret et al., 2015; Zhang et al., 2021; Azargoonjahromi, 2024). After dissociating from microtubules, hyperphosphorylated tau tends to oligomerize, then to form paired helical filaments (PHFs), and ultimately to develop into neurofibrillary tangles (Paula et al., 2009; Roda et al., 2022; Abyadeh et al., 2024). However, current experimental findings indicate that neuronal toxicity does not arise solely from mature tangles; soluble and mislocalized tau species also produce marked early damage to synaptic structure and plasticity (Rosa et al., 2016; Busche & Hyman, 2020; Capilla-López et al., 2025). For this reason, tau pathology should not be viewed as a static process of accumulation, but rather as an active process of dysregulation that progressively disrupts intracellular architecture, axon–dendrite polarization, synaptic protein organization, and

transcriptional responses (Bloom & Norambuena, 2024; Behroozi et al., 2026; Abdulkhaliq et al., 2026).

Although the relationship between Aβ and tau was long interpreted as a linear "amyloid cascade," current evidence indicates that it represents a more complex and bidirectional interaction (Roda et al., 2022; Zhang et al., 2021; Busche & Hyman, 2020; van der Kant et al., 2020; Abdulkhaliq et al., 2026). Aβ oligomers can increase tau hyperphosphorylation by activating GSK-3β, CDK5, p38 MAPK, Fyn, and related signaling nodes; this, in turn, facilitates the translocation of tau to the dendritic compartment, its accumulation in postsynaptic structures, and the progression of synaptic damage (Lloret et al., 2015; Zhang et al., 2021; Abdulkhaliq et al., 2026). Conversely, tau is not merely a passive recipient of Aβ toxicity; in experimental models, reduction of tau levels has been shown to alleviate Aβ-associated synaptic, network, and cognitive impairments, indicating that tau behaves as an influential mediator of Aβ neurotoxicity (Rosa et al., 2016; Busche & Hyman, 2020; Zhang et al., 2021). Indeed, the accumulation of Aβ and tau within the same pathological regions facilitates tau seeding and propagation, particularly around neuritic plaques; in the presence of Aβ, the spread of tau into cortical areas and throughout neural networks is accelerated (Busche & Hyman, 2020; van der Kant et al., 2020; Capilla-López et al., 2025). Therefore, it appears more explanatory to interpret the Aβ–tau relationship not simply through an "upstream/downstream" framework, but rather as a pathological positive feedback loop in which each reinforces the other (Roda et al., 2022; Zhang et al., 2021; Busche & Hyman, 2020; Abdulkhaliq et al., 2026).

One of the most visible consequences of this bidirectional interaction is synaptic disruption. Aβ oligomers impair transmission mediated by AMPA and NMDA receptors, increase AMPA receptor internalization, suppress long-term potentiation (LTP), and lead to

dendritic spine loss (Guntupalli et al., 2016; Behroozi et al., 2026). Tau, in turn, disrupts postsynaptic density proteins, excitatory network activity, and presynaptic organization; in particular, pathological dendritic tau, together with Aβ, renders synaptic function and plasticity even more vulnerable (Busche & Hyman, 2020; Behroozi et al., 2026; Capilla-López et al., 2025). It has been reported that Aβ and tau may exert differential effects with varying weights across distinct circuits in regions such as the hippocampus and amygdala, yet when present together, they produce especially synergistic damage in hippocampal LTP and memory circuits (Capilla-López et al., 2025). In addition, the ability of tau to reduce BDNF expression, together with the capacity of Aβ to decrease BDNF transcript IV levels through tau-dependent mechanisms, suggests that the axis of synaptic resilience and neurotrophic support is weakened by the combined presence of Aβ and tau (Rosa et al., 2016). In this way, protein accumulation is not limited to the formation of deposits, but is transformed into cognitive destruction by targeting the mechanisms of plasticity, which constitute the most critical physiological basis of neuronal communication (Behroozi et al., 2026; Santos et al., 2024; Capilla-López et al., 2025).

Another important dimension of the interaction between Aβ and tau is its integration with neuroinflammation and cellular stress networks (Garbuz et al., 2021; Aranda-Abreu et al., 2026; Zhuang et al., 2024). It has been shown that Aβ activates microglia and astrocytes, thereby increasing the production of cytokines, complement components, and oxidants, and that this pro-inflammatory environment further facilitates tau pathology (Aranda-Abreu et al., 2026; Zhuang et al., 2024). In the opposite direction, tau accumulation may also exacerbate glial responses and synaptic pruning, thereby expanding and chronicizing Aβ-induced damage (Zhang et al., 2021; Roda et al., 2022; Aranda-Abreu et al., 2026). Emerging approaches that consider PANoptosis together with

pyroptosis, necroptosis, and apoptosis suggest that the accumulation of Aβ and tau, in the presence of inflammation, may trigger neuronal death programs through multiple mechanisms (Zhuang et al., 2024). When defects in autophagy and mitophagy are added to this picture, intracellular waste clearance capacity declines even further, thereby strengthening the cycle of pathological accumulation for both Aβ and hyperphosphorylated tau (Reddy & Oliver, 2019; Haghi et al., 2025). Therefore, Aβ and tau accumulation should not be regarded as two independent deposition phenomena, but rather as components of a shared degenerative ecosystem intertwined with inflammation, proteostasis impairment, and programmed cell death networks (Garbuz et al., 2021; Zhuang et al., 2024; Aranda-Abreu et al., 2026).

Mitochondrial and metabolic dysfunction also constitute components that cannot be overlooked within this context. Aβ accumulation increases oxidative stress, disrupts mitochondrial dynamics, and reduces energy production, while tau pathology further deepens this dysfunction by promoting calcium overload, increased ROS generation, and neuronal vulnerability (Lloret et al., 2015; Reddy & Oliver, 2019; Ebrahimi et al., 2026). Recent evidence suggests that Aβ oligomers can aberrantly redirect mTORC1 signaling in a tau-dependent manner, linking this process to early pathogenic events such as inappropriate cell-cycle re-entry and disrupted mitochondria–lysosome communication (Bloom & Norambuena, 2024). In addition, data from Drosophila models have shown that tau and Aβ can impair autophagy-related genes through oxidative stress, and that antioxidant intervention may partially alleviate these effects (Haghi et al., 2025). Recent studies investigating mitochondrial calcium dysregulation have also emphasized that Aβ and tau synergistically disrupt calcium homeostasis, thereby increasing ATP depletion, ROS production, and the tendency toward neuronal death (Ebrahimi et al., 2026). These mechanisms explain the biochemical damage associated with

the Aβ–tau interaction not only through structural protein aggregation, but also through the collapse of the cellular control systems governing energy homeostasis and survival (Lloret et al., 2015; Bloom & Norambuena, 2024; Ebrahimi et al., 2026). Nevertheless, it is clear that the entire pathogenesis of Alzheimer's disease cannot be fully explained by a linear scheme of "Aβ first, tau later." Human PET and neuropathological data suggest that the initial regions of Aβ and tau deposition do not fully overlap; tau may be observed in medial temporal structures even in the absence of Aβ, whereas its neocortical spread appears to become more pronounced in most cases in the presence of Aβ (Ashford, 2019; van der Kant et al., 2020; Busche & Hyman, 2020). This indicates that Aβ may act as a potent accelerator facilitating tau pathology, but that, particularly in sporadic Alzheimer's disease, Aβ-independent or partially Aβ-independent upstream regulatory pathways, such as lipid metabolism, the endosomal system, microglial responses, and cholesterol homeostasis, also shape tau biology (van der Kant et al., 2020; Polanco et al., 2018). Similarly, proteomic analyses have revealed the presence of novel proteins associated with Aβ pathology and broad alterations in molecular networks, supporting the view that the tissue-level impact of amyloid burden extends beyond the classical plaque-centered narrative (Montero-Calle et al., 2023). For this reason, the current synthesis requires that Aβ and tau be evaluated not as part of a single-axis chain of causality, but within a framework of shared upstream regulators, reciprocal feedback loops, and region-specific patterns of vulnerability (Busche & Hyman, 2020; van der Kant et al., 2020; Capilla-López et al., 2025).

In conclusion, amyloid-β accumulation and tau protein dysregulation in Alzheimer's disease are not two disconnected histopathological events; rather, they constitute a unified pathobiological axis positioned at the center of interwoven processes, including the shift in APP processing, disruption of the

production–clearance balance, formation of toxic oligomers, tau hyperphosphorylation, loss of synaptic plasticity, reduction in neurotrophic support, glial activation, defects in autophagy/mitophagy, and disturbances in mTOR and calcium homeostasis (Paula et al., 2009; Roda et al., 2022; Zhang et al., 2021; Abdulkhaliq et al., 2026). Although Aβ often serves as an early trigger and facilitating factor, tau pathology is more closely associated with the clinical severity of the disease, its regional spread, and neuronal destruction; however, the most devastating effect emerges when these two proteins behave synergistically within the same biological network (Ashford, 2019; Busche & Hyman, 2020; van der Kant et al., 2020; Capilla-López et al., 2025). Therefore, an effective biochemical approach must move beyond strategies targeting Aβ alone or tau alone, and instead necessitates the development of integrated strategies that simultaneously address Aβ–tau crosstalk, kinase/phosphatase balance, clearance mechanisms, synaptic resilience, and the neuroinflammatory environment (Zhang et al., 2021; Roda et al., 2022; Busche & Hyman, 2020; Aranda-Abreu et al., 2026; Zhuang et al., 2024).

References

Abdulkhaliq, A. A., Kim, B., Almoghrabi, Y. M., Khan, J., Ajoolabady, A., Ren, J., Bahijri, S., Tuomilehto, J., Borai, A., & Pratico, D. (2026). Amyloid-β and tau in Alzheimer's disease: Pathogenesis, mechanisms, and interplay. *Cell Death & Disease, 17*(1), 21. https://doi.org/10.1038/s41419-025-08186-8

Abyadeh, M., Gupta, V., Paulo, J. A., Gohari Mahmoudabad, A., Shadfar, S., Mirshahvaladi, S., Gupta, V., Nguyen, C. T. O., Finkelstein, D. I., You, Y., Haynes, P. A., Salekdeh, G. H., Graham, S. L., & Mirzaei, M. (2024). Amyloid-beta and tau protein beyond Alzheimer's disease. *Neural Regeneration Research, 19*(6), 1262–1276. https://doi.org/10.4103/1673-5374.386406

Aranda-Abreu, G. E., Rojas-Durán, F., Hernández-Aguilar, M. E., Herrera-Covarrubias, D., Tlapa-Monge, L. R., & Mestizo-Gutiérrez, S. L. (2026). Alzheimer's disease as a disorder of neuroimmune dysregulation. *Neurology International, 18*, 37. https://doi.org/10.3390/neurolint18020037

Ashford, J. W. (2019). The dichotomy of Alzheimer's disease pathology: Amyloid-β and tau. *Journal of Alzheimer's Disease, 68*(1), 77–83. https://doi.org/10.3233/JAD-181198

Azargoonjahromi, A. (2024). The duality of amyloid-β: Its role in normal and Alzheimer's disease states. *Molecular Brain, 17*, 44. https://doi.org/10.1186/s13041-024-01118-1

Behroozi, Z., Askarpour, H., & Baghcheghi, Y. (2026). Molecular sabotage of hippocampal synaptic plasticity by amyloid-β in Alzheimer's disease: A narrative review. *Journal of Alzheimer's Disease Reports, 10*, 25424823261415807. https://doi.org/10.1177/25424823261415807

Bloom, G. S., & Norambuena, A. (2024). Dysregulation of mTOR by tau in Alzheimer's disease. *Cytoskeleton, 81*(1), 30–34. https://doi.org/10.1002/cm.21782

Busche, M. A., & Hyman, B. T. (2020). Synergy between amyloid-β and tau in Alzheimer's disease. *Nature Neuroscience, 23*(10), 1183–1193. https://doi.org/10.1038/s41593-020-0687-6

Capilla-López, M. D., Deprada, A., Andrade-Talavera, Y., Martínez-Gallego, I., Coatl-Cuaya, H., Sotillo, P., Rodríguez-Alvarez, J., Rodríguez-Moreno, A., Parra-Damas, A., & Saura, C. A. (2025). Synaptic vulnerability to amyloid-β and tau pathologies differentially disrupts emotional and memory neural circuits. *Molecular Psychiatry, 30*(7), 2966–2979. https://doi.org/10.1038/s41380-025-02901-9

Ebrahimi, R., Hatami, S., Hashempoor, A., Oraee, S., Salarvandian, S., Faramarzi, A., & Esmaeilpour, K. (2026). Role of mitochondrial calcium dysregulation in Alzheimer's disease pathogenesis. *Molecular Neurobiology, 63*(1), 214. https://doi.org/10.1007/s12035-025-05465-5

Garbuz, D. G., Zatsepina, O. G., & Evgen'ev, M. B. (2021). Beta amyloid, tau protein, and neuroinflammation: An attempt to integrate different hypotheses of Alzheimer's disease pathogenesis. *Molecular Biology, 55*(5), 670–682. https://doi.org/10.1134/S002689332104004X

Guntupalli, S., Widagdo, J., & Anggono, V. (2016). Amyloid-β-induced dysregulation of AMPA receptor trafficking. *Neural Plasticity, 2016*, 3204519. https://doi.org/10.1155/2016/3204519

Haghi, M., Masoudi, R., Ataellahi, F., Yousefi, R., & Najibi, S. M. (2025). Role of tau and amyloid-beta in autophagy gene dysregulation through oxidative stress. *Tissue and Cell, 93*, 102765. https://doi.org/10.1016/j.tice.2025.102765

Kikuchi, K., Kidana, K., Tatebe, T., & Tomita, T. (2017). Dysregulated metabolism of the amyloid-β protein and therapeutic approaches in Alzheimer disease. *Journal of Cellular Biochemistry, 118*, 4183–4190. https://doi.org/10.1002/jcb.26129

Lloret, A., Fuchsberger, T., Giraldo, E., & Viña, J. (2015). Molecular mechanisms linking amyloid β toxicity and tau hyperphosphorylation in Alzheimer's disease. *Free Radical Biology and Medicine, 83*, 186–191. https://doi.org/10.1016/j.freeradbiomed.2015.02.028

Montero-Calle, A., Coronel, R., Garranzo-Asensio, M., Solís-Fernández, G., Rábano, A., de Los Ríos, V., Fernández-Aceñero, M. J., Mendes, M. L., Martínez-Useros, J., Megías, D., Moreno-Casbas, M. T., Peláez-García, A., Liste, I., & Barderas, R. (2023). Proteomics analysis of prefrontal cortex of Alzheimer's disease patients revealed dysregulated proteins in the disease and novel proteins associated with amyloid-β pathology. *Cellular and Molecular Life Sciences, 80*(6), 141. https://doi.org/10.1007/s00018-023-04791-y

Paula, V. de J. R. de, Guimarães, F. M., Diniz, B. S., & Forlenza, O. V. (2009). Neurobiological pathways to Alzheimer's disease: Amyloid-beta, tau protein or both? *Dementia & Neuropsychologia, 3*(3), 188–194. https://doi.org/10.1590/S1980-57642009DN30300003

Polanco, J. C., Li, C., Bodea, L.-G., Martinez-Marmol, R., Meunier, F. A., & Götz, J. (2018). Amyloid-β and tau complexity—Towards improved biomarkers and targeted therapies. *Nature Reviews Neurology, 14*, 22–39. https://doi.org/10.1038/nrneurol.2017.162

Reddy, P. H., & Oliver, D. M. (2019). Amyloid beta and phosphorylated tau-induced defective autophagy and mitophagy in

Alzheimer's disease. *Cells, 8*(5), 488. https://doi.org/10.3390/cells8050488

Roda, A. R., Serra-Mir, G., Montoliu-Gaya, L., Tiessler, L., & Villegas, S. (2022). Amyloid-beta peptide and tau protein crosstalk in Alzheimer's disease. *Neural Regeneration Research, 17*(8), 1666–1674. https://doi.org/10.4103/1673-5374.332127

Rosa, E., Mahendram, S., Ke, Y. D., Ittner, L. M., Ginsberg, S. D., & Fahnestock, M. (2016). Tau downregulates BDNF expression in animal and cellular models of Alzheimer's disease. *Neurobiology of Aging, 48*, 135–142. https://doi.org/10.1016/j.neurobiolaging.2016.08.020

Santos, H. M. dos, Bertollo, A. G., Mingoti, M. E. D., Grolli, R. E., Kreuz, K. M., & Ignácio, Z. M. (2024). Dementia and depression: Biological connections with amyloid β protein. *Basic & Clinical Pharmacology & Toxicology, 134*(5), 563–573. https://doi.org/10.1111/bcpt.13996

van der Kant, R., Goldstein, L. S. B., & Ossenkoppele, R. (2020). Amyloid-β-independent regulators of tau pathology in Alzheimer disease. *Nature Reviews Neuroscience, 21*, 21–35. https://doi.org/10.1038/s41583-019-0240-3

Xin, S.-H., Tan, L., Cao, X., Yu, J.-T., & Tan, L. (2018). Clearance of amyloid beta and tau in Alzheimer's disease: From mechanisms to therapy. *Neurotoxicity Research, 34*(3), 733–748. https://doi.org/10.1007/s12640-018-9895-1

Zhang, H., Wei, W., Zhao, M., Ma, L., Jiang, X., Pei, H., Cao, Y., & Li, H. (2021). Interaction between Aβ and tau in the pathogenesis of Alzheimer's disease. *International Journal of Biological Sciences, 17*(9), 2181–2192. https://doi.org/10.7150/ijbs.57078

Zhuang, X., Lin, J., Song, Y., Ban, R., Zhao, X., Xia, Z., Wang, Z., & Zhang, G. (2024). The interplay between accumulation of amyloid-beta and tau proteins, PANoptosis, and inflammation in Alzheimer's disease. *NeuroMolecular Medicine, 27*(1), 2. https://doi.org/10.1007/s12017-024-08815-z

CHAPTER 3

THE ROLE OF OXIDATIVE STRESS IN ALZHEIMER'S DISEASE

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In Alzheimer's disease, oxidative stress is no longer regarded merely as a secondary biochemical event that emerges after disease progression, but rather as one of the principal pathogenic axes that, from the early stages onward, increases neuronal vulnerability, impairs synaptic function, collapses energy metabolism, and accelerates the neurodegenerative process (Tchekalarova and Tzoneva, 2023; Plascencia-Villa and Perry, 2023; Alkhalifa et al., 2025; Dhapola et al., 2024). Oxidative stress refers to the redox imbalance that develops when the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) exceeds the cellular capacity to detoxify them or to repair the damage they cause (Reddy, 2023; Houldsworth, 2024; Alkhalifa et al., 2025). In Alzheimer's disease, this imbalance leads to the oxidative modification of fundamental biomolecules such as lipids, proteins, DNA, and RNA, disruption of membrane integrity, disturbance of calcium homeostasis, mitochondrial failure, and ultimately loss of

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neuronal function (Nikolenko et al., 2022; Plascencia-Villa and Perry, 2023; Ekundayo et al., 2024). Therefore, oxidative stress is not merely an accompanying component of Alzheimer's pathology, but rather an active determinant that shapes the biochemical and cellular architecture of the disease in a multidirectional manner (Rummel and Butterfield, 2022; Firdous et al., 2024; Dhapola et al., 2024).

The susceptibility of the brain to oxidative damage explains why this mechanism is so central in the context of Alzheimer's disease (Houldsworth, 2024; Alkhalifa et al., 2025; Foret et al., 2024). Neural tissue provides an environment that is systemically vulnerable to oxidative attack because of its high oxygen consumption, membrane structure rich in polyunsaturated fatty acids, the presence of redox-active metals such as iron and copper, and limited antioxidant reserves (Nikolenko et al., 2022; Houldsworth, 2024; Foret et al., 2024). The fact that neurons are long-lived and non-proliferative cells further complicates the compensation of accumulated oxidative damage over time (Plascencia-Villa and Perry, 2023; Foret et al., 2024). With aging, the reduction in the glutathione pool, weakening of defense systems such as superoxide dismutase, catalase, and glutathione peroxidase, and the decline of endogenous antioxidants such as melatonin further accentuate the redox environment favorable to the development of Alzheimer's disease (Tchekalarova and Tzoneva, 2023; Kamaljeet et al., 2024; Naik et al., 2025). Thus, advanced age functions not only as an epidemiological risk factor, but also as a sensitization phase that reinforces the biochemical basis of oxidative stress (Tchekalarova and Tzoneva, 2023; Nikolenko et al., 2022; Houldsworth, 2024).

In Alzheimer's disease, the production of ROS and RNS does not arise from a single source, but rather from numerous cellular mechanisms that reinforce one another (Reddy, 2023; Das and

Ganesh, 2023; Dhapola et al., 2024). Within the mitochondrial electron transport chain, electron leakage, particularly at complexes I and III, constitutes one of the principal sources of superoxide production; this is accompanied by monoamine oxidases, nitric oxide synthase isoforms, NADPH oxidases, xanthine oxidase, cytochrome P450 systems, lipoxygenases, and peroxisomal reactions (Das and Ganesh, 2023; Dhapola et al., 2024; Alkhalifa et al., 2025). In the presence of redox-active iron and copper, hydroxyl radical formation is enhanced through Fenton and Haber–Weiss reactions, and these highly reactive species produce damage to membranes, proteins, and nucleic acids that is difficult to reverse (Nikolenko et al., 2022; Das and Ganesh, 2023; Firdous et al., 2024). In addition, disruption of endothelial and neuronal NO production, together with the increase in peroxynitrite and other nitrative products, generates not only an oxidative burden but also a nitrosative stress load (Reddy, 2023; Houldsworth, 2024; Dhapola et al., 2024). This multi-source oxidant production constitutes the fundamental biochemical framework explaining why redox balance remains chronically disrupted in the Alzheimer's brain (Plascencia-Villa and Perry, 2023; Alkhalifa et al., 2025; Firdous et al., 2024).

Mitochondrial dysfunction represents one of the strongest links between oxidative stress and the pathogenesis of Alzheimer's disease (Alqahtani et al., 2023; Rummel and Butterfield, 2022; Alkhalifa et al., 2025). In the Alzheimer's brain, impairments in electron transport chain enzymes, reduced oxidative phosphorylation efficiency, decreased ATP synthesis, and loss of membrane potential render the mitochondrion a central site of both energy deficiency and excessive ROS production (Alqahtani et al., 2023; Alkhalifa et al., 2025; Rummel and Butterfield, 2022). Disruption of mitochondrial dynamics, namely a shift in the fusion–fission balance, contributes to the chronicity of the oxidative burden by causing the accumulation of damaged organelles and the

insufficiency of mitophagy (Alqahtani et al., 2023; Naik et al., 2025; Rummel and Butterfield, 2022). In this context, the relationship between oxidative stress and mitochondrial dysfunction is not linear, but rather constitutes a self-perpetuating vicious cycle, because dysfunctional mitochondria generate greater amounts of ROS, while elevated ROS levels further damage mitochondrial proteins, membranes, and mtDNA (Tchekalarova and Tzoneva, 2023; Alqahtani et al., 2023; Alkhalifa et al., 2025). As a result, the neuron is simultaneously exposed to both an energy crisis and intensified oxidative attack, thereby being driven into a synaptically and metabolically unsustainable state (Plascencia-Villa and Perry, 2023; Rummel and Butterfield, 2022; Alkhalifa et al., 2025).

One of the most striking consequences of oxidative stress in Alzheimer's disease is the large-scale reprogramming of brain metabolism (Rummel and Butterfield, 2022; Dhapola et al., 2024). Oxidative damage disrupts neuronal homeostasis at multiple levels by exerting deleterious effects on glycolysis, the pentose phosphate pathway, lipid metabolism, calcium signaling, and mTOR-associated anabolic processes (Rummel and Butterfield, 2022; Dhapola et al., 2024). In particular, the decline in glucose utilization causes a reduction in energy production in cognitively critical regions such as the hippocampus and cortex, thereby weakening the biochemical infrastructure required for synaptic function (Rummel and Butterfield, 2022; Alkhalifa et al., 2025). In the aging and oxidatively burdened brain, these metabolic defects also adversely affect neuron–astrocyte metabolic cooperation, disrupting lactate support, neurotransmitter recycling, and ionic balance (Rummel and Butterfield, 2022; Das and Ganesh, 2023). In this way, oxidative stress ceases to be merely a process that inflicts damage at the molecular level and instead becomes a fundamental metabolic disturbance that collapses the energy economy of the Alzheimer's

brain (Alqahtani et al., 2023; Rummel and Butterfield, 2022; Naik et al., 2025).

One of the most prominent cellular imprints of oxidative stress is lipid peroxidation, and in Alzheimer's disease this event causes severe biophysical disturbances particularly in synaptic membranes, mitochondrial membrane structures, and myelin-associated components (Nikolenko et al., 2022; Plascencia-Villa and Perry, 2023; Houldsworth, 2024). Peroxidation products such as malondialdehyde, 4-hydroxynonenal, and F2-isoprostanes are not merely passive indicators of damage, but also active toxic mediators that covalently modify proteins, thereby disrupting enzymatic function, membrane fluidity, and receptor stability (Das and Ganesh, 2023; Plascencia-Villa and Perry, 2023; Naik et al., 2025). In particular, adducts formed by 4-hydroxynonenal on neuronal proteins adversely affect processes related to synaptic plasticity, ion transport, and cytoskeletal organization (Nikolenko et al., 2022; Foret et al., 2024; Alkhalifa et al., 2025). Membrane structures damaged by lipid peroxidation strengthen the biochemical basis of cognitive impairment by leading to disrupted mitochondrial permeability, calcium imbalance, and impaired neurotransmitter release (Plascencia-Villa and Perry, 2023; Ekundayo et al., 2024; Naik et al., 2025). Therefore, lipid oxidation in Alzheimer's disease should be regarded not only as a laboratory marker of oxidative stress, but also as a direct mediator of neuronal dysfunction (Tchekalarova and Tzoneva, 2023; Firdous et al., 2024; Foret et al., 2024).

Protein oxidation and glyco-oxidative modifications also constitute important manifestations of oxidative stress in the pathogenesis of Alzheimer's disease (Reddy, 2023; Firdous et al., 2024; Dhapola et al., 2024). Modifications such as protein carbonyls, 3-nitrotyrosine, and advanced glycation end products (AGEs) lead to loss of enzymatic activity, defects in protein folding, increased

burden on the proteasome and autophagy systems, and ultimately disruption of proteostasis (Reddy, 2023; Das and Ganesh, 2023; Plascencia-Villa and Perry, 2023). The AGE–RAGE axis is particularly noteworthy, because the interaction of AGEs with RAGE activates NF-κB and related inflammatory pathways, thereby establishing a vicious cycle that further amplifies both oxidative and inflammatory burden (Reddy, 2023; Foret et al., 2024). This process not only deepens intracellular damage, but also gives rise to broader pathophysiological consequences, including vascular dysfunction, blood–brain barrier disruption, and glial activation (Reddy, 2023; Alkhalifa et al., 2025; Firdous et al., 2024). Accordingly, protein oxidation in Alzheimer's disease should be understood not merely as damage to individual proteins, but rather as a systemic biochemical stress field in which redox imbalance and inflammation converge (Plascencia-Villa and Perry, 2023; Dhapola et al., 2024; Houldsworth, 2024).

Nucleic acid oxidation represents another layer of damage in Alzheimer's disease that has been observed from the early stages and has gained increasing attention in recent years (Nikolenko et al., 2022; Plascencia-Villa and Perry, 2023; Foret et al., 2024). Oxidative stress can induce base modifications, single-strand breaks, and double-strand breaks in both nuclear DNA and mitochondrial DNA; at the same time, through RNA oxidation, it can impair ribosomal function, translation, and cellular stress responses (Plascencia-Villa and Perry, 2023; Foret et al., 2024; Sanders, 2023). The detection of increased expression of Sod2, Gsr, Idh1, and DNA repair genes in hippocampal neurons carrying an intraneuronal Aβ burden at the pre-plaque stage suggests that oxidative stress may be engaged even before the onset of clinical symptoms and plaque formation (Foret et al., 2024). In the same study, the demonstration of increased XPD, γH2AX foci, and dysregulation of DNA repair genes indicates that early neuronal damage in Alzheimer's disease is not limited to the

level of membranes or proteins, but also affects genomic integrity (Foret et al., 2024). Beyond this, the recognition of oxidatively damaged cytosolic or extracellular nucleic acids by the innate immune system as virus-like DAMPs establishes a novel link among inflammation, synaptotoxicity, and neuronal death (Sanders, 2023).

Synaptic dysfunction is one of the most clinically consequential effects of oxidative stress in Alzheimer's disease, because it constitutes the direct biological basis of memory and learning loss (Plascencia-Villa and Perry, 2023; Naik et al., 2025; Houldsworth, 2024). Oxidative burden adversely affects long-term potentiation and network-level synaptic coordination by disrupting postsynaptic membrane components, glutamatergic transmission proteins, calcium channel balance, and cytoskeletal support (Plascencia-Villa and Perry, 2023; Dhapola et al., 2024). While mitochondrial ATP depletion reduces the energy required for the synaptic vesicle cycle, neurotransmitter release, and ion pump activity, elevated ROS levels further aggravate this impairment by causing carbonylation and nitration of synaptic proteins (Alqahtani et al., 2023; Rummel and Butterfield, 2022; Naik et al., 2025). Therefore, oxidative stress should be viewed not as a terminal event that emerges only immediately before cell death, but rather as a mechanism that weakens the efficiency of neuronal circuits at a much earlier stage and lays the foundation for cognitive insufficiency (Plascencia-Villa and Perry, 2023; Sharma and Kim, 2023; Alkhalifa et al., 2025). In other words, forgetfulness, disorientation, and learning difficulty in Alzheimer's disease represent the macroscopic clinical manifestations of the wear imposed by oxidative stress on synaptic biology (Plascencia-Villa and Perry, 2023; Firdous et al., 2024; Houldsworth, 2024).

The mutually reinforcing relationship between oxidative stress and neuroinflammation also occupies a central position in Alzheimer's pathology (Firdous et al., 2024; Das and Ganesh, 2023;

Naik et al., 2025). Chronic activation of microglia and astrocytes increases NADPH oxidase-mediated superoxide production, the release of pro-inflammatory cytokines, and nitric oxide derivatives, thereby elevating the oxidative burden in the neuronal microenvironment (Das and Ganesh, 2023; Firdous et al., 2024). Conversely, increased oxidative stress further strengthens glial activation by stimulating NF-κB-, complement-, chemokine-, and inflammasome-associated pathways, thereby rendering neuroinflammation chronic (Firdous et al., 2024; Dhapola et al., 2024). Impairment of the glutamate-clearing capacity of astrocytes, together with the resulting excitotoxic calcium influx, further intensifies oxidative stress and contributes to the weakening of synaptic function (Das and Ganesh, 2023; Ekundayo et al., 2024). Therefore, in Alzheimer's disease, oxidative stress and inflammation should not be regarded as two separate entities, but rather as two interconnected nodes of a common pathobiological network that reinforce one another (Naik et al., 2025; Firdous et al., 2024; Alkhalifa et al., 2025).

This inflammatory-redox network is not limited solely to cells within the brain; the gut microbiota and the gut–brain axis may also contribute to the oxidative stress background in Alzheimer's disease (Das and Ganesh, 2023). Dysbiosis disrupts intestinal permeability and blood–brain barrier integrity, thereby allowing microbial products, alterations in short-chain fatty acids, lipopolysaccharides, and immune signals to exert a more pronounced influence on the central nervous system (Das and Ganesh, 2023). Through Toll-like receptor activation, peripheral and central inflammation, glial responses, and ROS/RNS production, this process may generate a redox burden associated with the progression of Alzheimer's disease (Das and Ganesh, 2023). In addition, nitrate/nitrite and sulfur metabolites accompanying dysbiosis, together with bacteria-derived pro-oxidant signals, may

reinforce nitrative stress and inflammation in neural tissue (Das and Ganesh, 2023). Although this field is still under development, the connection between gut microbiota and oxidative stress indicates that Alzheimer's disease should be evaluated within a multi-axis systems biology framework rather than as a condition confined solely to the brain (Das and Ganesh, 2023; Naik et al., 2025).

Endoplasmic reticulum stress and apoptosis are also important mechanisms that complement the destructive effects of oxidative stress in Alzheimer's disease (Ekundayo et al., 2024; Dhapola et al., 2024). Oxidative burden increases the accumulation of misfolded proteins, leading to elevated levels of ER stress markers such as PERK, eIF2α, IRE1α, Grp78, and CHOP; in cells whose protein-processing capacity is exceeded, this condition activates death signaling pathways (Ekundayo et al., 2024). At the same time, events such as increased mitochondrial permeability, cytochrome c release, caspase activation, and calcium overload further facilitate the apoptotic response (Ekundayo et al., 2024; Plascencia-Villa and Perry, 2023). The convergence of ER stress with oxidative stress drives the neuronal cell to a breaking point at which it can no longer sustain both proteostatic and redox burden simultaneously (Ekundayo et al., 2024; Rummel and Butterfield, 2022). Thus, neuronal loss in Alzheimer's disease is not merely the passive consequence of accumulated damage, but rather the product of an active degenerative process in which oxidative stress, protein misfolding, and cellular death programs are triggered together (Plascencia-Villa and Perry, 2023; Ekundayo et al., 2024; Firdous et al., 2024).

One of the reasons why oxidative stress remains so persistent in Alzheimer's disease is that the physiological defense networks that normally suppress it are also impaired (Tchekalarova and Tzoneva, 2023; Kamaljeet et al., 2024; Dhapola et al., 2024). Weakening of the Nrf2/ARE axis, decreased glutathione synthesis,

epigenetic or functional insufficiency of antioxidant enzymes, and reduced mitochondrial antioxidant capacity make it more difficult to neutralize the oxidative burden emerging in the brain (Houldsworth, 2024; Dhapola et al., 2024; Naik et al., 2025). A broad experimental literature has emerged on the restoration of antioxidant defense through melatonin, EGCG, astaxanthin, catalase, glutathione support, resveratrol, and certain pharmacological agents (Tchekalarova and Tzoneva, 2023; Kamaljeet et al., 2024; Valverde-Salazar et al., 2023). In particular, the age-related decline in endogenous melatonin levels suggests that it should be regarded not only as a circadian hormone, but also as a redox buffer and mitochondrial protector (Tchekalarova and Tzoneva, 2023). In addition, EGCG has been reported to support neuronal survival pathways through both antioxidant and anti-inflammatory effects and to slow degenerative events associated with oxidative stress (Valverde-Salazar et al., 2023).

Nevertheless, the degree of clinical success achieved by antioxidant-based therapies has not been as high as the promise suggested by experimental findings, and this highlights how complex oxidative stress is in Alzheimer's disease (Kamaljeet et al., 2024; Sharma and Kim, 2023; Alkhalifa et al., 2025). While many antioxidants have produced favorable outcomes in animal models, they have shown limited or inconsistent effects in human studies; the principal reasons include low bioavailability, insufficient passage across the blood–brain barrier, intervention at overly advanced stages of the disease, and the inadequacy of single agents against a multilayered pathology (Kamaljeet et al., 2024; Naik et al., 2025; Alkhalifa et al., 2025). For this reason, the current approach has shifted away from merely suppressing oxidative stress in isolation and toward more refined interventions, such as mitochondria-targeted antioxidants, combination strategies that simultaneously regulate multiple signaling pathways, nanozymes, and redox-

sensitive targeted delivery systems (Reddy, 2023; Dhapola et al., 2024; Alkhalifa et al., 2025). In particular, mitochondria-targeted antioxidants are thought to produce more meaningful effects on ATP synthesis, ROS generation, and neuronal resilience by acting closer to the source of the problem (Alqahtani et al., 2023; Alkhalifa et al., 2025). Thus, although oxidative stress remains an important therapeutic target in Alzheimer's disease, achieving clinical success requires the reconsideration of timing, delivery systems, target selection, and combination logic (Sharma and Kim, 2023; Dhapola et al., 2024; Alkhalifa et al., 2025).

In conclusion, oxidative stress in Alzheimer's disease should be regarded as a central biochemical node linking aging-associated weakening of antioxidant defense, mitochondrial insufficiency, lipid and protein oxidation, DNA/RNA damage, neuroinflammation, ER stress, synaptic collapse, and neuronal death (Tchekalarova and Tzoneva, 2023; Plascencia-Villa and Perry, 2023; Alkhalifa et al., 2025). This redox imbalance, which may emerge even in the early stages of the disease, subsequently combines with metabolic collapse, inflammatory activation, and cellular death programs, thereby facilitating the transition of Alzheimer's pathology into a phase that is difficult to reverse (Foret et al., 2024; Sanders, 2023; Ekundayo et al., 2024). For this reason, the role of oxidative stress in Alzheimer's disease is too profound to be confined within the dilemma of "cause or consequence"; the more accurate perspective is to recognize oxidative stress as a process that is simultaneously initiating, reinforcing, and propagating (Sharma and Kim, 2023; Rummel and Butterfield, 2022; Firdous et al., 2024). Future strategies with real promise will depend not merely on the general suppression of oxidative stress, but on the simultaneous regulation of interconnected targets such as mitochondria, inflammation, redox-sensitive signaling pathways, biomolecular damage repair,

and brain barrier systems (Dhapola et al., 2024; Alkhalifa et al., 2025; Das and Ganesh, 2023).

References

Alkhalifa, A. E., Alkhalifa, O., Durdanovic, I., Ibrahim, D. R., & Maragkou, S. (2025). Oxidative stress and mitochondrial dysfunction in Alzheimer's disease: Insights into pathophysiology and treatment. *Journal of Dementia and Alzheimer's Disease, 2*(2), 17. https://doi.org/10.3390/jdad2020017

Alqahtani, T., Deore, S. L., Kide, A. A., Shende, B. A., Sharma, R., Chakole, R. D., Nemade, L. S., Kale, N. K., Borah, S., Deokar, S. S., Behera, A., Bhandari, D. D., Gaikwad, N., Azad, A. K., & Ghosh, A. (2023). Mitochondrial dysfunction and oxidative stress in Alzheimer's disease, and Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis—An updated review. *Mitochondrion, 71*, 83–92. https://doi.org/10.1016/j.mito.2023.05.007

Das, T. K., & Ganesh, B. P. (2023). Interlink between the gut microbiota and inflammation in the context of oxidative stress in Alzheimer's disease progression. *Gut Microbes, 15*(1), 2206504. https://doi.org/10.1080/19490976.2023.2206504

Dhapola, R., Beura, S. K., Sharma, P., Singh, S. K., & HariKrishnaReddy, D. (2024). Oxidative stress in Alzheimer's disease: Current knowledge of signaling pathways and therapeutics. *Molecular Biology Reports, 51*(1), 48. https://doi.org/10.1007/s11033-023-09021-z

Ekundayo, B. E., Obafemi, T. O., Adewale, O. B., Obafemi, B. A., Oyinloye, B. E., & Ekundayo, S. K. (2024). Oxidative stress, endoplasmic reticulum stress and apoptosis in the pathology of Alzheimer's disease. *Cell Biochemistry and Biophysics, 82*(2), 457–477. https://doi.org/10.1007/s12013-024-01248-2

Firdous, S. M., Khan, S. A., & Maity, A. (2024). Oxidative stress-mediated neuroinflammation in Alzheimer's disease. *Naunyn-*

Schmiedeberg's Archives of Pharmacology, 397(11), 8189–8209. https://doi.org/10.1007/s00210-024-03188-3

Foret, M. K., Orciani, C., Welikovitch, L. A., Huang, C., Cuello, A. C., & Do Carmo, S. (2024). Early oxidative stress and DNA damage in Aβ-burdened hippocampal neurons in an Alzheimer's-like transgenic rat model. *Communications Biology, 7*, 861. https://doi.org/10.1038/s42003-024-06552-4

Houldsworth, A. (2024). Role of oxidative stress in neurodegenerative disorders: A review of reactive oxygen species and prevention by antioxidants. *Brain Communications, 6*(1), fcad356. https://doi.org/10.1093/braincomms/fcad356

Kamaljeet, Singh, S., Gupta, G. D., & Aran, K. R. (2024). Emerging role of antioxidants in Alzheimer's disease: Insight into physiological, pathological mechanisms and management. *Pharmaceutical Science Advances, 2*(1), 100021. https://doi.org/10.1016/j.pscia.2023.100021

Naik, R. A., Mir, M. N., Malik, I. A., Bhardwaj, R., Alshabrmi, F. M., Mahmoud, M. A., Alhomrani, M., Alamri, A. S., Alsanie, W. F., Hjazi, A., Ghatak, T., Poeggeler, B., Singh, M. P., Gopenath, T. S., & Singh, S. K. (2025). The potential mechanism and the role of antioxidants in mitigating oxidative stress in Alzheimer's disease. *Frontiers in Bioscience-Landmark, 30*(2), 25551. https://doi.org/10.31083/FBL25551

Nikolenko, V. N., Rizaeva, N. A., Bulygin, K. V., Anokhina, V. M., & Bolotskaya, A. A. (2022). The role of oxidative stress in the development of Alzheimer's disease. *Neurology, Neuropsychiatry, Psychosomatics, 14*(4), 68–74. https://doi.org/10.14412/2074-2711-2022-4-68-74

Plascencia-Villa, G., & Perry, G. (2023). Roles of oxidative stress in synaptic dysfunction and neuronal cell death in Alzheimer's

disease. *Antioxidants, 12*(8), 1628. https://doi.org/10.3390/antiox12081628

Reddy, V. P. (2023). Oxidative stress in health and disease. *Biomedicines, 11*(11), 2925. https://doi.org/10.3390/biomedicines11112925

Rummel, N. G., & Butterfield, D. A. (2022). Altered metabolism in Alzheimer disease brain: Role of oxidative stress. *Antioxidants & Redox Signaling, 36*(16–18), 1289–1305. https://doi.org/10.1089/ars.2021.0177

Sanders, O. D. (2023). Virus-like cytosolic and cell-free oxidatively damaged nucleic acids likely drive inflammation, synapse degeneration, and neuron death in Alzheimer's disease. *Journal of Alzheimer's Disease Reports, 7*(1), 1–19. https://doi.org/10.3233/ADR-220047

Sharma, C., & Kim, S. R. (2023). Oxidative stress: Culprit or consequence in Alzheimer's amyloidopathy. *Neural Regeneration Research, 18*(9), 1948–1949. https://doi.org/10.4103/1673-5374.367843

Tchekalarova, J., & Tzoneva, R. (2023). Oxidative stress and aging as risk factors for Alzheimer's disease and Parkinson's disease: The role of the antioxidant melatonin. *International Journal of Molecular Sciences, 24*(3), 3022. https://doi.org/10.3390/ijms24033022

Valverde-Salazar, V., Ruiz-Gabarre, D., & García-Escudero, V. (2023). Alzheimer's disease and green tea: Epigallocatechin-3-gallate as a modulator of inflammation and oxidative stress. *Antioxidants, 12*(7), 1460. https://doi.org/10.3390/antiox12071460

CHAPTER 4

MITOCHONDRIAL DYSFUNCTION AND BIOENERGETIC FAILURE IN ALZHEIMER'S DISEASE

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1. Introduction

Mitochondria are the principal bioenergetic organelles of the neuron, generating the majority of cellular adenosine triphosphate (ATP) through oxidative phosphorylation while simultaneously governing calcium buffering, reactive oxygen species (ROS) production, and the intrinsic apoptotic pathway. Because neurons are post mitotic, highly polarized, and among the most energy-demanding cells in the body, they are exceptionally dependent on a continuous and well regulated supply of mitochondrial ATP to sustain synaptic transmission, axonal transport, and ionic homeostasis, and are correspondingly vulnerable when mitochondrial function declines (Pinky et al., 2026). In Alzheimer's disease (AD),

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mitochondrial abnormalities are among the earliest detectable cellular changes, preceding overt amyloid-β (Aβ) plaque deposition and neurofibrillary tangle formation by years, and encompass impaired electron transport chain (ETC) activity, reduced ATP synthesis, disrupted mitochondrial dynamics, defective mitophagy, and increased mitochondrial ROS generation (Wang et al., 2025). These changes are not merely a downstream consequence of amyloid and tau pathology; a substantial body of evidence indicates that mitochondrial dysfunction actively contributes to, and may in certain respects precede and drive, the accumulation of both Aβ and hyperphosphorylated tau, positioning bioenergetic failure as a central and mechanistically interconnected node within the broader pathophysiological network of AD rather than a passive bystander of neurodegeneration (Swerdlow, 2023). Mitochondrial Aβ accumulation, aberrant interactions between Aβ and mitochondrial proteins such as amyloid binding alcohol dehydrogenase (ABAD), and cyclophilin D-dependent opening of the mitochondrial permeability transition pore (mPTP) collectively compromise mitochondrial membrane potential, respiratory capacity, and calcium handling, while tau itself associates with mitochondrial membranes and impairs axonal mitochondrial trafficking (D'Alessandro et al., 2025). These mitochondrial insults are compounded by disrupted communication between mitochondria and the endoplasmic reticulum at mitochondria associated membranes (MAMs), by imbalanced mitochondrial fission and fusion, and by impaired mitochondrial quality control through defective mitophagy, all of which converge on a self-amplifying cycle of bioenergetic decline, oxidative damage, and synaptic degeneration (Area-Gomez & Schon, 2024; Ahmad & Sachdeva, 2022). This chapter

examines mitochondrial dysfunction and bioenergetic failure as a distinct and mechanistically central axis of AD pathophysiology, situating it alongside and in direct molecular dialogue with the amyloidogenic, tauopathic, oxidative, and neuroinflammatory processes addressed elsewhere in this volume, and considering the therapeutic strategies that specifically target mitochondrial bioenergetics as a route toward disease modification.

2. Mitochondrial Bioenergetics in the Healthy Neuron

The human brain accounts for only about 2% of total body mass yet consumes approximately 20% of resting oxygen intake, a disproportionate energy demand that is met almost entirely by the aerobic oxidation of glucose within neuronal and glial mitochondria (Faria-Pereira & Morais, 2022). At the cellular level, glucose is first metabolized to pyruvate through cytosolic glycolysis; pyruvate is then imported into the mitochondrial matrix, converted to acetyl CoA, and fed into the tricarboxylic acid (TCA) cycle, which generates the reduced electron carriers NADH and $FADH_2$ that donate electrons to the mitochondrial electron transport chain (ETC) embedded in the inner mitochondrial membrane (Faria-Pereira & Morais, 2022). Sequential electron transfers through ETC Complexes I–IV pumps protons across the inner membrane, establishing an electrochemical gradient the protonmotive force that drives ATP synthase (Complex V) to phosphorylate ADP into ATP, the principal energetic currency supporting ion pumping, synaptic vesicle cycling, axonal transport, and the restoration of resting membrane potential after neurotransmission (Faria-Pereira & Morais, 2022). Because neurons are highly polarized, post mitotic cells with energy demands that are especially

concentrated at synaptic terminals, mitochondria must be efficiently trafficked along axons and dendrites and anchored at sites of high metabolic need, such that even localized mitochondrial impairment can produce a synaptic energy deficit long before it manifests as global neuronal dysfunction (Pinky et al., 2026). Beyond ATP generation, neuronal mitochondria serve two further functions that are of particular relevance to AD: they act as the principal intracellular calcium buffer, taking up cytosolic Ca^{2+} during neurotransmission and thereby shaping synaptic signaling, and they are the main intracellular source of ROS, generated as an unavoidable by product of electron leakage from the ETC, which under physiological conditions is neutralized by mitochondrial antioxidant systems but which becomes pathogenic when this balance is disrupted (Faria-Pereira & Morais, 2022). This tight coupling of bioenergetics, calcium handling, and redox regulation within a single organelle means that a disturbance in any one of these functions is readily transmitted to the others, providing the physiological basis for the self amplifying bioenergetic failure that characterizes the AD brain, as detailed in the following section.

3. Evidence for Bioenergetic Failure in the AD Brain

A substantial and converging body of clinical, neuroimaging, and post mortem evidence demonstrates that cerebral bioenergetic failure is a consistent and early feature of AD rather than a late or incidental finding. In vivo, fluorodeoxyglucose positron emission tomography (FDG-PET) studies reliably show a characteristic pattern of reduced cerebral glucose metabolism in the posterior cingulate, temporo parietal, and precuneus regions of AD patients, a hypometabolic signature that correlates with cognitive and functional

impairment and that can be detected years before the onset of overt clinical symptoms, and whose severity independently predicts the rate of subsequent conversion from normal cognition or mild cognitive impairment to dementia (Alhasan et al., 2025). This in vivo glucose hypometabolism is paralleled at the biochemical level by well-documented deficits in the activity of multiple ETC complexes in AD brain tissue and platelets, most consistently affecting cytochrome c oxidase (Complex IV), the terminal and rate-limiting enzyme of the respiratory chain, alongside reductions in the activity of pyruvate dehydrogenase and other key TCA cycle enzymes that collectively constrain the supply of reducing equivalents to the ETC (D'Alessandro et al., 2025). These respiratory chain deficits translate directly into reduced mitochondrial ATP output, decreased mitochondrial membrane potential, and impaired calcium buffering capacity, findings that have been replicated across post mortem AD brain tissue, transgenic mouse models, and induced pluripotent stem cell derived neurons carrying AD-associated mutations (Wang et al., 2025). Critically, several of these bioenergetic abnormalities have been shown to precede detectable Aβ plaque deposition in animal models and to be present in the brains of cognitively normal individuals who are at genetic risk for AD, most notably APOE ε4 carriers, arguing that bioenergetic failure is not simply a secondary consequence of amyloid and tau accumulation but an early and potentially initiating feature of the disease process, consistent with the broader mitochondrial cascade hypothesis discussed in Section 7 (Swerdlow, 2023). Taken together, this evidence establishes that the AD brain is a chronically energy deficient organ, and that the resulting shortfall in ATP availability is sufficient, on its own, to compromise the energy

intensive processes of synaptic transmission, protein clearance, and ionic homeostasis that underlie cognitive function.

4. Amyloid-β, Tau, and Mitochondrial Toxicity

Both of the canonical protein lesions of AD converge directly on mitochondria, providing a molecular bridge between the amyloid cascade and tauopathy hypotheses on one hand and bioenergetic failure on the other. Rather than acting solely as an extracellular plaque-forming peptide, Aβ accumulates intracellularly within mitochondria themselves, entering through the translocase of the outer mitochondrial membrane and reaching concentrations inside the organelle that precede and exceed the appearance of extracellular plaques in both AD brain tissue and transgenic mouse models (D'Alessandro et al., 2025). Once inside the mitochondrion, Aβ interacts directly with amyloid binding alcohol dehydrogenase (ABAD), a matrix enzyme whose interaction with Aβ potentiates mitochondrial and neuronal oxidative stress and impairs mitochondrial respiratory function, and with cyclophilin D (CypD), a key regulatory component of the mitochondrial permeability transition pore (mPTP) (Du & Yan, 2010). Aβ driven formation of the CypDAβ complex sensitizes the mPTP to opening under conditions of calcium or oxidative stress, producing a sudden collapse of mitochondrial membrane potential, release of cytochrome c and other pro apoptotic factors, and a further surge in ROS generation, effectively creating a self reinforcing cycle of mitochondrial and neuronal injury that has been substantially attenuated in animal models by genetic or pharmacological CypD inhibition, underscoring its causal contribution to Aβ induced toxicity (Du & Yan, 2010). Tau exerts a parallel, and in some respects complementary, toxicity toward mitochondria.

Pathologically hyperphosphorylated and caspase cleaved tau species associate directly with mitochondrial membranes and with the protein adaptors that couple mitochondria to microtubule-based motor proteins, thereby impairing the anterograde and retrograde axonal transport of mitochondria and causing their pathological accumulation in the neuronal soma at the expense of energy demanding axonal and synaptic compartments (Pérez et al., 2018). Beyond its effects on trafficking, pathological tau also disrupts mitochondrial morphology, promoting a shift toward a fragmented, fission favoring phenotype, and directly impairs mitochondrial bioenergetics, producing measurable reductions in membrane potential and ATP output that mirror, and likely compound, the bioenergetic deficits attributable to Aβ (Pérez et al., 2018). Because these two lesions act on overlapping mitochondrial targets respiratory function, membrane integrity, and axonal transport their combined presence in the AD brain is thought to produce a bioenergetic insult substantially greater than either pathology alone, reinforcing the view that mitochondrial dysfunction functions as a convergence point rather than a downstream bystander of amyloid and tau pathology.

5. Mitochondrial Dynamics and Mitophagy: Fission, Fusion, and Quality Control Failure

Under physiological conditions, mitochondria exist as a dynamic, interconnected network whose shape, distribution, and functional quality are continuously remodeled by two opposing processes: fission, mediated principally by the cytosolic GTPase dynamin-related protein 1 (DRP1) recruited to the outer mitochondrial membrane, and fusion, mediated by the mitofusins MFN1 and MFN2 at the outer membrane and by

optic atrophy 1 (OPA1) at the inner membrane (Wang et al., 2025). This fission fusion equilibrium allows healthy mitochondria to exchange metabolites and mitochondrial DNA, permits their transport to sites of high energy demand such as synaptic terminals, and critically allows severely damaged mitochondrial segments to be isolated by fission and subsequently cleared by mitophagy, the selective autophagic degradation pathway orchestrated principally by the PINK1-Parkin axis (Wang et al., 2025). In AD, this equilibrium is consistently shifted toward excessive fission and fragmentation. Using APP/PS1 transgenic mice, de la Cueva et al. (2022) demonstrated that imbalanced mitochondrial dynamics in the cerebral cortex and hippocampus, together with a compensatory increase in mitophagy, represent a very early consequence of Aβ accumulation that precedes overt neurodegeneration, directly linking amyloid pathology to disordered mitochondrial quality control. Mechanistically, Aβ and oxidative stress promote the translocation and activation of DRP1 while simultaneously suppressing OPA1-mediated fusion, producing a population of small, fragmented, and functionally compromised mitochondria that are less efficient at ATP generation and more prone to ROS leakage and mPTP opening (Wang et al., 2025). Paradoxically, while this fragmentation initially favors the removal of damaged mitochondria via mitophagy, the PINK1-Parkin pathway itself becomes progressively impaired as AD advances, with reduced expression and activity of both proteins reported in AD models and post-mortem brain tissue, so that fragmented, ROS producing mitochondria increasingly persist rather than being cleared, accumulating at synaptic terminals where they most acutely compromise neurotransmission (Pinky et al., 2026). The net effect is a mitochondrial population that is simultaneously

overproduced through excessive fission and undercleared through defective mitophagy, a combination that compounds bioenergetic failure, amplifies oxidative damage, and disrupts calcium buffering at precisely the subcellular sites synapses where energy supply is most urgently needed, thereby providing a direct mechanistic link between defective mitochondrial quality control and the synaptic degeneration that closely tracks cognitive decline in AD (Pinky et al., 2026; D'Alessandro et al., 2025).

6. Mitochondria Endoplasmic Reticulum Crosstalk: The MAM Hypothesis and Calcium Dyshomeostasis

Mitochondria do not function in isolation but are physically and biochemically tethered to the endoplasmic reticulum (ER) at specialized contact sites known as mitochondria-associated membranes (MAMs), a subdomain of the ER that regulates lipid biosynthesis and trafficking, glucose metabolism, and, most importantly for AD, the transfer of calcium from the ER to the mitochondrial matrix (Eysert et al., 2020). Under physiological conditions, this ER-to-mitochondria calcium flux, mediated by inositol trisphosphate receptors on the ER membrane and voltage-dependent anion channels on the outer mitochondrial membrane, stimulates calcium-sensitive TCA cycle dehydrogenases and thereby couples cellular signaling demand to mitochondrial ATP output; when this coupling becomes excessive or dysregulated, however, mitochondrial calcium overload directly promotes mPTP opening and bioenergetic collapse (Eysert et al., 2020). A substantial body of work has shown that MAM function is markedly upregulated in AD, both because presenilin-1 and presenilin-2 the catalytic components of γ-secretase are

enriched at MAMs rather than diffusely distributed throughout the ER, and because familial AD associated mutations in these presenilins, along with sporadic AD pathology more broadly, increase ER mitochondria contact and MAM associated lipid and calcium signaling in a manner that parallels the biochemical abnormalities long observed in AD, including aberrant cholesterol and phospholipid metabolism (Area-Gomez & Schon, 2024). Because γ-secretase activity and, consequently, amyloidogenic processing of the amyloid precursor protein are concentrated at this ER mitochondria interface, increased MAM function has been proposed to causally link the amyloidogenic pathway to the calcium and lipid abnormalities that are otherwise difficult to reconcile with the amyloid cascade hypothesis alone, an integrative framework that Area-Gomez and Schon (2024) have termed a unitary hypothesis of AD pathogenesis centered on MAM dysregulation. Downstream of this excessive ER mitochondria coupling, sustained mitochondrial calcium overload desensitizes and eventually damages the ETC, promotes chronic mPTP opening, and synergizes with Aβ and tau driven toxicity described in Section 4 to accelerate bioenergetic failure, while simultaneously disrupting the ER's own protein folding capacity and triggering the unfolded protein response, linking mitochondrial calcium dyshomeostasis to a second, ER centered arm of cellular stress that is increasingly recognized as integral to AD pathogenesis (Eysert et al., 2020). Together, these findings position the MAM interface not as a peripheral biochemical curiosity but as a structural hub at which amyloidogenic processing, calcium signaling, lipid metabolism, and mitochondrial bioenergetics are mechanistically and spatially unified.

7. Mitochondrial DNA, the Mitochondrial Cascade Hypothesis, and Cybrid Evidence

Because each mitochondrion carries its own genome (mitochondrial DNA, or mtDNA), which encodes core subunits of the ETC complexes and is replicated independently of nuclear DNA, mitochondrial genomic integrity is itself a distinct and clinically relevant dimension of AD pathophysiology. mtDNA is markedly more vulnerable to oxidative damage than nuclear DNA, owing to its proximity to the ROS generating ETC, its lack of protective histones, and its comparatively limited repair capacity, and in the AD brain this vulnerability manifests as reduced mtDNA copy number and an accumulation of oxidative mtDNA lesions that further impair OXPHOS protein synthesis, creating a self reinforcing cycle of genomic damage and bioenergetic decline (McGill Percy et al., 2025). This cycle carries a further inflammatory consequence: damaged mitochondria increasingly release mtDNA fragments into the cytoplasm through VDAC1 and the mPTP, where the released mtDNA is sensed by the cGAS-STING pathway, triggering a type I interferon response that links mitochondrial genomic instability directly to the chronic neuroinflammation addressed elsewhere in this volume (McGill Percy et al., 2025). The heritability and systemic nature of mtDNA related mitochondrial dysfunction in sporadic, late onset AD is most directly demonstrated by cybrid (cytoplasmic hybrid) studies, in which mitochondria from platelets of AD patients are transferred into mtDNA depleted neuronal cell lines that otherwise share an identical nuclear genome: cybrid lines carrying AD patient mitochondria consistently reproduce the reduced cytochrome c oxidase activity, altered ROS production, and abnormal calcium handling characteristic of AD, and these abnormalities have

been shown to progressively worsen with continued passage in culture, indicating that the mitochondrial genotype itself, independent of the nuclear genetic and cellular environment, is sufficient to transmit key features of AD associated mitochondrial dysfunction (Trimmer et al., 2004). These cybrid findings constitute the empirical foundation of the mitochondrial cascade hypothesis, which proposes that inherited mitochondrial and mtDNA characteristics establish a person's baseline mitochondrial durability, that this durability determines how mitochondrial function changes with advancing age, and that once age related mitochondrial decline crosses a critical threshold it becomes sufficient to initiate the amyloidogenic, tauopathic, and neuroinflammatory cascades that produce the clinical and histopathological phenotype of sporadic AD (Swerdlow, 2023). Under this framework, mitochondrial and mtDNA changes are not merely a downstream consequence of amyloid and tau pathology but can, in a substantial proportion of late onset, nonfamilial cases, function as the primary upstream driver of the disease process, a reframing with direct implications for the therapeutic strategies discussed in Section 9.

8. Bioenergetic Failure, Synaptic Dysfunction, and the Vicious Cycle with Oxidative Stress and Neuroinflammation

The mitochondrial abnormalities described in the preceding sections converge with particular force at the synapse, the subcellular compartment with the highest and most acute energetic demand in the neuron and the structure whose loss correlates most closely with cognitive decline in AD. Synaptic transmission requires substantial, rapidly mobilized ATP to

support neurotransmitter vesicle cycling, restoration of ionic gradients after depolarization, and local protein synthesis, and because presynaptic terminals are populated by a distinct pool of synaptic mitochondria that must be trafficked there and maintained in a functional state, the combination of impaired mitochondrial transport (Section 4), excessive fission and defective mitophagy (Section 5), and ETC deficits (Section 3) is disproportionately damaging to synaptic, rather than somatic, bioenergetics (Faria-Pereira & Morais, 2022). The resulting local ATP shortfall directly compromises synaptic vesicle recycling and the maintenance of resting membrane potential, producing measurable synaptic dysfunction and loss that precedes overt neuronal death and tracks closely with the severity of cognitive impairment (Pinky et al., 2026). This bioenergetic failure does not occur in isolation but is mechanistically inseparable from two further pathological processes that are treated in dedicated detail elsewhere in this volume: dysfunctional mitochondria are the principal cellular source of the excess ROS that drives the oxidative stress addressed in the following chapter, while mtDNA released from damaged mitochondria activates the cGAS-STING and NLRP3 inflammasome pathways that sustain the chronic microglial neuroinflammation described in the chapter on neuroinflammatory mechanisms (McGill Percy et al., 2025). These three processes bioenergetic failure, oxidative stress, and neuroinflammation are therefore best understood not as sequential stages but as a single, tightly coupled and mutually reinforcing cycle: ROS generated by failing mitochondria further damages ETC complexes and mtDNA, amplifying bioenergetic decline, while the resulting mtDNA release and metabolic stress signals sustain a neuroinflammatory

microenvironment that itself impairs mitochondrial function in neighboring neurons and glia, so that once initiated, this cycle is capable of self perpetuation largely independent of any further increase in amyloid or tau burden (D'Alessandro et al., 2025). Understood in this way, mitochondrial bioenergetic failure functions as both a cause and a consequence of oxidative and inflammatory stress in AD, providing the mechanistic rationale for therapeutic strategies that target mitochondrial function directly, rather than amyloid or tau alone, as discussed in the following section.

9. Therapeutic Strategies Targeting Mitochondrial Bioenergetics

The recognition that mitochondrial dysfunction is an early, mechanistically central, and potentially causal feature of AD has motivated a growing pipeline of therapeutic strategies aimed directly at restoring mitochondrial quality control and bioenergetic capacity, positioned as complementary to, rather than a replacement for, the amyloid and tau targeted approaches discussed elsewhere in this volume. One major translational direction seeks to enhance mitophagy and thereby clear the fragmented, ROS producing mitochondria described in Section 5; candidate approaches include the sirtuin activator resveratrol, which promotes mitophagy through the Sirt1-Parkin pathway, the mTOR inhibitor rapamycin and the AMPK activator EGCG, both of which enhance autophagic clearance of damaged mitochondria, and the nicotinamide adenine dinucleotide (NAD^+) precursor nicotinamide riboside, currently under early-phase clinical investigation for its effects on mitochondrial bioenergetics in mild cognitive impairment and AD (Wang et al., 2025). A second, longer standing strategy employs mitochondria

targeted antioxidants compounds conjugated to lipophilic cations that concentrate selectively within the mitochondrial matrix to scavenge ROS at its principal site of production before it can further damage the ETC, mtDNA, and surrounding cellular structures, an approach supported by consistent preclinical evidence of reduced oxidative damage and improved mitochondrial respiratory function (Ahmad & Sachdeva, 2022). A third avenue targets mitochondrial biogenesis itself, most notably through activation of PGC-1α, the master transcriptional regulator of mitochondrial biogenesis, with the aim of increasing the overall population of healthy, functional mitochondria to offset losses from excessive fission and defective mitophagy, complementing pharmacological approaches with strategies such as exercise and caloric restriction that are known to upregulate PGC-1α signaling through physiological rather than pharmacological means (McGill Percy et al., 2025). More recent efforts have also begun to explore gene therapy approaches designed to directly correct mitochondrial quality control defects or restore ETC subunit expression, reflecting a broader shift toward precision, mechanism based mitochondrial interventions rather than symptomatic or purely antioxidant strategies (McGill Percy et al., 2025). Because bioenergetic failure, oxidative stress, and neuroinflammation form a single interlocking cycle (Section 8), the most promising translational strategies increasingly favor combination approaches for example, pairing a mitophagy enhancer with a mitochondria targeted antioxidant over single agent interventions, on the rationale that addressing only one node of this cycle is unlikely to be sufficient to durably interrupt it (D'Alessandro et al., 2025). While no mitochondria targeted agent has yet achieved regulatory approval for AD, the consistency of preclinical

efficacy across multiple independent mechanistic classes lends considerable support to mitochondrial bioenergetics as a viable and complementary therapeutic target, one whose translational progress will be considered further, alongside natural compound and multitarget strategies, in the concluding chapters of this volume.

10. Conclusion

Mitochondrial dysfunction and bioenergetic failure occupy a distinctive and mechanistically central position within the pathophysiology of Alzheimer's disease. As this chapter has outlined, the healthy neuron depends on an uninterrupted supply of mitochondrially generated ATP to sustain synaptic transmission, and in AD this supply is compromised at nearly every level: cerebral glucose hypometabolism and ETC complex deficits reduce ATP output; Aβ and tau directly poison mitochondrial membranes, enzymes, and transport machinery; excessive fission and defective mitophagy allow damaged, ROS producing mitochondria to accumulate rather than being cleared; dysregulated ER–mitochondria crosstalk at MAMs drives calcium overload and couples mitochondrial dysfunction to amyloidogenic processing itself; and oxidative mtDNA damage both deepens the bioenergetic deficit and activates innate immune signaling that sustains chronic neuroinflammation. Cybrid studies demonstrate that mitochondrial and mitochondrial genomic characteristics are, on their own, sufficient to transmit key features of AD associated dysfunction, lending substantial support to the mitochondrial cascade hypothesis and its proposal that mitochondrial decline can function as a primary driver, rather than a mere downstream consequence, of sporadic, late onset disease. Critically, none of

these mitochondrial processes operates in isolation: they are mechanistically and spatially interwoven with the amyloidogenic, tauopathic, oxidative, and neuroinflammatory pathways examined in the chapters that precede and follow this one, such that bioenergetic failure is best understood not as a discrete pathological event but as a hub through which several of AD's principal pathogenic mechanisms are functionally connected. This integrative view carries a direct translational implication: therapeutic strategies that target mitochondrial quality control, bioenergetic capacity, and mitochondrial organellar crosstalk directly rather than amyloid or tau alone offer a mechanistically well supported and increasingly active avenue for disease modifying intervention, one whose further pharmacological and natural compound based development is taken up in the remaining chapters of this volume.

References

Ahmad, F., & Sachdeva, P. (2022). Critical appraisal on mitochondrial dysfunction in Alzheimer's disease. *Aging Medicine, 5*(4), 272–280. https://doi.org/10.1002/agm2.12217

Alhasan, M. S., Alhasan, A. S., Milburn, J., Khalil, M., Almaghraby, A., Alharthi, O., Hamoud, S., Essibayi, M. A., Elhassan, Y. H., Feltrin, F., Singh, S., & Azzam, A. Y. (2025). FDG-PET brain glucose hypometabolism predicts Alzheimer's disease progression pathways in cognitively normal adults: A longitudinal competing risks modeling. *Metabolism Open, 28*, 100400. https://doi.org/10.1016/j.metop.2025.100400

Area-Gomez, E., & Schon, E. A. (2024). Towards a unitary hypothesis of Alzheimer's disease pathogenesis. *Journal of Alzheimer's Disease, 98*(4), 1243–1275. https://doi.org/10.3233/JAD-231318

D'Alessandro, M. C. B., Kanaan, S., Geller, M., Praticò, D., & Daher, J. P. L. (2025). Mitochondrial dysfunction in Alzheimer's disease. *Ageing Research Reviews, 107*, 102713. https://doi.org/10.1016/j.arr.2025.102713

de la Cueva, M., Antequera, D., Ordoñez-Gutiérrez, L., Wandosell, F., Camins, A., Carro, E., & Bartolomé, F. (2022). Amyloid-β impairs mitochondrial dynamics and autophagy in Alzheimer's disease experimental models. *Scientific Reports, 12*, 10092. https://doi.org/10.1038/s41598-022-13683-3

Du, H., & Yan, S. S. (2010). Mitochondrial permeability transition pore in Alzheimer's disease: Cyclophilin D

and amyloid beta. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, 1802*(1), 198–204. https://doi.org/10.1016/j.bbadis.2009.07.005

Eysert, F., Kinoshita, P. F., Mary, A., Vaillant-Beuchot, L., Checler, F., & Chami, M. (2020). Molecular dysfunctions of mitochondria-associated membranes (MAMs) in Alzheimer's disease. *International Journal of Molecular Sciences, 21*(24), 9521. https://doi.org/10.3390/ijms21249521

Faria-Pereira, A., & Morais, V. A. (2022). Synapses: The brain's energy-demanding sites. *International Journal of Molecular Sciences, 23*(7), 3627. https://doi.org/10.3390/ijms23073627

McGill Percy, K. C., Liu, Z., & Qi, X. (2025). Mitochondrial dysfunction in Alzheimer's disease: Guiding the path to targeted therapies. *Neurotherapeutics, 22*(3), e00525. https://doi.org/10.1016/j.neurot.2025.e00525

Pérez, M. J., Jara, C., & Quintanilla, R. A. (2018). Contribution of tau pathology to mitochondrial impairment in neurodegeneration. *Frontiers in Neuroscience, 12*, 441. https://doi.org/10.3389/fnins.2018.00441

Pinky, Wali, Z., Neha, Tiwari, P., El-Tanani, M., Rabbani, S. A., & Parvez, S. (2026). Aging and Alzheimer's: The critical role of mitochondrial dysfunction and synaptic alterations. *Frontiers in Synaptic Neuroscience, 17*, 1676317. https://doi.org/10.3389/fnsyn.2025.1676317

Swerdlow, R. H. (2023). The Alzheimer's disease mitochondrial cascade hypothesis: A current overview. *Journal of*

Alzheimer's Disease, 92(3), 751–768. https://doi.org/10.3233/JAD-221286

Trimmer, P. A., Keeney, P. M., Borland, M. K., Simon, F. A., Almeida, J., Swerdlow, R. H., Parks, J. P., Parker, W. D., Jr., & Bennett, J. P., Jr. (2004). Mitochondrial abnormalities in cybrid cell models of sporadic Alzheimer's disease worsen with passage in culture. *Neurobiology of Disease, 15*(1), 29–39. https://doi.org/10.1016/j.nbd.2003.09.011

Wang, S., Liao, Z., Zhang, Q., Han, X., Liu, C., & Wang, J. (2025). Mitochondrial dysfunction in Alzheimer's disease: A key frontier for future targeted therapies. *Frontiers in Immunology, 15*, 1484373. https://doi.org/10.3389/fimmu.2024.1484373

CHAPTER 5

THE GUT-BRAIN AXIS: MOLECULAR INSIGHTS INTO THE MICROBIOME IN ALZHEIMER'S DISEASE

ELİF AYDIN[1]

1. Introduction: The role of the microbiome in Alzheimer's disease

For many years, Alzheimer's disease has largely been explained through the accumulation of amyloid-β in the brain, dysregulation of tau protein, synaptic loss, and progressive neuronal damage. This framework remains essential for understanding the core pathological features of the disease; however, it does not fully explain why Alzheimer's progresses more rapidly in some individuals, why it is closely associated with systemic inflammation, or how a wide range of environmental factors, from metabolic status to immune responses, may influence disease trajectory (Heneka et al., 2015; Long & Holtzman, 2019; Scheltens et al., 2021). The growing interest in the gut microbiota has emerged from this unresolved space. The gut is no longer viewed merely as an organ system involved in digestion, but rather as a dynamic biological

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ecosystem that continuously interacts with immunity, metabolism, neuroendocrine responses, and the central nervous system (Cryan et al., 2019).

The gut microbiota is a complex community composed of bacteria, viruses, fungi, archaea, and other microorganisms. This community can influence host immune balance, energy metabolism, the production of neuroactive metabolites such as short-chain fatty acids, and the intensity of inflammatory responses. In Alzheimer's disease, the importance of the microbiome does not primarily arise from the direct migration of microorganisms to the brain, but from their ability to shape central nervous system function indirectly and persistently through microbial metabolites and immune-mediated pathways. For this reason, the gut-brain axis should not be considered only a complementary topic in Alzheimer's pathogenesis; rather, it represents an important biological link that helps explain the systemic nature of the disease (Faulin & Estadella, 2023; Murray et al., 2022).

Human studies increasingly suggest that the gut microbiota of individuals with Alzheimer's disease differs from that of healthy controls. Vogt et al. (2017) reported reduced gut microbial diversity in patients diagnosed with Alzheimer's disease and demonstrated that the overall microbiota composition differed markedly from that of the control group. This finding does not establish causality on its own; nevertheless, it indicates that Alzheimer's disease should not be viewed as a closed process beginning exclusively in the brain, but rather as a condition that needs to be interpreted together with the peripheral biological environment.

2. The Gut-Brain Axis and Neurodegenerative Processes

The gut-brain axis refers to a bidirectional communication network between the gastrointestinal tract and the central nervous system. This interaction is mediated through several interconnected

routes, including the vagus nerve, the enteric nervous system, the hypothalamic-pituitary-adrenal axis, immune signaling, intestinal barrier integrity, and microbial metabolites. Within this framework, the gut microbiota should not be viewed as a passive microbial community functioning separately from the brain. It acts more like a biological regulatory interface, capable of influencing stress responses, inflammatory tone, neurotransmitter-related pathways, and metabolic homeostasis (Cryan et al., 2019; Kowalski & Mulak, 2019).

In Alzheimer's disease, the relevance of this axis becomes especially apparent when aging, inflammation, and barrier dysfunction are considered together. Aging may reduce microbial diversity and weaken the resilience of the intestinal ecosystem, allowing pro-inflammatory microbial patterns to become more prominent. When intestinal barrier function is impaired, microbial products such as lipopolysaccharide can more easily enter the circulation and contribute to systemic inflammation. This inflammatory signal may then influence blood-brain barrier permeability and create a biological environment in which microglial cells are more readily activated. In this context, neuronal injury in Alzheimer's disease is not shaped only by amyloid-β deposition or tau pathology; it may also be amplified by a chronic inflammatory microenvironment that connects peripheral immune activity with central neurodegeneration (Claesson et al., 2012; Heneka et al., 2015; Liu et al., 2020; Zenaro et al., 2017).

Experimental studies have made this connection more visible. Harach et al. (2017) showed that amyloid pathology was reduced in germ-free APPPS1 transgenic mice lacking gut microbiota. When these germ-free Alzheimer's model mice were colonized with microbiota obtained from conventionally raised Alzheimer's model mice, amyloid burden increased. This finding does not prove that the microbiome alone initiates Alzheimer's

pathology. It does, however, strongly suggest that gut microbiota can act as a biological modulator capable of accelerating or attenuating disease-related pathological processes (Harach et al., 2017).

3. Microbiota Alterations and Dysbiosis in Alzheimer's Disease

Dysbiosis refers to the disruption of microbial balance within the gut ecosystem. In Alzheimer's disease, this concept should not be reduced simply to the increase or decrease of particular bacterial taxa. The more important issue is the loss of microbial functions that normally support immune regulation, metabolic stability, mucosal barrier integrity, and anti-inflammatory signaling. Reduced microbial diversity, shifts toward inflammation-associated bacterial profiles, and impaired production of beneficial metabolites may all intersect with neurodegenerative mechanisms. For this reason, dysbiosis is increasingly interpreted not as an isolated intestinal abnormality, but as part of the systemic biological environment that may influence Alzheimer's disease progression (Kowalski & Mulak, 2019; Liu et al., 2020; Murray et al., 2022).

Human studies support the presence of altered gut microbial profiles in Alzheimer's disease, although the clinical meaning of these alterations remains under discussion. Vogt et al. (2017) reported reduced microbial diversity in individuals with Alzheimer's disease and showed that their gut microbiota composition differed from that of age- and sex-matched controls. These findings should be interpreted cautiously, because gut microbiota is strongly influenced by age, diet, medication use, antibiotic exposure, lifestyle, and comorbid diseases. Therefore, microbiota alterations in Alzheimer's disease should not be presented as direct evidence of causality. A more balanced interpretation is that dysbiosis may modify the biological terrain in which Alzheimer's pathology develops and progresses (Murray et al., 2022; Vogt et al., 2017).

4. Molecular Mechanisms: Inflammation, the Blood-Brain Barrier, and Microbial Metabolites

The relationship between the gut microbiota and Alzheimer's disease is best understood through indirect biological pathways rather than through the simple migration of microorganisms into the brain. A healthy intestinal ecosystem contributes to immune regulation, mucosal barrier integrity, metabolic balance, and the production of bioactive metabolites. When this ecosystem becomes disrupted, its regulatory function may weaken. Microbial products can then more easily interact with the host immune system, creating a low-grade but persistent inflammatory environment. In Alzheimer's disease, such systemic inflammatory pressure may not initiate neurodegeneration by itself, but it can shape the biological conditions under which neuronal injury progresses (Cryan et al., 2019; Kowalski & Mulak, 2019).

Lipopolysaccharide is one of the microbial products most frequently discussed in this context. As a component of the outer membrane of Gram-negative bacteria, lipopolysaccharide can activate innate immune pathways, particularly through pattern-recognition receptors such as Toll-like receptors. This activation may increase the release of pro-inflammatory mediators, including IL-1β, IL-6, and TNF-α. Once peripheral inflammation becomes sustained, it can influence endothelial function, alter blood-brain barrier permeability, and facilitate microglial activation within the central nervous system. Microglia may initially act as protective cells by clearing damaged proteins and cellular debris; however, chronic activation can shift them toward a state that promotes synaptic dysfunction, oxidative injury, and neuronal vulnerability (Heneka et al., 2015; Liu et al., 2020; Zhao et al., 2017).

The blood-brain barrier occupies a central position in this network. It is not merely a physical wall between the brain and peripheral circulation, but a highly active interface composed of

endothelial cells, tight junction proteins, pericytes, astrocytic endfeet, and basement membrane components. Aging, vascular dysfunction, metabolic stress, and systemic inflammation can weaken this barrier. When barrier integrity is compromised, inflammatory cytokines, microbial molecules, and other circulating mediators may gain greater access to the brain microenvironment. In Alzheimer's disease, blood-brain barrier dysfunction is increasingly considered an important contributor to disease progression, particularly because it can impair amyloid-β clearance and intensify neuroinflammatory signaling (Sweeney et al., 2018; Zenaro et al., 2017).

Microbial metabolites add another layer to this mechanism. Short-chain fatty acids, especially acetate, propionate, and butyrate, are produced through bacterial fermentation of dietary fibers. These metabolites can support intestinal barrier function, regulate immune responses, and influence neuroactive signaling pathways. Butyrate is particularly important because of its potential effects on anti-inflammatory responses and gene regulation. Yet the biological consequences of short-chain fatty acids should not be interpreted as universally beneficial or harmful. Their effects depend on concentration, host metabolic status, microbial community structure, and the inflammatory context in which they act (Dalile et al., 2019; Liu et al., 2020).

Taken together, these mechanisms suggest that the microbiota may influence Alzheimer's disease through a connected biological network involving intestinal permeability, systemic inflammation, blood-brain barrier integrity, microglial activation, and microbial metabolites. This perspective moves the discussion beyond the search for a single causative bacterium. A more realistic interpretation is that gut dysbiosis may alter the host environment in ways that either intensify or attenuate the molecular processes

already involved in Alzheimer's pathology (Murray et al., 2022; Cryan et al., 2019).

5. Relationship of the Microbiome with Amyloid-β, Tau Pathology, and Oxidative Stress

Amyloid-β accumulation and tau dysregulation remain central pathological features of Alzheimer's disease. The microbiome does not replace these classical mechanisms; rather, it provides a broader biological context in which they may develop and progress. Amyloid-β deposition is influenced not only by increased production, but also by impaired clearance. Chronic inflammation can modify microglial activity and reduce the efficiency with which amyloid-β is removed from the brain. In this sense, gut-derived inflammatory signals may indirectly contribute to the persistence of amyloid pathology by changing the immune environment surrounding plaques (Heneka et al., 2015; Long & Holtzman, 2019).

Experimental evidence has strengthened this interpretation. Harach et al. (2017) showed that germ-free APPPS1 transgenic mice developed reduced amyloid-β pathology compared with conventionally raised Alzheimer's model mice. When germ-free mice were colonized with microbiota obtained from Alzheimer's model mice, amyloid burden increased. Other studies using antibiotic-induced microbiome perturbation have also reported changes in amyloid deposition, neuroinflammation, and microglial phenotypes in murine models. These findings should not be translated directly into clinical recommendations, but they indicate that the gut microbiota can act as a disease-modifying factor in experimental Alzheimer's pathology (Dodiya et al., 2019; Harach et al., 2017; Minter et al., 2016).

Another possible connection involves microbial amyloids. Some bacteria produce amyloid-like proteins, such as curli fibers, which may interact with host immune pathways and contribute to

inflammatory priming. This idea has led to the hypothesis that microbial amyloids could influence protein misfolding or immune responses through molecular mimicry-like mechanisms. The concept remains biologically plausible but should be presented cautiously, because the strongest evidence comes from mechanistic and experimental work rather than definitive human causal studies. Even so, it offers a meaningful explanation for how peripheral microbial products might influence central protein aggregation indirectly (Friedland, 2015; Zhao et al., 2017).

The relationship between the microbiome and tau pathology appears to be less direct, but still important. Tau hyperphosphorylation is influenced by inflammatory signaling, oxidative stress, mitochondrial dysfunction, and altered kinase-phosphatase activity. Gut dysbiosis may contribute to these processes by sustaining peripheral inflammation and increasing the burden of circulating microbial products. In such an environment, inflammatory cytokines and glial activation may promote intracellular stress pathways that favor tau phosphorylation and synaptic instability. Therefore, the microbiome-tau connection should not be described as a simple linear pathway; it is more accurately understood as part of an inflammatory and metabolic network that influences neuronal vulnerability (Heneka et al., 2015; Liu et al., 2020; Sun et al., 2019).

Oxidative stress forms another bridge between dysbiosis and neurodegeneration. Alzheimer’s disease is associated with mitochondrial dysfunction, excessive production of reactive oxygen species, lipid peroxidation, protein oxidation, and impaired antioxidant defense. Dysbiosis may aggravate this state by promoting systemic inflammation and reducing the production of metabolites that normally support barrier and immune homeostasis. Conversely, a more balanced microbial ecosystem may help maintain anti-inflammatory and antioxidant signaling through

metabolite-mediated pathways. The clinical importance of this relationship lies in its cumulative effect: oxidative stress may not explain Alzheimer's disease alone, but it can intensify amyloid toxicity, tau pathology, and synaptic failure (Dalile et al., 2019; Liu et al., 2020).

Human studies also point toward a relationship between gut microbial profiles, amyloid pathology, and peripheral inflammation. Cattaneo et al. (2017) reported an association between brain amyloidosis, pro-inflammatory gut bacterial taxa, and inflammatory markers in cognitively impaired elderly individuals. This does not prove that dysbiosis causes amyloid deposition. It does, however, reinforce the idea that Alzheimer's disease develops within a broader immune-metabolic environment. For this reason, the microbiome should be viewed not as an isolated cause, but as a biological modifier that may influence the tempo and severity of neurodegenerative change (Cattaneo et al., 2017; Murray et al., 2022).

6. Microbiota Targeted Therapeutic Approaches and Future Perspectives

Microbiota targeted strategies are not yet established as standard treatments for Alzheimer's disease. Their current value lies in their potential to modify the inflammatory and metabolic background that may contribute to disease progression. These approaches include dietary interventions, probiotics, prebiotics, synbiotics, postbiotic metabolites, and, at an experimental level, fecal microbiota transplantation. The aim is not to eliminate amyloid-β or tau pathology directly, but to reshape the intestinal ecosystem in a way that may reduce systemic inflammation, improve metabolic signaling, and support gut-brain communication (Liu et al., 2020; Murray et al., 2022).

Diet is one of the most practical ways to influence the gut microbiota. Dietary patterns rich in fiber, polyphenols, unsaturated fatty acids, and plant-based foods may support microbial diversity and promote the production of beneficial metabolites. Mediterranean style and MIND dietary patterns have been associated with lower Alzheimer's disease risk and slower cognitive decline in observational studies. These effects are usually discussed in relation to vascular health, oxidative stress, and metabolic regulation, but microbiota-mediated anti-inflammatory pathways may also contribute. The important point is that dietary influence should be understood as a long term biological pattern, not as the effect of a single nutrient or supplement (Morris et al., 2015; Scarmeas et al., 2006).

Probiotic and prebiotic interventions have attracted particular attention because they offer a direct way to modify microbial composition and function. Probiotics are live microorganisms that may provide health benefits when administered in adequate amounts, while prebiotics are substrates that support the growth or activity of beneficial microbes. In a randomized controlled trial, Akbari et al. (2016) reported that probiotic supplementation for 12 weeks improved cognitive scores and some metabolic parameters in patients with Alzheimer's disease. Leblhuber et al. (2018) also explored probiotic supplementation in Alzheimer's dementia and reported changes in inflammatory and metabolic markers. These findings are promising, but they remain preliminary because sample sizes are limited, probiotic strains vary across studies, and intervention durations are generally short.

Fecal microbiota transplantation represents a more experimental approach. In animal models, transplantation of microbiota from healthy donors has been reported to improve cognitive performance and reduce Alzheimer's-like pathological changes. Sun et al. (2019), for example, found that fecal microbiota

transplantation reduced amyloid-β deposition, tau phosphorylation, synaptic dysfunction, and neuroinflammation in APP/PS1 transgenic mice. Despite these encouraging findings, clinical translation remains uncertain. Donor selection, long-term safety, microbial engraftment, immune responses in older adults, and standardization of protocols are major issues that must be resolved before fecal microbiota transplantation can be considered a realistic therapeutic option for Alzheimer's disease.

Future work in this field will likely move toward personalized microbiome medicine. Alzheimer's disease is biologically heterogeneous, and it is unlikely that all patients share a single microbial signature. Age, diet, medications, antibiotic exposure, comorbidities, frailty, disease stage, and genetic background may all influence the microbiome. For this reason, future studies should integrate metagenomics, metabolomics, inflammatory markers, neuroimaging, and cognitive outcomes. Such multi-layered designs may help distinguish microbial changes that merely accompany Alzheimer's disease from those that actively contribute to its progression (Cryan et al., 2019; Kowalski & Mulak, 2019; Murray et al., 2022).

The major limitation of the field remains causality. Many studies show associations between dysbiosis and Alzheimer's disease, but association does not establish directionality. Microbiota changes may contribute to neurodegeneration, but they may also arise as a consequence of aging, reduced mobility, altered diet, medication use, institutional care, or disease-related lifestyle changes. Therefore, microbiota-targeted strategies should be presented as complementary and investigational, not as independent curative treatments. With stronger longitudinal studies and well-designed clinical trials, the microbiome may become useful not only as a therapeutic target, but also as a source of biomarkers for early

diagnosis, risk stratification, and individualized disease monitoring (Liu et al., 2020; Murray et al., 2022).

References

Akbari, E., Asemi, Z., Daneshvar Kakhaki, R., Bahmani, F., Kouchaki, E., Tamtaji, O. R., Hamidi, G. A., & Salami, M. (2016). Effect of probiotic supplementation on cognitive function and metabolic status in Alzheimer's disease: A randomized, double-blind and controlled trial. *Frontiers in Aging Neuroscience, 8*, 256. https://doi.org/10.3389/fnagi.2016.00256

Cattaneo, A., Cattane, N., Galluzzi, S., Provasi, S., Lopizzo, N., Festari, C., Ferrari, C., Guerra, U. P., Paghera, B., Muscio, C., Bianchetti, A., Dalla Volta, G., Turla, M., Cotelli, M. S., Gennuso, M., Prelle, A., Zanetti, O., Lussignoli, G., Mirabile, D., . . . Frisoni, G. B. (2017). Association of brain amyloidosis with pro-inflammatory gut bacterial taxa and peripheral inflammation markers in cognitively impaired elderly. *Neurobiology of Aging, 49*, 60–68. https://doi.org/10.1016/j.neurobiolaging.2016.08.019

Claesson, M. J., Jeffery, I. B., Conde, S., Power, S. E., O'Connor, E. M., Cusack, S., Harris, H. M. B., Coakley, M., Lakshminarayanan, B., O'Sullivan, O., Fitzgerald, G. F., Deane, J., O'Connor, M., Harnedy, N., O'Connor, K., O'Mahony, D., van Sinderen, D., Wallace, M., Brennan, L., . . . O'Toole, P. W. (2012). Gut microbiota composition correlates with diet and health in the elderly. *Nature, 488*(7410), 178–184. https://doi.org/10.1038/nature11319

Cryan, J. F., O'Riordan, K. J., Cowan, C. S. M., Sandhu, K. V., Bastiaanssen, T. F. S., Boehme, M., Codagnone, M. G., Cussotto, S., Fulling, C., Golubeva, A. V., Guzzetta, K. E., Jaggar, M., Long-Smith, C. M., Lyte, J. M., Martin, J. A., Molinero-Perez, A., Moloney, G., Morelli, E., Morillas, E., . . . Dinan, T. G. (2019). The microbiota-gut-brain axis.

Physiological Reviews, 99(4), 1877–2013. https://doi.org/10.1152/physrev.00018.2018

Dalile, B., Van Oudenhove, L., Vervliet, B., & Verbeke, K. (2019). The role of short-chain fatty acids in microbiota-gut-brain communication. *Nature Reviews Gastroenterology & Hepatology, 16*(8), 461–478. https://doi.org/10.1038/s41575-019-0157-3

Dodiya, H. B., Kuntz, T., Shaik, S. M., Baufeld, C., Leibowitz, J., Zhang, X., Gottel, N., Zhang, X., Butovsky, O., Gilbert, J. A., & Sisodia, S. S. (2019). Sex-specific effects of microbiome perturbations on cerebral Aβ amyloidosis and microglia phenotypes. *Journal of Experimental Medicine, 216*(7), 1542–1560. https://doi.org/10.1084/jem.20182386

Faulin, T. do E. S., & Estadella, D. (2023). Alzheimer's disease and its relationship with the microbiota-gut-brain axis. *Arquivos de Gastroenterologia, 60*(1), 144–154. https://doi.org/10.1590/S0004-2803.202301000-17

Friedland, R. P. (2015). Mechanisms of molecular mimicry involving the microbiota in neurodegeneration. *Journal of Alzheimer's Disease, 45*(2), 349–362. https://doi.org/10.3233/JAD-142841

Harach, T., Marungruang, N., Duthilleul, N., Cheatham, V., Mc Coy, K. D., Frisoni, G., Neher, J. J., Fåk, F., Jucker, M., Lasser, T., & Bolmont, T. (2017). Reduction of Aβ amyloid pathology in APPPS1 transgenic mice in the absence of gut microbiota. *Scientific Reports, 7*, 41802. https://doi.org/10.1038/srep41802

Heneka, M. T., Carson, M. J., El Khoury, J., Landreth, G. E., Brosseron, F., Feinstein, D. L., Jacobs, A. H., Wyss-Coray, T., Vitorica, J., Ransohoff, R. M., Herrup, K., Frautschy, S.

A., Finsen, B., Brown, G. C., Verkhratsky, A., Yamanaka, K., Koistinaho, J., Latz, E., Halle, A., . . . Kummer, M. P. (2015). Neuroinflammation in Alzheimer's disease. *The Lancet Neurology, 14*(4), 388–405. https://doi.org/10.1016/S1474-4422(15)70016-5

Kowalski, K., & Mulak, A. (2019). Brain-gut-microbiota axis in Alzheimer's disease. *Journal of Neurogastroenterology and Motility, 25*(1), 48–60. https://doi.org/10.5056/jnm18087

Leblhuber, F., Steiner, K., Schuetz, B., Fuchs, D., & Gostner, J. M. (2018). Probiotic supplementation in patients with Alzheimer's dementia: An explorative intervention study. *Current Alzheimer Research, 15*(12), 1106–1113. https://doi.org/10.2174/1389200219666180813144834

Liu, S., Gao, J., Zhu, M., Liu, K., & Zhang, H.-L. (2020). Gut microbiota and dysbiosis in Alzheimer's disease: Implications for pathogenesis and treatment. *Molecular Neurobiology, 57*(12), 5026–5043. https://doi.org/10.1007/s12035-020-02073-3

Long, J. M., & Holtzman, D. M. (2019). Alzheimer disease: An update on pathobiology and treatment strategies. *Cell, 179*(2), 312–339. https://doi.org/10.1016/j.cell.2019.09.001

Minter, M. R., Zhang, C., Leone, V., Ringus, D. L., Zhang, X., Oyler-Castrillo, P., Musch, M. W., Liao, F., Ward, J. F., Holtzman, D. M., Chang, E. B., Tanzi, R. E., & Sisodia, S. S. (2016). Antibiotic-induced perturbations in gut microbial diversity influences neuro-inflammation and amyloidosis in a murine model of Alzheimer's disease. *Scientific Reports, 6*, 30028. https://doi.org/10.1038/srep30028

Morris, M. C., Tangney, C. C., Wang, Y., Sacks, F. M., Bennett, D. A., & Aggarwal, N. T. (2015). MIND diet associated with

reduced incidence of Alzheimer's disease. *Alzheimer's & Dementia, 11*(9), 1007–1014. https://doi.org/10.1016/j.jalz.2014.11.009

Murray, E. R., Kemp, M., & Nguyen, T. T. (2022). The microbiota-gut-brain axis in Alzheimer's disease: A review of taxonomic alterations and potential avenues for interventions. *Archives of Clinical Neuropsychology, 37*(3), 595–607. https://doi.org/10.1093/arclin/acac008

Scarmeas, N., Stern, Y., Tang, M.-X., Mayeux, R., & Luchsinger, J. A. (2006). Mediterranean diet and risk for Alzheimer's disease. *Annals of Neurology, 59*(6), 912–921. https://doi.org/10.1002/ana.20854

Scheltens, P., De Strooper, B., Kivipelto, M., Holstege, H., Chételat, G., Teunissen, C. E., Cummings, J., & van der Flier, W. M. (2021). Alzheimer's disease. *The Lancet, 397*(10284), 1577–1590. https://doi.org/10.1016/S0140-6736(20)32205-4

Sun, J., Xu, J., Ling, Y., Wang, F., Gong, T., Yang, C., Ye, S., Ye, K., Wei, D., Song, Z., Chen, D., Liu, J., & Wang, B. (2019). Fecal microbiota transplantation alleviated Alzheimer's disease-like pathogenesis in APP/PS1 transgenic mice. *Translational Psychiatry, 9*, 189. https://doi.org/10.1038/s41398-019-0525-3

Sweeney, M. D., Sagare, A. P., & Zlokovic, B. V. (2018). Blood-brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. *Nature Reviews Neurology, 14*(3), 133–150. https://doi.org/10.1038/nrneurol.2017.188

Vogt, N. M., Kerby, R. L., Dill-McFarland, K. A., Harding, S. J., Merluzzi, A. P., Johnson, S. C., Carlsson, C. M., Asthana, S., Zetterberg, H., Blennow, K., Bendlin, B. B., & Rey, F. E. (2017). Gut microbiome alterations in Alzheimer's disease.

Scientific Reports, 7, 13537. https://doi.org/10.1038/s41598-017-13601-y

Zenaro, E., Piacentino, G., & Constantin, G. (2017). The blood-brain barrier in Alzheimer's disease. *Neurobiology of Disease, 107*, 41–56. https://doi.org/10.1016/j.nbd.2016.07.007

Zhao, Y., Cong, L., Jaber, V., & Lukiw, W. J. (2017). Microbiome-derived lipopolysaccharide enriched in the perinuclear region of Alzheimer's disease brain. *Frontiers in Immunology, 8*, 1064. https://doi.org/10.3389/fimmu.2017.01064

CHAPTER 6

BIOCHEMICAL BIOMARKERS IN ALZHEIMER'S DISEASE

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1. Alzheimer's Disease Biomarkers: From Pathophysiology to Early Detection

Alzheimer's disease (AD) is a progressive age-related neurodegenerative disorder and the leading cause of dementia worldwide, representing a major and growing global health burden (World Health Organization, 2021). Although the clinical manifestation of AD is characterized by an insidious and progressive deterioration of cognitive and functional abilities, the underlying neuropathological alterations emerge years, and potentially decades, before the onset of overt clinical symptoms. The classical pathological hallmarks of AD are defined by the extracellular accumulation of amyloid-β (Aβ) plaques and the intracellular deposition of hyperphosphorylated tau proteins, while increasing evidence implicates a broader network of

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pathological mechanisms, including neuroinflammation, metabolic dysfunction, and cerebrovascular abnormalities, in disease initiation and progression (Mahaman et al., 2022; Scheltens et al., 2016; Zetterberg & Mattsson, 2014). The recognition that these pathological processes precede clinical manifestations has accelerated efforts to identify reliable biomarkers capable of detecting disease-related changes at preclinical and early symptomatic stages. In this context, advances in molecular profiling, neuroimaging, proteomics, and multiplex analytical technologies have substantially expanded the biomarker landscape for AD, supporting earlier detection, biological characterization, and longitudinal monitoring of disease progression. The National Institute on Aging–Alzheimer's Association (NIA-AA) Amyloid/Tau/Neurodegeneration (ATN) research framework provides a biologically grounded classification system in which AD-related biomarkers are organized according to amyloid pathology, pathological tau accumulation, and neurodegeneration, thereby offering an integrated framework for characterizing and staging the disease in living individuals (Georgakas et al., 2023; Jack et al., 2018).

The rapidly increasing prevalence and socioeconomic burden of AD have made it a major global health crisis, and the literature reports that the number of affected individuals is expected to reach 139 million by 2050 (Georgakas et al., 2023). Given that therapeutic interventions may be less effective once substantial and irreversible pathological changes have occurred, the early detection of AD through reliable and accessible biomarkers is critical for improving treatment outcomes (Morris & Selkoe, 2011; Salloway et al., 2014; Sperling et al., 2011). Therefore, the development of sensitive, cost-effective, and

minimally invasive biomarkers remains essential for early diagnosis, disease monitoring, patient stratification, and the advancement of disease-modifying therapies.

Accordingly, the identification and validation of robust biomarkers that accurately reflect the underlying pathological processes of AD are essential for enabling earlier and more precise disease characterization. In this context, key biomarkers associated with amyloid pathology, tau accumulation, neurodegeneration, and glial activation—including amyloid-β (Aβ), phosphorylated tau (p-tau), neurofilament light chain (NfL), and glial fibrillary acidic protein (GFAP)—have emerged as promising tools for improving early diagnosis, disease staging, and monitoring. The following section therefore focuses on the most clinically relevant biochemical biomarkers of AD and their potential contribution to advancing early detection and precision-oriented therapeutic strategies (Georgakas et al., 2023).

2. Clinical Factors

Alzheimer's disease is the most prevalent cause of dementia, a clinical syndrome characterized by progressive impairments in memory, cognition, language, and daily functioning. Although dementia encompasses several neurodegenerative disorders, including Lewy body dementia, vascular dementia, and frontotemporal dementia, AD accounts for approximately 60–80% of all dementia cases (Wu et al., 2018). The pathological progression of AD is primarily associated with the accumulation of amyloid-β, tau hyperphosphorylation, and subsequent aggregation of phosphorylated tau, particularly in brain regions involved in

learning and memory, such as the hippocampus (Chen et al., 2017; Jack et al., 2011; Selkoe & Hardy, 2016).

Clinically, AD progresses through a continuum beginning with a presymptomatic, cognitively unimpaired stage, followed by mild cognitive impairment and ultimately clinically manifest dementia. While pathological alterations may develop years before the appearance of overt cognitive symptoms, progressive neuronal dysfunction and loss during the MCI stage contribute to memory impairment and subsequent functional decline. In advanced disease, severe cognitive deterioration and neuropsychiatric manifestations may emerge, imposing a substantial psychological, physical, and socioeconomic burden on patients and caregivers. Therefore, the identification of AD-related pathological changes before the onset of irreversible cognitive decline is of critical importance. Accordingly, the development and validation of sensitive and reliable biomarkers capable of detecting these alterations at preclinical and early symptomatic stages have become a major focus of AD research (Gunes et al., 2022).

3. Conventional and Advanced Biomarkers

3.1. Conventional biomarkers

Biomarker-based approaches have become increasingly central to the identification of individuals at elevated risk for AD, particularly during the preclinical stages preceding the emergence of overt cognitive impairment and dementia. In addition to facilitating early disease detection, well-established AD biomarkers provide essential benchmark measures for the assessment, validation, and clinical positioning of newly emerging biomarkers identified in human biological fluids. The contemporary biomarker landscape in AD has been substantially

shaped by the 2018 research framework proposed by the National NIA-AA. Unlike conventional diagnostic paradigms that were predominantly grounded in clinical symptomatology, this framework established a biologically oriented model in which AD is defined and staged according to objective evidence of disease-specific pathological processes, as reflected by validated biomarkers (Jack et al., 2018).

Since its initial establishment in the early 1990s, the Braak staging framework has remained an important neuropathological model for delineating the temporal evolution and anatomical distribution of AD-related pathology throughout the brain. More recently, a 2021 investigation combined Braak-based staging with positron emission tomography (PET) biomarkers to assess the relative prognostic utility of tau-PET and amyloid-β (Aβ)-PET imaging. The findings suggested that integrating tau-PET measures with Braak staging may provide a more informative estimate of patient-level risk for subsequent clinical progression of AD compared with Aβ-PET imaging considered in isolation (Biel et al., 2021; Braak & Braak, 1991).

3.1.1. Brain imaging

Single-photon emission computed tomography (SPECT) provides a functional imaging approach for assessing cerebral perfusion by characterizing the distribution of radiotracers in the brain relative to their delivery through the arterial circulation. Through the application of appropriate kinetic modeling approaches, SPECT can be used to estimate regional cerebral blood flow (rCBF), thereby providing information on regional alterations in cerebral perfusion. Beyond perfusion assessment, SPECT can also employ radioligands targeting the dopamine transporter (DAT). These DAT-specific tracers may provide

additional functional information and have demonstrated clinical value in the differential evaluation of dementia syndromes, particularly when distinguishing between clinically overlapping forms of neurodegenerative dementia. (Ferrando & Damian, 2021).

Functional neuroimaging modalities, including magnetic resonance imaging (MRI), positron emission tomography (PET), and single-photon emission computed tomography (SPECT), play an important role in the biomarker-based assessment of Alzheimer's disease (AD). These techniques have demonstrated clinically relevant accuracy in identifying individuals with mild cognitive impairment (MCI) who are at increased risk of subsequent conversion to AD. In a meta-analysis encompassing 24 studies and 1,112 participants, Yuan et al. reported modality-specific sensitivity and specificity estimates for predicting progression from MCI to AD of 89% and 85% for PET, 84% and 70% for SPECT, and 73% and 81% for MRI, respectively (Yuan et al., 2009). Collectively, these findings indicate that functional and structural neuroimaging can provide useful discriminatory information across the MCI–AD continuum. Nevertheless, the interpretation of amyloid PET requires particular caution, especially in older populations. The prevalence of amyloid-positive (A+) PET findings increases with advancing age, and amyloid positivity alone does not necessarily imply the presence of clinically meaningful memory impairment or broader cognitive dysfunction. Consequently, reliance on visual assessment of structural or conventional imaging findings may be insufficient for detecting individuals in the presymptomatic phase preceding MCI, highlighting the need for biomarker strategies capable of identifying AD-related

pathology before the emergence of overt clinical symptoms. (Mathis et al., 2013).

3.2.2. Highly invasive biomarkers

The National Institute on Aging–Alzheimer's Association has proposed the ATN framework, which integrates biomarkers of amyloid-β (Aβ) deposition, pathological tau, and neurodegeneration to support the biological characterization and diagnosis of Alzheimer's disease (AD) (Jack et al., 2016). The complementary use of cerebrospinal fluid (CSF) and neuroimaging biomarkers can enhance the diagnostic accuracy and prognostic assessment of AD, particularly in complex clinical presentations. Among CSF biomarkers, Aβ42 represents the 42-amino-acid isoform of Aβ and is one of the most extensively investigated biomarkers associated with AD pathology. CSF Aβ42 concentrations are inversely associated with cerebral Aβ deposition; thus, reduced CSF Aβ42 levels generally reflect an increased burden of Aβ plaques in the brain, as detected by amyloid-PET imaging. In addition, the CSF Aβ42/Aβ40 ratio has been proposed as a clinically relevant biomarker for assessing the risk of disease progression, including the transition from the presymptomatic stage to mild MCI and from MCI to clinically manifest AD (Gunes et al., 2022).

Tau is a microtubule-associated protein predominantly localized within neuronal axons and plays an essential role in maintaining neuronal structure and function. Increased concentrations of total tau and phosphorylated tau in CSF are closely associated with AD-related neuropathology. Elevated CSF tau levels may reflect neuronal injury and the subsequent release of tau proteins from affected neurons, potentially

occurring in the context of Aβ accumulation and downstream neurodegenerative processes. Phosphorylated tau, commonly referred to as p-tau, comprises several disease-associated isoforms, including p-tau181, p-tau217, and p-tau231. The diagnostic and prognostic performance of these individual p-tau species may vary depending on the clinical context, with differences observed in their ability to distinguish AD from non-AD tauopathies and to characterize disease progression across distinct stages of AD. In contrast, CSF t-tau is generally considered an indicator of the intensity of neuronal injury and neurodegeneration. The pathological accumulation and aggregation of tau contribute to the formation of neurofibrillary tangles, which disrupt neuronal integrity, synaptic transmission, and normal mechanisms of neuronal plasticity (Gunes et al., 2022).

Morinaga et al., (2010) evaluated the diagnostic performance of MRI, SPECT, FDG-PET, and CSF biomarkers in a cohort of 207 patients with AD. AD-associated abnormalities were identified in 77.4% of patients using MRI, 81.6% using SPECT, 93.1% using fluorodeoxyglucose (FDG)-PET, and 94.0% using CSF biomarkers. Among individuals classified as having a Clinical Dementia Rating (CDR) score of 0.5, corresponding to questionable cognitive impairment, CSF biomarkers demonstrated the highest sensitivity (90.0%), followed by SPECT (80.8%), FDG-PET (71.4%), and MRI (65.5%). In patients with a CDR score of 1, representing mild dementia, FDG-PET (96.7%) and CSF biomarkers (95.5%) exhibited the greatest diagnostic sensitivity. At a CDR score of 2, corresponding to moderate dementia, all evaluated biomarkers demonstrated high sensitivity. Nevertheless, the relationship between AD pathology and these established

biomarkers has not been consistently confirmed in all reported cases, highlighting potential limitations in their diagnostic interpretation and clinical applicability (Morinaga et al., 2010).

Despite their proven clinical benefits, conventional assessments of Alzheimer's disease (AD) biomarkers have significant practical limitations. CSF-based analyses require a lumbar puncture—an invasive procedure that limits their feasibility for routine screening and repeated monitoring—while advanced neuroimaging techniques are often associated with high costs, specialized infrastructure, and significant time requirements. Consequently, the widespread implementation of these approaches in primary care and routine clinical settings remains challenging. These limitations have intensified efforts to identify more affordable, accessible, and minimally invasive biomarkers that can be measured in easily obtainable biological fluids, particularly blood and urine. Accordingly, there is growing interest in metabolite- and molecular factor-based biomarkers that could facilitate the earlier and more accessible detection of AD pathology.

4. Blood-Based Biomarkers

Blood-based biomarkers have emerged as one of the most promising approaches for the broad clinical implementation of Alzheimer’s disease assessment, primarily owing to their accessibility, relatively low invasiveness, and feasibility across both tertiary and non-tertiary healthcare settings. Compared with CSF analysis and neuroimaging modalities, blood-based assays offer substantial advantages in terms of cost, procedural burden, and time requirements. Recent advances in analytical and bioanalytical technologies—including enzyme-linked immunosorbent assays (ELISA),

electrochemiluminescence-based immunoassays such as Meso Scale Discovery (MSD), single-molecule array (Simoa) platforms, and mass spectrometry—have considerably enhanced the analytical sensitivity and clinical potential of circulating AD biomarkers. Although a broad spectrum of blood-derived biomarkers has been investigated, current research has predominantly focused on circulating amyloid-β (Aβ), phosphorylated and total tau species, neurofilament light chain and glial fibrillary acidic protein, which collectively provide complementary information on amyloid pathology, tau-related neurodegeneration, axonal injury, and astroglial activation, respectively.

4.1. Aβ isoforms

Early investigations of plasma amyloid biomarkers yielded inconsistent findings, with substantial variability reported across (Blennow et al., 2010; Mahaman et al., 2022; Mayeux & Schupf, 2011). Consistent with these early observations, a 2016 meta-analysis found no significant differences in plasma Aβ42 or Aβ40 concentrations between individuals with Alzheimer's disease (AD) and cognitively healthy controls (Olsson et al., 2016; Teunissen et al., 2022). The limited reproducibility of these measurements has been partly attributed to the complex peripheral biology of Aβ peptides, which are present in multiple tissues and can interact with circulating plasma proteins, thereby potentially affecting their recovery and analytical quantification (Kuo et al., 1999; Mahaman et al., 2022; Teunissen et al., 2022). However, the implementation of highly sensitive analytical technologies, particularly mass spectrometry and automated ultrasensitive immunoassays, has substantially improved the consistency and

diagnostic performance of plasma Aβ measurements (Teunissen et al., 2022). The increasing analytical sensitivity of these approaches has strengthened the evidence supporting plasma Aβ42/40 as a surrogate marker of cerebral amyloid pathology. In a population-based British 1946 birth cohort at approximately 70 years of age, Keshavan et al. (2021) evaluated plasma Aβ using liquid chromatography–mass spectrometry, Aβ measured by single-molecule array (Simoa), and plasma phosphorylated tau181 (p-tau181) measured by Simoa for their ability to predict amyloid positron emission tomography (Aβ-PET) status. Among the evaluated biomarkers, the mass spectrometry-derived Aβ42/40 ratio provided the greatest incremental predictive value over a baseline model incorporating age, sex, and APOE genotype, whereas the other biomarker modalities did not demonstrate comparable improvement (Keshavan et al., 2021). Moreover, studies validated against CSF Aβ and Aβ-PET measurements have consistently supported an inverse association between plasma Aβ concentrations and cerebral amyloid burden, further reinforcing the potential diagnostic relevance of plasma Aβ profiling (Nakamura et al., 2018; Olsson et al., 2016; Ovod et al., 2017; Teunissen et al., 2022).

As observed with CSF-based assessment, the plasma Aβ42/40 ratio has emerged as a more informative measure than either Aβ isoform alone. Across several studies, this ratio has demonstrated diagnostic accuracies ranging from approximately 82% to 97% for identifying AD-related amyloid pathology (Georgakas et al., 2023; Nakamura et al., 2018; Ovod et al., 2017; Schindler et al., 2019; Teunissen et al., 2022; West et al., 2021). Importantly, incorporating apolipoprotein E (APOE) genotype into biomarker-based models appears to further enhance diagnostic performance. Multiple investigations have

reported improved discrimination following stratification according to APOE status, particularly APOE ε4 carrier status, highlighting the potential value of integrating genetic and biochemical information within plasma-based diagnostic algorithms (Chatterjee et al., 2021; De Meyer et al., 2020; de Rojas et al., 2018; Janelidze et al., 2016; W.-W. Li et al., 2019; Palmqvist et al., 2019; Risacher et al., 2019; Teunissen et al., 2022; Verberk et al., 2018, 2020; Vergallo et al., 2019; West et al., 2021).

Further support for this combined biomarker strategy was provided by Li et al. (Y. Li et al., 2022), who employed high-precision immunoprecipitation mass spectrometry (IPMS) to quantify the plasma Aβ42/40 ratio. The resulting area under the curve (AUC) for predicting CSF amyloid status was 0.85 (95% CI: 0.78–0.91), increasing substantially to 0.93 (95% CI: 0.89–0.97) after stratification according to APOE ε4 status. These findings illustrate the added diagnostic value of incorporating genetic risk information into plasma amyloid-based assessment. Accordingly, commercially available assays have been developed that combine the Aβ42/40 ratio with APOE genotype to generate an amyloid probability score for clinical decision support (West et al., 2021). Such an integrated score has demonstrated an AUC of 0.90 and an overall diagnostic accuracy of 86% for predicting Aβ-PET-defined amyloid status (Georgakas et al., 2023; West et al., 2021).

Beyond the extensively investigated Aβ1–42 and Aβ1–40 species, additional circulating Aβ isoforms—particularly Aβ1–17—have been suggested as potential biomarkers for the diagnosis of AD. Notably, the ratio of free to cell-associated Aβ1–17 in peripheral blood has been reported to effectively distinguish cognitively healthy individuals from patients with

MCI or mild AD, demonstrating high diagnostic sensitivity and specificity. The clinical utility of less extensively characterized Aβ isoforms may extend beyond diagnosis, as changes in their circulating or central concentrations could provide insight into the biochemical response to therapeutic interventions. For instance, a clinical investigation of the γ-secretase inhibitor LY450139 (semagacestat) demonstrated a dose-dependent increase in shorter Aβ species in CSF, whereas no significant alterations were observed in plasma Aβ concentrations or in CSF Aβ1–42 and Aβ1–40 levels. These findings suggest that specific Aβ isoform profiles may provide complementary information for pharmacodynamic monitoring and treatment-response assessment. Several analytical platforms have subsequently been developed to quantify Aβ species in peripheral blood. Among these, Araclon Biotech Ltd. developed colorimetric ELISA platforms for the measurement of blood Aβ1–40 and Aβ1–42, enabling the assessment of both direct biomarker concentrations and derived indices associated with an increased probability of MCI (Georgakas et al., 2023). In parallel, alternative immunoassay strategies, including additional ELISA-based methods and electrochemiluminescence-based platforms, have been investigated to improve the sensitivity and analytical performance of blood-based Aβ detection. Collectively, these developments underscore the growing potential of circulating Aβ isoforms as accessible biomarkers for AD diagnosis and therapeutic monitoring (Snyder et al., 2014).

Despite these advances, several barriers currently limit the widespread clinical adoption of plasma amyloid testing. Although commercially available, some assays rely on technically demanding and relatively costly mass spectrometry workflows and may not be routinely reimbursed by insurance

systems. For example, the reported list price of approximately $1,250 represents a substantial economic barrier to broader implementation (West et al., 2021). In addition, although plasma Aβ-based approaches show considerable promise for identifying amyloid pathology, their ability to reliably distinguish AD from other causes of dementia remains insufficiently established. Further large-scale, prospective, and clinically representative studies are therefore required to determine their diagnostic specificity and establish their role within routine dementia assessment (Georgakas et al., 2023).

4.2. Plasma tau biomarkers

Similar to Aβ, early investigations of circulating tau biomarkers reported limited diagnostic performance and substantial inter-study variability (Mahaman et al., 2022; Teunissen et al., 2022). This heterogeneity is partly attributable to the molecular complexity of tau, which contains more than 70 post-translational modification sites, over 40 phosphorylation sites, and multiple truncated forms, complicating the identification of disease-specific circulating species (Teunissen et al., 2022; Wesseling et al., 2020) Consequently, research has increasingly focused on phosphorylated tau isoforms, particularly plasma P-tau181, P-tau217, and P-tau231. All three have been consistently reported to be elevated in AD compared with cognitively unimpaired controls and have demonstrated potential for reflecting disease severity and underlying AD pathology (Ashton et al., 2021; Barthélemy, Bateman, et al., 2020; Brickman et al., 2021; Palmqvist et al., 2019; Teunissen et al., 2022). Unlike the Aβ42/40 ratio, circulating phosphorylated tau concentrations generally increase with disease progression, further supporting their potential utility for longitudinal disease

monitoring (Mattsson-Carlgren et al., 2020). Their associations with both tau-PET and Aβ-PET measures have also been independently demonstrated (Barthélemy, Bateman, et al., 2020; Barthélemy, Horie, et al., 2020; Brickman et al., 2021; Janelidze et al., 2016; Mielke et al., 2018; Teunissen et al., 2022; Thijssen et al., 2020).

Beyond detecting AD-related pathology, disease-associated P-tau isoforms may improve the differentiation of AD from non-AD dementias (Georgakas et al., 2023). Among these candidates, P-tau217 has attracted particular attention because of its strong association with tau-PET and its potentially superior ability to predict amyloid positivity compared with P-tau181, particularly when measured by mass spectrometry (Janelidze et al., 2020). Notably, in autosomal dominant AD, plasma P-tau217 has been detected approximately 20 years before clinical symptom onset and before measurable changes on tau-PET, highlighting its potential value for very early disease detection (Janelidze et al., 2021; Teunissen et al., 2022). P-tau231 demonstrates a diagnostic performance comparable to P-tau181 for distinguishing AD from non-AD pathology, although some evidence suggests that P-tau231 alterations may emerge earlier in relation to Aβ-PET positivity and Braak tau stages; however, this temporal advantage has not been consistently replicated (Ashton et al., 2021; Mielke et al., 2021; Teunissen et al., 2022).

Clinical evidence further supports the prognostic utility of plasma P-tau. In participants from the Swedish BioFINDER-2 and ADNI cohorts, Palmqvist et al. (Palmqvist et al., 2021) reported that plasma P-tau217 predicted conversion to AD dementia within four years with an AUC of 0.83. Combining P-tau217 with selected cognitive measures and APOE genotype substantially improved predictive performance, yielding an

AUC of 0.91. Notably, adding CSF P-tau, CSF Aβ42/40, or plasma NfL did not significantly improve prediction beyond the more accessible biomarker-based model (Palmqvist et al., 2021). Similarly, Karikari et al. (2021) demonstrated that plasma P-tau181 is already elevated during preclinical AD and progressively increases with disease advancement. Among individuals with subjective cognitive decline, higher baseline P-tau181 predicted subsequent conversion to AD and demonstrated an AUC of 0.85 for AD diagnosis (Karikari et al., 2021). Collectively, these findings indicate that plasma P-tau181 and particularly P-tau217 may enable the detection of AD-related pathology before overt clinical symptoms, supporting their potential application in early diagnosis, risk stratification, longitudinal monitoring, and the identification of suitable participants for clinical trials.

4.3. Neurofilament light chain and glial fibrillary acidic protein

Neurofilament light chain (NfL) is an essential structural component of the axonal cytoskeleton and is predominantly expressed in large, myelinated axons (Schlaepfer & Lynch, 1977). Axonal injury leads to the release of NfL into the cerebrospinal fluid and subsequently into the bloodstream, resulting in increased concentrations in both compartments. The association between elevated CSF NfL and neurodegenerative disorders was first reported in 1996 (Rosengren et al., 1996). Notably, CSF and blood NfL concentrations exhibit a strong correlation, and increased levels have been consistently observed in individuals with MCI and AD dementia (Mattsson et al., 2017; Zetterberg et al., 2016). Accumulating evidence therefore supports circulating NfL as a sensitive indicator of

neuroaxonal injury. However, its diagnostic utility is constrained by limited disease specificity, as elevated blood NfL has been documented across a broad range of neurological conditions, including different forms of dementia, traumatic brain injury, cerebrovascular disorders, and multiple sclerosis (Hampel et al., 2023).

Glial fibrillary acidic protein (GFAP), a type III intermediate filament protein, is selectively expressed by astrocytes within the central nervous system (CNS), where these cells provide critical structural and functional support to neurons. Following CNS injury, GFAP and its degradation products are rapidly released into biofluids, providing an accessible molecular signature of astroglial activation. Importantly, plasma GFAP concentrations increase progressively across the AD continuum, with elevated levels detectable in cognitively unimpaired individuals exhibiting preclinical cerebral Aβ pathology and further increases during symptomatic stages of AD (Benedet et al., 2021; Chatterjee et al., 2021). Comparative analyses of paired plasma and CSF samples have additionally demonstrated that plasma GFAP may provide superior biomarker performance relative to CSF GFAP, characterized by a greater magnitude of change and improved discrimination between Aβ-positive and Aβ-negative individuals (Benedet et al., 2021). Within the ATX(N) biomarker framework, NfL is primarily assigned to the neurodegeneration component (N), whereas GFAP represents the neuroinflammatory "X" component (Hampel et al., 2023).

The analytical assessment of NfL and GFAP has benefited from a relatively standardized methodological landscape. Most recent AD studies evaluating these biomarkers have employed commercially available, well-established and

validated single-molecule array (Simoa)-based research-use-only (RUO) assays, while comparable GFAP findings have also been reproduced using fully automated analytical platforms (Palmqvist et al., 2019). Compared with Aβ42/40 and phosphorylated tau (p-tau) isoforms, for which a wide range of assays and analytical platforms are currently available, the greater methodological consistency of NfL and GFAP measurements facilitates the comparison and harmonization of findings across studies and may support their more rapid progression toward clinical implementation. Notably, a clinical NfL assay has already been developed as a Clinical Laboratory Improvement Amendments (CLIA)-approved laboratory-developed test (LDT) and has received Breakthrough Device designation from the U.S. Food and Drug Administration (FDA) for clinical management of multiple sclerosis, underscoring the increasing translational potential of blood-based NfL testing (Hampel et al., 2023).

5. Emerging Biomarker Strategies in Alzheimer's Disease

Several emerging strategies are currently being investigated to address the need for biomarkers that are both accurate and readily accessible for the detection and monitoring of Alzheimer's disease (AD). Beyond established central nervous system–derived biomarkers, growing attention has been directed toward less invasive and more readily obtainable sources of disease-related information. Among these approaches, retinal imaging, the analysis of peripheral biological fluids such as saliva, tears, and nasal secretions, and the profiling of small non-coding RNAs (sncRNAs) represent promising avenues for biomarker discovery and clinical translation (Mahaman et al., 2022).

5.1. Retinal imaging

Over the past decade, retinal imaging has emerged as an increasingly investigated avenue for the detection of AD-associated pathological signatures within the human retina. Techniques such as optical coherence tomography (OCT) offer several practical advantages over conventional advanced neuroimaging modalities, owing to their non-invasive nature, relatively broad accessibility, and lower cost. These characteristics have positioned retinal imaging as a particularly attractive strategy for the development of scalable biomarkers, with considerable potential for facilitating accessible and clinically relevant approaches to AD detection and monitoring.

Evidence from both experimental and human studies indicates that Alzheimer's disease (AD) is accompanied by alterations in retinal architecture, microvasculature, and neuropathological features, with such changes reported in transgenic mouse models as well as in older individuals with amnestic mild cognitive impairment (MCI) or AD (Alber et al., 2020; Chidlow et al., 2017; Georgakas et al., 2023; Mirzaei et al., 2020). Among the pathological features investigated in the retina, amyloid-β (Aβ) deposition has been consistently demonstrated in transgenic models of AD; however, evidence for comparable retinal Aβ pathology in humans remains relatively scarce and has yielded inconsistent findings across studies (Jiang et al., 2016). Histological examination of whole-retina preparations provided the initial evidence of substantial intracellular and extracellular Aβ accumulation in retinal tissue obtained from patients with AD (Koronyo et al., 2017). Subsequent efforts extended these observations to living patients through the development of an in vivo approach for detecting retinal Aβ; nevertheless, the reproducibility of this finding has

remained uncertain, as several independent studies have subsequently failed to confirm the original observations (Georgakas et al., 2023).

Evidence concerning the presence and distribution of tau pathology within the retina remains relatively limited compared with the more extensively investigated retinal Aβ abnormalities. Early histopathological investigations did not detect phosphorylated tau in retinal specimens obtained from individuals with AD, indicating that retinal tau alterations may be difficult to characterize consistently (Ho et al., 2014). Subsequent evidence has provided support for retinal P-tau pathology, with den Haan et al. reporting enhanced P-tau immunoreactivity in both the inner and outer plexiform retinal layers in post-mortem specimens from six individuals with AD compared with control tissues (den Haan et al., 2018)

Among the structural alterations of the retina, reduction in retinal nerve fiber layer (RNFL) thickness has emerged as a potential imaging-based biomarker of Alzheimer's disease (AD). Quantification by optical coherence tomography has consistently demonstrated thinner RNFL measurements in older individuals with mild cognitive impairment (MCI) or AD compared with cognitively healthy controls (Alber et al., 2020; Chan et al., 2019). Nevertheless, the longitudinal trajectory of RNFL degeneration across the AD continuum remains insufficiently characterized, particularly with respect to the preclinical and prodromal phases, underscoring the need for prospective longitudinal investigations. Of particular interest, RNFL thickness has been linked to cerebrospinal fluid (CSF) concentrations of amyloid-β (Aβ) and tau in older adults with biomarker-defined preclinical AD (Asanad et al., 2020). Associations have also been reported between RNFL thickness

and hippocampal volume among cognitively unimpaired older adults (Shi et al., 2014, 2020). Collectively, these observations raise the possibility that retinal neuroaxonal alterations may emerge early during AD pathogenesis and support further evaluation of RNFL thickness as a non-invasive marker for the early identification and screening of AD.

Beyond structural and neuropathological alterations, retinal microvascular features have also attracted attention as potential indicators of neurodegeneration and AD pathology (Czakó et al., 2020; Georgakas et al., 2023). Early evidence supporting this association was provided by Frost et al., who observed narrower retinal vessels and reduced vascular branching in asymptomatic older individuals exhibiting increased cerebral amyloid-β (Aβ) burden compared with healthy controls (Frost et al., 2013). More recent advances in optical coherence tomography angiography (OCTA) have enabled quantitative characterization of retinal microvascular alterations. In cognitively unimpaired older adults, a larger foveal avascular zone (FAZ) was associated with higher cerebral Aβ levels, further supporting a potential relationship between retinal vascular alterations and preclinical amyloid pathology (O'Bryhim et al., 2018). Importantly, receiver operating characteristic (ROC) analysis demonstrated that FAZ area differentiated Aβ-positive from Aβ-negative individuals with an area under the curve (AUC) of 0.80, indicating its potential utility as a vascular imaging measure for the identification of preclinical AD. Overall, retinal imaging has generated a diverse range of candidate biomarkers encompassing structural, vascular, and neuropathological features, although this field remains at a relatively early stage of development. Before these measures can be incorporated into routine clinical practice,

several methodological and translational challenges must be resolved. In particular, differences among OCT platforms and image-analysis software developed by different manufacturers may introduce substantial variability in biomarker measurements and limit comparability across studies. Furthermore, longitudinal studies involving repeated assessments within the same individuals are needed to clarify the temporal evolution of retinal biomarkers and to determine how their trajectories relate to established AD biomarkers and cognitive changes throughout disease progression (Alber et al., 2020).

5.2. sncRNAs

Small non-coding RNAs (sncRNAs), particularly microRNAs (miRNAs), have emerged as important post-transcriptional regulators of gene expression and cellular programming. Increasing evidence suggests that alterations in miRNA-mediated regulatory pathways may contribute to the molecular mechanisms underlying Alzheimer's disease (AD) as well as other neurodegenerative disorders (Mahaman et al., 2022; Zendjabil, 2018; Zhang et al., 2020) The potential of miRNAs as circulating biomarkers has been supported by studies demonstrating abnormal miRNA profiles in extracellular vesicles of brain origin that are detectable in both blood and CSF from individuals with AD compared with cognitively healthy controls (Cha et al., 2019; Müller et al., 2016). Further evidence has emerged from studies of amnestic mild cognitive impairment (MCI), a clinical stage associated with increased risk of AD progression. De Felice et al. reported significantly elevated serum levels of hsa-miR-567 in individuals with amnestic MCI compared with controls. This differential

expression pattern suggests that hsa-miR-567 may warrant further investigation as a minimally invasive biomarker for the early detection and monitoring of disease progression (De Felice et al., 2020).

5.3. Biomarkers in peripheral bodily fluids

Readily accessible peripheral biological fluids, including nasal secretions, saliva, and tears, are increasingly being considered as potential sources of non-invasive biomarkers for Alzheimer's disease (AD). In particular, nasal sampling using swab-based approaches is currently being investigated as a practical strategy for the detection and characterization of AD-associated molecular alterations. Protein-based analyses of samples collected from distinct anatomical regions of the nasal cavity and passages have revealed region-specific differences in phosphorylated tau (P-tau) relative to total tau (t-tau), with significantly higher P-tau/t-tau ratios detected in selected nasal regions of individuals with AD compared with healthy controls. These findings support the possibility that nasal secretions may provide a minimally invasive source of biomarkers reflecting AD-related pathology.

Salivary biomarkers have likewise attracted considerable interest, particularly through metabolomic approaches aimed at identifying disease-associated metabolic signatures. Several studies have reported metabolite panels comprising two or three metabolites that were capable of discriminating individuals with AD from those with MCI and cognitively healthy controls, with their performance demonstrated during both discovery and independent validation phases. Such findings highlight the potential of salivary metabolomics as a relatively accessible approach for the development of peripheral biomarker panels.

Tear fluid represents another potentially informative biological matrix, given its accessibility and previous utility in biomarker research across other disease conditions. Preliminary evidence indicates that ocular tear samples from individuals with AD may exhibit alterations in tau and Aβ profiles. In one small-scale study, the concentrations of total tau and Aβ42 in tear samples were reported to differ in association with AD status compared with control participants, suggesting a possible relationship between tear-based molecular measures and AD pathology. Nevertheless, these findings require confirmation in larger and independently characterized cohorts before their diagnostic relevance can be established. Beyond nasal secretions, saliva, and tears, other peripheral biofluids, including urine, represent additional sources that warrant systematic investigation for the identification of accessible and minimally invasive AD biomarkers. (Georgakas et al., 2023).

References

Alber, J., Goldfarb, D., Thompson, L. I., Arthur, E., Hernandez, K., Cheng, D., DeBuc, D. C., Cordeiro, F., Provetti-Cunha, L., den Haan, J., Van Stavern, G. P., Salloway, S. P., Sinoff, S., & Snyder, P. J. (2020). Developing retinal biomarkers for the earliest stages of Alzheimer's disease: What we know, what we don't, and how to move forward. *Alzheimer's & Dementia, 16*(1), 229–243. https://doi.org/10.1002/alz.12006

Asanad, S., Fantini, M., Sultan, W., Nassisi, M., Felix, C. M., Wu, J., Karanjia, R., Ross-Cisneros, F. N., Sagare, A. P., Zlokovic, B. V., Chui, H. C., Pogoda, J. M., Arakaki, X., Fonteh, A. N., Sadun, A. A., & Harrington, M. G. (2020). Retinal nerve fiber layer thickness predicts CSF amyloid/tau before cognitive decline. *PLOS ONE, 15*(5), e0232785. https://doi.org/10.1371/journal.pone.0232785

Ashton, N. J., Pascoal, T. A., Karikari, T. K., Benedet, A. L., Lantero-Rodriguez, J., Brinkmalm, G., Snellman, A., Schöll, M., Troakes, C., Hye, A., Gauthier, S., Vanmechelen, E., Zetterberg, H., Rosa-Neto, P., & Blennow, K. (2021). Plasma p-tau231: A new biomarker for incipient Alzheimer's disease pathology. *Acta Neuropathologica, 141*(5), 709–724. https://doi.org/10.1007/s00401-021-02275-6

Barthélemy, N. R., Bateman, R. J., Hirtz, C., Marin, P., Becher, F., Sato, C., Gabelle, A., & Lehmann, S. (2020). Cerebrospinal fluid phospho-tau T217 outperforms T181 as a biomarker for the differential diagnosis of Alzheimer's disease and PET amyloid-positive patient identification. *Alzheimer's Research & Therapy, 12*(1), 26. https://doi.org/10.1186/s13195-020-00596-4

Barthélemy, N. R., Horie, K., Sato, C., & Bateman, R. J. (2020). Blood plasma phosphorylated-tau isoforms track CNS change in Alzheimer's disease. *Journal of Experimental Medicine, 217*(11), e20200861. https://doi.org/10.1084/jem.20200861

Benedet, A. L., Milà-Alomà, M., Vrillon, A., Ashton, N. J., Pascoal, T. A., Lussier, F., Karikari, T. K., Hourregue, C., Cognat, E., Dumurgier, J., Stevenson, J., Rahmouni, N., Pallen, V., Poltronetti, N. M., Salvadó, G., Shekari, M., Operto, G., Gispert, J. D., Minguillon, C., . . . Suárez-Calvet, M. (2021). Differences between plasma and cerebrospinal fluid glial fibrillary acidic protein levels across the Alzheimer disease continuum. *JAMA Neurology, 78*(12), 1471–1483. https://doi.org/10.1001/jamaneurol.2021.3671

Biel, D., Brendel, M., Rubinski, A., Buerger, K., Janowitz, D., Dichgans, M., & Franzmeier, N. (2021). Tau-PET and in vivo Braak-staging as prognostic markers of future cognitive decline in cognitively normal to demented individuals. *Alzheimer's Research & Therapy, 13*(1), 137. https://doi.org/10.1186/s13195-021-00880-x

Blennow, K., Hampel, H., Weiner, M., & Zetterberg, H. (2010). Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. *Nature Reviews Neurology, 6*(3), 131–144. https://doi.org/10.1038/nrneurol.2010.4

Braak, H., & Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathologica, 82*(4), 239–259. https://doi.org/10.1007/BF00308809

Brickman, A. M., Manly, J. J., Honig, L. S., Sanchez, D., Reyes-Dumeyer, D., Lantigua, R. A., Lao, P. J., Stern, Y.,

Vonsattel, J. P., Teich, A. F., Airey, D. C., Proctor, N. K., Dage, J. L., & Mayeux, R. (2021). Plasma p-tau181, p-tau217, and other blood-based Alzheimer's disease biomarkers in a multi-ethnic, community study. *Alzheimer's & Dementia, 17*(8), 1353–1364. https://doi.org/10.1002/alz.12301

Cha, D. J., Mengel, D., Mustapic, M., Liu, W., Selkoe, D. J., Kapogiannis, D., Galasko, D., Rissman, R. A., Bennett, D. A., & Walsh, D. M. (2019). miR-212 and miR-132 are downregulated in neurally derived plasma exosomes of Alzheimer's patients. *Frontiers in Neuroscience, 13*, 1208. https://doi.org/10.3389/fnins.2019.01208

Chan, V. T. T., Sun, Z., Tang, S., Chen, L. J., Wong, A., Tham, C. C., Wong, T. Y., Chen, C., Ikram, M. K., Whitson, H. E., Lad, E. M., Mok, V. C. T., & Cheung, C. Y. (2019). Spectral-domain OCT measurements in Alzheimer's disease. *Ophthalmology, 126*(4), 497–510. https://doi.org/10.1016/j.ophtha.2018.08.009

Chatterjee, P., Pedrini, S., Stoops, E., Goozee, K., Villemagne, V. L., Asih, P. R., Verberk, I. M. W., Dave, P., Taddei, K., Sohrabi, H. R., Zetterberg, H., Blennow, K., Teunissen, C. E., Vanderstichele, H. M., & Martins, R. N. (2021). Plasma glial fibrillary acidic protein is elevated in cognitively normal older adults at risk of Alzheimer's disease. *Translational Psychiatry, 11*(1), 27. https://doi.org/10.1038/s41398-020-01137-1

Chen, G., Xu, T., Yan, Y., Zhou, Y., Jiang, Y., Melcher, K., & Xu, H. E. (2017). Amyloid beta: Structure, biology and structure-based therapeutic development. *Acta Pharmacologica Sinica, 38*(9), 1205–1235. https://doi.org/10.1038/aps.2017.28

Chidlow, G., Wood, J. P. M., Manavis, J., Finnie, J., & Casson, R. J. (2017). Investigations into retinal pathology in the early stages of a mouse model of Alzheimer's disease. *Journal of Alzheimer's Disease, 56*(2), 655–675. https://doi.org/10.3233/JAD-160823

Czakó, C., Kovács, T., Ungvari, Z., Csiszar, A., Yabluchanskiy, A., Conley, S., Csipo, T., Lipecz, A., Horváth, H., Sándor, G. L., István, L., Logan, T., Nagy, Z. Z., & Kovács, I. (2020). Retinal biomarkers for Alzheimer's disease and vascular cognitive impairment and dementia (VCID): Implication for early diagnosis and prognosis. *GeroScience, 42*(6), 1499–1525. https://doi.org/10.1007/s11357-020-00252-7

De Felice, B., Montanino, C., Oliva, M., Bonavita, S., Di Onofrio, V., & Coppola, C. (2020). MicroRNA expression signature in mild cognitive impairment due to Alzheimer's disease. *Molecular Neurobiology, 57*(11), 4408–4416. https://doi.org/10.1007/s12035-020-02029-7

De Meyer, S., Schaeverbeke, J. M., Verberk, I. M. W., Gille, B., De Schaepdryver, M., Luckett, E. S., Gabel, S., Bruffaerts, R., Mauroo, K., Thijssen, E. H., Stoops, E., Vanderstichele, H. M., Teunissen, C. E., Vandenberghe, R., & Poesen, K. (2020). Comparison of ELISA- and SIMOA-based quantification of plasma Aβ ratios for early detection of cerebral amyloidosis. *Alzheimer's Research & Therapy, 12*(1), 162. https://doi.org/10.1186/s13195-020-00728-w

de Rojas, I., Romero, J., Rodríguez-Gomez, O., Pesini, P., Sanabria, A., Pérez-Cordon, A., Abdelnour, C., Hernández, I., Rosende-Roca, M., Mauleón, A., Vargas, L., Alegret, M., Espinosa, A., Ortega, G., Gil, S., Guitart, M., Gailhajanet, A., Santos-Santos, M. A., Moreno-Grau, S., . . . Boada, M. (2018).

Correlations between plasma and PET beta-amyloid levels in individuals with subjective cognitive decline: The Fundació ACE Healthy Brain Initiative (FACEHBI). *Alzheimer's Research & Therapy, 10*(1), 119. https://doi.org/10.1186/s13195-018-0444-1

den Haan, J., Morrema, T. H. J., Verbraak, F. D., de Boer, J. F., Scheltens, P., Rozemuller, A. J., Bergen, A. A. B., Bouwman, F. H., & Hoozemans, J. J. (2018). Amyloid-beta and phosphorylated tau in post-mortem Alzheimer's disease retinas. *Acta Neuropathologica Communications, 6*(1), 147. https://doi.org/10.1186/s40478-018-0650-x

Ferrando, R., & Damian, A. (2021). Brain SPECT as a biomarker of neurodegeneration in dementia in the era of molecular imaging: Still a valid option? *Frontiers in Neurology, 12*, 629442. https://doi.org/10.3389/fneur.2021.629442

Frost, S., Kanagasingam, Y., Sohrabi, H., Vignarajan, J., Bourgeat, P., Salvado, O., Villemagne, V., Rowe, C. C., Macaulay, S. L., Szoeke, C., Ellis, K. A., Ames, D., Masters, C. L., Rainey-Smith, S., & Martins, R. N. (2013). Retinal vascular biomarkers for early detection and monitoring of Alzheimer's disease. *Translational Psychiatry, 3*(2), e233. https://doi.org/10.1038/tp.2012.150

Georgakas, J. E., Howe, M. D., Thompson, L. I., Riera, N. M., & Riddle, M. C. (2023). Biomarkers of Alzheimer's disease: Past, present and future clinical use. *Biomarkers in Neuropsychiatry, 8*, 100063. https://doi.org/10.1016/j.bionps.2023.100063

Gunes, S., Aizawa, Y., Sugashi, T., Sugimoto, M., & Rodrigues, P. P. (2022). Biomarkers for Alzheimer's disease in

the current state: A narrative review. *International Journal of Molecular Sciences, 23*(9), 4962. https://doi.org/10.3390/ijms23094962

Hampel, H., Hu, Y., Cummings, J., Mattke, S., Iwatsubo, T., Nakamura, A., Vellas, B., O'Bryant, S., Shaw, L. M., Cho, M., Batrla, R., Vergallo, A., Blennow, K., Dage, J., & Schindler, S. E. (2023). Blood-based biomarkers for Alzheimer's disease: Current state and future use in a transformed global healthcare landscape. *Neuron, 111*(18), 2781–2799. https://doi.org/10.1016/j.neuron.2023.05.017

Ho, C.-Y., Troncoso, J. C., Knox, D., Stark, W., & Eberhart, C. G. (2014). Beta-amyloid, phospho-tau and alpha-synuclein deposits similar to those in the brain are not identified in the eyes of Alzheimer's and Parkinson's disease patients. *Brain Pathology, 24*(1), 25–32. https://doi.org/10.1111/bpa.12070

Jack, C. R., Albert, M. S., Knopman, D. S., McKhann, G. M., Sperling, R. A., Carrillo, M. C., Thies, B., & Phelps, C. H. (2011). Introduction to the recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & Dementia, 7*(3), 257–262. https://doi.org/10.1016/j.jalz.2011.03.004

Jack, C. R., Bennett, D. A., Blennow, K., Carrillo, M. C., Feldman, H. H., Frisoni, G. B., Hampel, H., Jagust, W. J., Johnson, K. A., Knopman, D. S., Petersen, R. C., Scheltens, P., Sperling, R. A., & Dubois, B. (2016). A/T/N: An unbiased descriptive classification scheme for Alzheimer disease biomarkers. *Neurology, 87*(5), 539–547. https://doi.org/10.1212/WNL.0000000000002923

Jack, C. R., Bennett, D. A., Blennow, K., Carrillo, M. C., Dunn, B., Haeberlein, S. B., Holtzman, D. M., Jagust, W., Jessen, F., Karlawish, J., Liu, E., Molinuevo, J. L., Montine, T., Phelps, C., Rankin, K. P., Rowe, C. C., Scheltens, P., Siemers, E., Snyder, H. M., . . . Silverberg, N. (2018). NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimer's & Dementia, 14*(4), 535–562. https://doi.org/10.1016/j.jalz.2018.02.018

Janelidze, S., Berron, D., Smith, R., Strandberg, O., Proctor, N. K., Dage, J. L., Stomrud, E., Palmqvist, S., Mattsson-Carlgren, N., & Hansson, O. (2021). Associations of plasma phospho-tau217 levels with tau positron emission tomography in early Alzheimer disease. *JAMA Neurology, 78*(2), 149–156. https://doi.org/10.1001/jamaneurol.2020.4201

Janelidze, S., Mattsson, N., Palmqvist, S., Smith, R., Beach, T. G., Serrano, G. E., Chai, X., Proctor, N. K., Eichenlaub, U., Zetterberg, H., Blennow, K., Reiman, E. M., Stomrud, E., Dage, J. L., & Hansson, O. (2020). Plasma P-tau181 in Alzheimer's disease: Relationship to other biomarkers, differential diagnosis, neuropathology and longitudinal progression to Alzheimer's dementia. *Nature Medicine, 26*(3), 379–386. https://doi.org/10.1038/s41591-020-0755-1

Janelidze, S., Stomrud, E., Palmqvist, S., Zetterberg, H., van Westen, D., Jeromin, A., Song, L., Hanlon, D., Tan Hehir, C. A., Baker, D., Blennow, K., & Hansson, O. (2016). Plasma β-amyloid in Alzheimer's disease and vascular disease. *Scientific Reports, 6*(1), 26801. https://doi.org/10.1038/srep26801

Jiang, J., Wang, H., Li, W., Cao, X., & Li, C. (2016). Amyloid plaques in retina for diagnosis in Alzheimer's patients:

A meta-analysis. *Frontiers in Aging Neuroscience, 8*, 267. https://doi.org/10.3389/fnagi.2016.00267

Karikari, T. K., Benedet, A. L., Ashton, N. J., Lantero-Rodriguez, J., Snellman, A., Suárez-Calvet, M., Saha-Chaudhuri, P., Lussier, F., Kvartsberg, H., Rial, A. M., Pascoal, T. A., Andreasson, U., Schöll, M., Weiner, M. W., Rosa-Neto, P., Trojanowski, J. Q., Shaw, L. M., Blennow, K., & Zetterberg, H. (2021). Diagnostic performance and prediction of clinical progression of plasma phospho-tau181 in the Alzheimer's Disease Neuroimaging Initiative. *Molecular Psychiatry, 26*(2), 429–442. https://doi.org/10.1038/s41380-020-00923-z

Keshavan, A., Pannee, J., Karikari, T. K., Lantero Rodriguez, J., Ashton, N. J., Nicholas, J. M., Cash, D. M., Coath, W., Lane, C. A., Parker, T. D., Lu, K., Buchanan, S. M., Keuss, S. E., James, S.-N., Murray-Smith, H., Wong, A., Barnes, A., Dickson, J. C., Heslegrave, A., … Schott, J. M. (2021). Population-based blood screening for preclinical Alzheimer's disease in a British birth cohort at age 70. *Brain, 144*(2), 434–449. https://doi.org/10.1093/brain/awaa403

Koronyo, Y., Biggs, D., Barron, E., Boyer, D. S., Pearlman, J. A., Au, W. J., Kile, S. J., Blanco, A., Fuchs, D.-T., Ashfaq, A., Frautschy, S., Cole, G. M., Miller, C. A., Hinton, D. R., Verdooner, S. R., Black, K. L., & Koronyo-Hamaoui, M. (2017). Retinal amyloid pathology and proof-of-concept imaging trial in Alzheimer's disease. *JCI Insight, 2*(16), e93621. https://doi.org/10.1172/jci.insight.93621

Kuo, Y.-M., Emmerling, M. R., Lampert, H. C., Hempelman, S. R., Kokjohn, T. A., Woods, A. S., Cotter, R. J., & Roher, A. E. (1999). High levels of circulating Aβ42 are sequestered by plasma proteins in Alzheimer's disease.

Biochemical and Biophysical Research Communications, 257(3), 787–791. https://doi.org/10.1006/bbrc.1999.0552

Li, W.-W., Shen, Y.-Y., Tian, D.-Y., Bu, X.-L., Zeng, F., Liu, Y.-H., Chen, Y., Yao, X.-Q., Li, H.-Y., Chen, D.-W., Zhou, F.-Y., Yang, H., Li, Q.-M., Bao, W.-Q., Guan, Y.-H., Zhou, H.-D., Jin, R.-B., & Wang, Y.-J. (2019). Brain amyloid-β deposition and blood biomarkers in patients with clinically diagnosed Alzheimer's disease. *Journal of Alzheimer's Disease, 69*(1), 169–178. https://doi.org/10.3233/JAD-190056

Li, Y., Schindler, S. E., Bollinger, J. G., Ovod, V., Mawuenyega, K. G., Weiner, M. W., Shaw, L. M., Masters, C. L., Fowler, C. J., Trojanowski, J. Q., Korecka, M., Martins, R. N., Janelidze, S., Hansson, O., & Bateman, R. J. (2022). Validation of plasma amyloid-β 42/40 for detecting Alzheimer disease amyloid plaques. *Neurology, 98*(7), e688–e699. https://doi.org/10.1212/WNL.0000000000013211

Mahaman, Y. A. R., Embaye, K. S., Huang, F., Li, L., Zhu, F., Wang, J.-Z., Liu, R., Feng, J., & Wang, X. (2022). Biomarkers used in Alzheimer's disease diagnosis, treatment, and prevention. *Ageing Research Reviews, 74*, 101544. https://doi.org/10.1016/j.arr.2021.101544

Mathis, C. A., Kuller, L. H., Klunk, W. E., Snitz, B. E., Price, J. C., Weissfeld, L. A., Rosario, B. L., Lopresti, B. J., Saxton, J. A., Aizenstein, H. J., McDade, E. M., Kamboh, M. I., DeKosky, S. T., & Lopez, O. L. (2013). In vivo assessment of amyloid-β deposition in nondemented very elderly subjects. *Annals of Neurology, 73*(6), 751–761. https://doi.org/10.1002/ana.23797

Mattsson-Carlgren, N., Janelidze, S., Palmqvist, S., Cullen, N., Svenningsson, A. L., Strandberg, O., Mengel, D., Walsh, D. M., Stomrud, E., Dage, J. L., & Hansson, O. (2020). Longitudinal plasma p-tau217 is increased in early stages of Alzheimer's disease. *Brain, 143*(11), 3234–3241. https://doi.org/10.1093/brain/awaa286

Mattsson, N., Andreasson, U., Zetterberg, H., & Blennow, K. (2017). Association of plasma neurofilament light with neurodegeneration in patients with Alzheimer disease. *JAMA Neurology, 74*(5), 557–566. https://doi.org/10.1001/jamaneurol.2016.6117

Mayeux, R., & Schupf, N. (2011). Blood-based biomarkers for Alzheimer's disease: Plasma Aβ40 and Aβ42, and genetic variants. *Neurobiology of Aging, 32*, S10–S19. https://doi.org/10.1016/j.neurobiolaging.2011.09.004

Mielke, M. M., Frank, R. D., Dage, J. L., Jeromin, A., Ashton, N. J., Blennow, K., Karikari, T. K., Vanmechelen, E., Zetterberg, H., Algeciras-Schimnich, A., Knopman, D. S., Lowe, V., Bu, G., Vemuri, P., Graff-Radford, J., Jack, C. R., & Petersen, R. C. (2021). Comparison of plasma phosphorylated tau species with amyloid and tau positron emission tomography, neurodegeneration, vascular pathology, and cognitive outcomes. *JAMA Neurology, 78*(9), 1108–1117. https://doi.org/10.1001/jamaneurol.2021.2293

Mielke, M. M., Hagen, C. E., Xu, J., Chai, X., Vemuri, P., Lowe, V. J., Airey, D. C., Knopman, D. S., Roberts, R. O., Machulda, M. M., Jack, C. R., Petersen, R. C., & Dage, J. L. (2018). Plasma phospho-tau181 increases with Alzheimer's disease clinical severity and is associated with tau- and amyloid-

positron emission tomography. *Alzheimer's & Dementia, 14*(8), 989–997. https://doi.org/10.1016/j.jalz.2018.02.013

Mirzaei, N., Shi, H., Oviatt, M., Doustar, J., Rentsendorj, A., Fuchs, D.-T., Sheyn, J., Black, K. L., Koronyo, Y., & Koronyo-Hamaoui, M. (2020). Alzheimer's retinopathy: Seeing disease in the eyes. *Frontiers in Neuroscience, 14*, 921. https://doi.org/10.3389/fnins.2020.00921

Morinaga, A., Ono, K., Ikeda, T., Ikeda, Y., Shima, K., Noguchi-Shinohara, M., Samuraki, M., Yanase, D., Yoshita, M., Iwasa, K., Mastunari, I., & Yamada, M. (2010). A comparison of the diagnostic sensitivity of MRI, CBF-SPECT, FDG-PET and cerebrospinal fluid biomarkers for detecting Alzheimer's disease in a memory clinic. *Dementia and Geriatric Cognitive Disorders, 30*(4), 285–292. https://doi.org/10.1159/000320265

Morris, J. C., & Selkoe, D. J. (2011). Recommendations for the incorporation of biomarkers into Alzheimer clinical trials: An overview. *Neurobiology of Aging, 32*, S1–S3. https://doi.org/10.1016/j.neurobiolaging.2011.09.005

Müller, M., Jäkel, L., Bruinsma, I. B., Claassen, J. A., Kuiperij, H. B., & Verbeek, M. M. (2016). MicroRNA-29a is a candidate biomarker for Alzheimer's disease in cell-free cerebrospinal fluid. *Molecular Neurobiology, 53*(5), 2894–2899. https://doi.org/10.1007/s12035-015-9156-8

Nakamura, A., Kaneko, N., Villemagne, V. L., Kato, T., Doecke, J., Doré, V., Fowler, C., Li, Q.-X., Martins, R., Rowe, C., Tomita, T., Matsuzaki, K., Ishii, K., Ishii, K., Arahata, Y., Iwamoto, S., Ito, K., Tanaka, K., Masters, C. L., & Yanagisawa, K. (2018). High performance plasma amyloid-β biomarkers for

Alzheimer's disease. *Nature, 554*(7691), 249–254. https://doi.org/10.1038/nature25456

O'Bryhim, B. E., Apte, R. S., Kung, N., Coble, D., & Van Stavern, G. P. (2018). Association of preclinical Alzheimer disease with optical coherence tomographic angiography findings. *JAMA Ophthalmology, 136*(11), 1242–1248. https://doi.org/10.1001/jamaophthalmol.2018.3556

Olsson, B., Lautner, R., Andreasson, U., Öhrfelt, A., Portelius, E., Bjerke, M., Hölttä, M., Rosén, C., Olsson, C., Strobel, G., Wu, E., Dakin, K., Petzold, M., Blennow, K., & Zetterberg, H. (2016). CSF and blood biomarkers for the diagnosis of Alzheimer's disease: A systematic review and meta-analysis. *The Lancet Neurology, 15*(7), 673–684. https://doi.org/10.1016/S1474-4422(16)00070-3

Ovod, V., Ramsey, K. N., Mawuenyega, K. G., Bollinger, J. G., Hicks, T., Schneider, T., Sullivan, M., Paumier, K., Holtzman, D. M., Morris, J. C., Benzinger, T., Fagan, A. M., Patterson, B. W., & Bateman, R. J. (2017). Amyloid β concentrations and stable isotope labeling kinetics of human plasma specific to central nervous system amyloidosis. *Alzheimer's & Dementia, 13*(8), 841–849. https://doi.org/10.1016/j.jalz.2017.06.2266

Palmqvist, S., Janelidze, S., Stomrud, E., Zetterberg, H., Karl, J., Zink, K., Bittner, T., Mattsson, N., Eichenlaub, U., Blennow, K., & Hansson, O. (2019). Performance of fully automated plasma assays as screening tests for Alzheimer disease-related β-amyloid status. *JAMA Neurology, 76*(9), 1060–1069. https://doi.org/10.1001/jamaneurol.2019.1632

Palmqvist, S., Tideman, P., Cullen, N., Zetterberg, H., Blennow, K., Dage, J. L., Stomrud, E., Janelidze, S., Mattsson-Carlgren, N., & Hansson, O. (2021). Prediction of future Alzheimer's disease dementia using plasma phospho-tau combined with other accessible measures. *Nature Medicine, 27*(6), 1034–1042. https://doi.org/10.1038/s41591-021-01348-z

Risacher, S. L., Fandos, N., Romero, J., Sherriff, I., Pesini, P., Saykin, A. J., & Apostolova, L. G. (2019). Plasma amyloid beta levels are associated with cerebral amyloid and tau deposition. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring, 11*(1), 510–519. https://doi.org/10.1016/j.dadm.2019.05.007

Rosengren, L. E., Karlsson, J.-E., Karlsson, J.-O., Persson, L. I., & Wikkelsø, C. (1996). Patients with amyotrophic lateral sclerosis and other neurodegenerative diseases have increased levels of neurofilament protein in CSF. *Journal of Neurochemistry, 67*(5), 2013–2018. https://doi.org/10.1046/j.1471-4159.1996.67052013.x

Salloway, S., Sperling, R., Fox, N. C., Blennow, K., Klunk, W., Raskind, M., Sabbagh, M., Honig, L. S., Porsteinsson, A. P., Ferris, S., Reichert, M., Ketter, N., Nejadnik, B., Guenzler, V., Miloslavsky, M., Wang, D., Lu, Y., Lull, J., Tudor, I. C., . . . Brashear, H. R. (2014). Two phase 3 trials of bapineuzumab in mild-to-moderate Alzheimer's disease. *The New England Journal of Medicine, 370*(4), 322–333. https://doi.org/10.1056/NEJMoa1304839

Scheltens, P., Blennow, K., Breteler, M. M. B., De Strooper, B., Frisoni, G. B., Salloway, S., & van der Flier, W. M. (2016). Alzheimer's disease. *The Lancet, 388*(10043), 505–517. https://doi.org/10.1016/S0140-6736(15)01124-1

Schindler, S. E., Bollinger, J. G., Ovod, V., Mawuenyega, K. G., Li, Y., Gordon, B. A., Holtzman, D. M., Morris, J. C., Benzinger, T. L. S., Xiong, C., Fagan, A. M., & Bateman, R. J. (2019). High-precision plasma β-amyloid 42/40 predicts current and future brain amyloidosis. *Neurology, 93*(17), e1647–e1659. https://doi.org/10.1212/WNL.0000000000008081

Schlaepfer, W., & Lynch, R. (1977). Immunofluorescence studies of neurofilaments in the rat and human peripheral and central nervous system. *The Journal of Cell Biology, 74*(1), 241–250. https://doi.org/10.1083/jcb.74.1.241

Selkoe, D. J., & Hardy, J. (2016). The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Molecular Medicine, 8*(6), 595–608. https://doi.org/10.15252/emmm.201606210

Shi, Z., Cao, X., Hu, J., Jiang, L., Mei, X., Zheng, H., Chen, Y., Wang, M., Cao, J., Li, W., Li, T., Li, C., & Shen, Y. (2020). Retinal nerve fiber layer thickness is associated with hippocampus and lingual gyrus volumes in nondemented older adults. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 99*, 109824. https://doi.org/10.1016/j.pnpbp.2019.109824

Shi, Z., Wu, Y., Wang, M., Cao, J., Feng, W., Cheng, Y., Li, C., & Shen, Y. (2014). Greater attenuation of retinal nerve fiber layer thickness in Alzheimer's disease patients. *Journal of Alzheimer's Disease, 40*(2), 277–283. https://doi.org/10.3233/JAD-131898

Snyder, H. M., Carrillo, M. C., Grodstein, F., Henriksen, K., Jeromin, A., Lovestone, S., Mielke, M. M., O'Bryant, S.,

Sarasa, M., Sjögren, M., Soares, H., Teeling, J., Trushina, E., Ward, M., West, T., Bain, L. J., Shineman, D. W., Weiner, M., & Fillit, H. M. (2014). Developing novel blood-based biomarkers for Alzheimer's disease. *Alzheimer's & Dementia, 10*(1), 109–114. https://doi.org/10.1016/j.jalz.2013.10.007

Sperling, R. A., Jack, C. R., & Aisen, P. S. (2011). Testing the right target and right drug at the right stage. *Science Translational Medicine, 3*(111), 111cm33. https://doi.org/10.1126/scitranslmed.3002609

Teunissen, C. E., Verberk, I. M. W., Thijssen, E. H., Vermunt, L., Hansson, O., Zetterberg, H., van der Flier, W. M., Mielke, M. M., & del Campo, M. (2022). Blood-based biomarkers for Alzheimer's disease: Towards clinical implementation. *The Lancet Neurology, 21*(1), 66–77. https://doi.org/10.1016/S1474-4422(21)00361-6

Thijssen, E. H., La Joie, R., Wolf, A., Strom, A., Wang, P., Iaccarino, L., Bourakova, V., Cobigo, Y., Heuer, H., Spina, S., VandeVrede, L., Chai, X., Proctor, N. K., Airey, D. C., Shcherbinin, S., Duggan Evans, C., Sims, J. R., Zetterberg, H., Blennow, K., . . . Boxer, A. L. (2020). Diagnostic value of plasma phosphorylated tau181 in Alzheimer's disease and frontotemporal lobar degeneration. *Nature Medicine, 26*(3), 387–397. https://doi.org/10.1038/s41591-020-0762-2

Verberk, I. M. W., Slot, R. E., Verfaillie, S. C. J., Heijst, H., Prins, N. D., van Berckel, B. N. M., Scheltens, P., Teunissen, C. E., & van der Flier, W. M. (2018). Plasma amyloid as prescreener for the earliest Alzheimer pathological changes. *Annals of Neurology, 84*(5), 648–658. https://doi.org/10.1002/ana.25334

Verberk, I. M. W., Thijssen, E., Koelewijn, J., Mauroo, K., Vanbrabant, J., de Wilde, A., Zwan, M. D., Verfaillie, S. C. J., Ossenkoppele, R., Barkhof, F., van Berckel, B. N. M., Scheltens, P., van der Flier, W. M., Stoops, E., Vanderstichele, H. M., & Teunissen, C. E. (2020). Combination of plasma amyloid beta(1-42/1-40) and glial fibrillary acidic protein strongly associates with cerebral amyloid pathology. *Alzheimer's Research & Therapy, 12*(1), 118. https://doi.org/10.1186/s13195-020-00682-7

Vergallo, A., Mégret, L., Lista, S., Cavedo, E., Zetterberg, H., Blennow, K., Vanmechelen, E., De Vos, A., Habert, M., Potier, M., Dubois, B., Neri, C., & Hampel, H. (2019). Plasma amyloid β 40/42 ratio predicts cerebral amyloidosis in cognitively normal individuals at risk for Alzheimer's disease. *Alzheimer's & Dementia, 15*(6), 764–775. https://doi.org/10.1016/j.jalz.2019.03.009

Wesseling, H., Mair, W., Kumar, M., Schlaffner, C. N., Tang, S., Beerepoot, P., Fatou, B., Guise, A. J., Cheng, L., Takeda, S., Muntel, J., Rotunno, M. S., Dujardin, S., Davies, P., Kosik, K. S., Miller, B. L., Berretta, S., Hedreen, J. C., Grinberg, L. T., . . . Steen, J. A. (2020). Tau PTM profiles identify patient heterogeneity and stages of Alzheimer's disease. *Cell, 183*(6), 1699–1713.e13. https://doi.org/10.1016/j.cell.2020.10.029

West, T., Kirmess, K. M., Meyer, M. R., Holubasch, M. S., Knapik, S. S., Hu, Y., Contois, J. H., Jackson, E. N., Harpstrite, S. E., Bateman, R. J., Holtzman, D. M., Verghese, P. B., Fogelman, I., Braunstein, J. B., & Yarasheski, K. E. (2021). A blood-based diagnostic test incorporating plasma Aβ42/40 ratio, ApoE proteotype, and age accurately identifies brain amyloid status: Findings from a multi cohort validity analysis.

Molecular Neurodegeneration, 16(1), 30. https://doi.org/10.1186/s13024-021-00451-6

World Health Organization. (2021). *Global status report on the public health response to dementia*. World Health Organization.

Wu, Y.-T., Clare, L., Hindle, J. V., Nelis, S. M., Martyr, A., & Matthews, F. E. (2018). Dementia subtype and living well: Results from the Improving the experience of Dementia and Enhancing Active Life (IDEAL) study. *BMC Medicine, 16*(1), 140. https://doi.org/10.1186/s12916-018-1135-2

Yuan, Y., Gu, Z.-X., & Wei, W.-S. (2009). Fluorodeoxyglucose-positron-emission tomography, single-photon emission tomography, and structural MR imaging for prediction of rapid conversion to Alzheimer disease in patients with mild cognitive impairment: A meta-analysis. *American Journal of Neuroradiology, 30*(2), 404–410. https://doi.org/10.3174/ajnr.A1357

Zendjabil, M. (2018). Circulating microRNAs as novel biomarkers of Alzheimer's disease. *Clinica Chimica Acta, 484*, 99–104. https://doi.org/10.1016/j.cca.2018.05.039

Zetterberg, H., & Mattsson, N. (2014). Understanding the cause of sporadic Alzheimer's disease. *Expert Review of Neurotherapeutics, 14*(6), 621–630. https://doi.org/10.1586/14737175.2014.915740

Zetterberg, H., Skillbäck, T., Mattsson, N., Trojanowski, J. Q., Portelius, E., Shaw, L. M., Weiner, M. W., & Blennow, K. (2016). Association of cerebrospinal fluid neurofilament light concentration with Alzheimer disease progression. *JAMA*

Neurology, *73*(1), 60–67. https://doi.org/10.1001/jamaneurol.2015.3037

Zhang, X., Trebak, F., Souza, L. A. C., Shi, J., Zhou, T., Kehoe, P. G., Chen, Q., & Feng Earley, Y. (2020). Small RNA modifications in Alzheimer's disease. *Neurobiology of Disease, 145*, 105058. https://doi.org/10.1016/j.nbd.2020.105058

CHAPTER 7

ACETYLCHOLINESTERASE IN ALZHEIMER'S DISEASE: PATHOPHYSIOLOGICAL ROLE AND THERAPEUTIC SIGNIFICANCE

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1. Introduction

Alzheimer's Disease (AD) is a progressive and irreversible neurodegenerative disorder (Kumar et al., 2025; Safiri et al., 2024). The disease follows an insidious course; initially characterized by memory loss, it progressively leads to deterioration in a range of cognitive and behavioral functions over time, including language, learning, visuospatial abilities, judgment, and behavior (Safiri et al., 2024). Therefore, memory impairment stands out as the most common and defining clinical feature of the disease (Apostolova, 2016). As the most common

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form of dementia, AD accounts for at least two-thirds of dementia cases in individuals aged 65 and over, making it a significant global public health problem (Corey-Bloom, 2002; Safiri et al., 2024). This profound clinical and epidemiological impact has driven decades of research, and one of the most consistent findings in the disease process has been a central failure of cholinergic neurotransmission.

Cognitive functions primarily impaired in AD—such as memory and learning—are supported by the cholinergic system, a widespread and vitally important network in the central nervous system (Hampel et al., 2018). Therefore, it is not surprising that disruption in this system constitutes a fundamental hypothesis in AD pathology. The basis of the cholinergic hypothesis of AD is the progressive degeneration of cholinergic innervation to limbic and neocortical regions; this presynaptic deficit, which can begin in the earliest stages of the disease, stems from the loss of basal forebrain neurons (Hampel et al., 2018; Schwarthoff et al., 2021). This impairment in cholinergic signaling, along with other neurotransmitter deficiencies, is the direct underlying cause of the defining cognitive dysfunctions of the disease. Consequently, the primary pharmacological strategy employed to address this deficit has been the use of cholinesterase inhibitors, which aim to increase synaptic acetylcholine levels and remain a cornerstone in the treatment of AD (Hampel et al., 2018). At the molecular heart of this system lies the enzyme acetylcholinesterase (AChE), whose critical biochemical role is to rapidly hydrolyze acetylcholine into choline and acetate immediately following neurotransmission. This action is essential for terminating the synaptic signal, thereby ensuring precise neural communication (Hung et al., 2025). Indeed, far from being merely a metabolic

enzyme, AChE is a crucial regulator of synaptic timing, and its dysregulation is closely linked to synaptic failure. Beyond this classical catalytic function, evidence suggests that AChE and related enzymes may also undertake non-hydrolytic, morphogenetic roles such as cell adhesion (Silman, 2021). The physiological indispensability of the enzyme is underscored by the lethality of its complete inhibition and the severe developmental abnormalities and early mortality observed in genetic models lacking AChE (Xie et al., 2000).

Given the fundamental role of AChE in cholinergic signaling, synaptic deficits in AD are characterized by a progressive decline in both cholinergic and glutamatergic neurotransmission, which directly underlies core cognitive dysfunctions such as memory loss (Schwarthoff et al., 2021). Notably, a pathological association is observed in the AD brain where AChE is frequently colocalized with β-amyloid plaques and neurofibrillary tangles, suggesting a complex interaction beyond the enzyme's classical function (García-Ayllón et al., 2011). This abnormal colocalization suggests that AChE may play a more multifaceted role in AD pathogenesis. Cholinergic neuron loss is a major contributor to the extensive synaptic loss observed during disease progression and exacerbates deficits in memory and attention (Ferreira-Vieira et al., 2016). Targeting this cholinergic imbalance has been a rational therapeutic strategy for decades, primarily through the use of acetylcholinesterase inhibitors (AChEIs). Drugs such as donepezil, galantamine, and rivastigmine enhance cholinergic neurotransmission by delaying the degradation of synaptic acetylcholine (Birks, 2006; Young et al., 2021). They constitute the cornerstone of current pharmacological treatment for AD and are often used in combination with the NMDA receptor

antagonist memantine (Young et al., 2021). The clinical rationale for this approach is supported by evidence demonstrating that these AChEIs can stabilize or slow decline in cognition, function, and overall clinical state in patients with mild-to-moderate AD compared to placebo (Hansen et al., 2008). Although their efficacy in producing substantial cognitive improvement is considered modest, their role in stabilizing overall disease status and potentially influencing long-term outcomes remains an important aspect of AD care (Moreta et al., 2021; Rees & Brimijoin, 2003). Thus, the history of AChE inhibition in AD stands as a crucial, albeit imperfect, chapter in translational neuroscience, illustrating both the promise and the limitations of targeting a single neurotransmitter system.

2. Role and Structure of AChE Enzyme

AChE, the primary enzyme that terminates neural transmission by hydrolyzing acetylcholine, possesses a structure comprising two main sites (Hung et al., 2025):

Catalytic Active Site (CAS): Acetylcholine is hydrolyzed at this site (Hung et al., 2025).

Peripheral Anionic Site (PAS): Located at the entrance of the enzyme, this site not only recognizes the substrate but also undertakes non-cholinergic functions, such as modulating amyloid-beta aggregation (Dinamarca et al., 2010). Through its PAS, AChE promotes amyloid plaque formation, a process that directly accelerates brain damage in AD (Dinamarca et al., 2010).

2.1. Postmortem and *in vivo* imaging studies

Quantitative detection of changes in AChE activity is conducted through postmortem tissue studies. In Alzheimer's patients, AChE activity has been found to be decreased in brain regions rich in cholinergic neurons, such as the hippocampus and temporal cortex (Pozzi et al., 2022). A key limitation of postmortem studies is that they only reflect the end-stage of the disease. Advanced in vivo imaging techniques are important tools that enable the measurement of AChE activity while patients are alive, allowing for the monitoring of disease pathology (Valotassiou et al., 2018). The progression of AD is marked by a gradual decline in AChE activity. Notably, there is a high correlation between the loss of AChE activity and hypoperfusion and atrophy occurring in the temporoparietal regions of the brain (Richter et al., 2025).

2.2. AChE isoforms and regional dynamics of deficiency

AChE deficiency is not uniform across all brain regions. In AD, while the occipital cortex and sensorimotor areas are largely preserved, the hippocampus and temporal cortex exhibit the most pronounced loss of AChE (Lane et al., 2006). This regional distribution pattern overlaps with early-stage memory impairment. The three distinct AChE isoforms found in the human brain are synaptic (AChE-T), erythrocytic (AChE-E), and embryonic/stress-related (AChE-R) (Gok et al., 2022). In AD, it has been reported that the AChE-R isoform is preserved, whereas the AChE-T isoform is decreased (Lane et al., 2006). This shift in isoforms indicates that the enzyme deficiency in the disease is not only quantitative but also qualitative.

2.3. Butyrylcholinesterase (BChE): A compensatory mechanism

Despite the decrease in AChE activity in AD, BChE—another enzyme belonging to the cholinesterase family—has been suggested to increase in activity and may serve as an important compensatory mechanism to ameliorate acetylcholine deficiency (Lane et al., 2006). This increase in BChE activity correlates with the formation of neurofibrillary tangles and amyloid plaques (Gok et al., 2022). In a healthy brain, BChE levels are considerably lower compared to AChE. However, in AD:

- BChE activity is markedly increased around amyloid plaques and neurofibrillary tangles (Ferjančič Benetik et al., 2024).
- The capacity of BChE to hydrolyze acetylcholine is enhanced (Lane et al., 2006).
- The activity of BChE-containing neurons in the frontal cortex is increased (Ferjančič Benetik et al., 2024).

These findings suggest that BChE acts as a compensatory mechanism in the context of AChE deficiency. It has been reported that while BChE partially compensates for the lack of AChE, the deficiency in certain brain regions evolves into symptomatic manifestations (Lane et al., 2006). The increase in BChE in AD positions this enzyme as a therapeutic target. BChE inhibitors may therefore be beneficial as an adjunct to AChE inhibition (Ferjančič Benetik et al., 2024).

3. Molecular Mechanisms of AChE Deficiency

The AChE deficiency observed in AD arises from complex regulatory disturbances at the molecular level. In the brains of Alzheimer's patients, the levels of transcripts encoding certain AChE isoforms have been found to be reduced (Gok et al., 2022). Oxidative stress directly impacts the maintenance of AChE function (Evans et al., 2024). In Alzheimer's brains, the glycosylation pattern of the enzyme is altered, leading to increased interaction with amyloid plaques (García-Ayllón et al., 2011).

4. Non-Catalytic Functions and AChE Deficiency

In addition to its enzymatic role, acetylcholinesterase possesses non-catalytic functions involved in processes such as cell adhesion and neuronal development (Small et al., 1996). These functions arise from different isoforms and structural features of the enzyme. The loss of the non-catalytic function of acetylcholinesterase may help elucidate the background of the disease (García-Ayllón et al., 2011). One of the non-catalytic functions of AChE is its contribution to cell adhesion. The structural similarity between AChE and adhesion proteins suggests that the enzyme may also play a role in cell adhesion (Small et al., 1996). Another non-catalytic function of AChE is its interaction with amyloid-beta (Aβ). A pioneering study found that the enzyme accelerates the formation of fibrils from Aβ peptides (Inestrosa et al., 1996). AChE exerts this effect through its peripheral anionic site (PAS) (Inestrosa et al., 2008). The discovery of non-catalytic functions has also opened avenues for the development of new therapeutic strategies. Dual-site inhibitors target both the catalytic active site (CAS) and the PAS of the enzyme, thereby interfering with enzyme activity and

slowing fibril formation with Aβ peptides (Grabowska et al., 2025).

In conclusion, the impairment of AChE's non-catalytic functions should be considered an independent pathophysiological mechanism in AD. In particular, its interaction with Aβ, its role in cell adhesion, and alterations in molecular isoforms reveal the multifaceted contribution of the enzyme to disease pathogenesis.

5. Relationship Between AChE Deficiency and Alzheimer's Pathology

Several hypotheses have been proposed to explain the etiology of AD:

- Cholinergic Hypothesis: Decreased acetylcholine levels in the nervous system impair learning and cognitive function, contributing to AD pathogenesis (Hampel et al., 2018).
  • Amyloid Hypothesis: Accumulation of extracellular amyloid-beta (Aβ) plaques triggers a cascade of events leading to AD (Kumar et al., 2025).
- Tau Hypothesis: Hyperphosphorylation of tau protein, resulting in the formation of intracellular neurofibrillary tangles (NFTs), is a central mechanism in AD pathology (Cacabelos et al., 2024).
- Other Factors: Additional factors including genetic predisposition, environmental influences (smoking, sleep disorders, obesity), oxidative stress, and neuroinflammation also contribute significantly to disease development (Evans et al., 2024).

5.1. Interaction with amyloid beta and tau

The colocalization of AChE with neurofibrillary tangles and amyloid plaques in Alzheimer's brains has been well documented (García-Ayllón et al., 2011). This colocalization of AChE with both Aβ and phosphorylated tau may accelerate disease progression (García-Ayllón et al., 2011). The peripheral anionic site (PAS) of AChE accelerates amyloid beta aggregation (Inestrosa et al., 1996). The resulting Aβ-AChE complexes exhibit enhanced neurotoxicity, exacerbating neuronal damage (Inestrosa et al., 2005). A 2022 study in patients with early-stage dementia reported a significant correlation between AChE activity and total tau/phosphorylated tau levels, suggesting that AChE may be closely linked to tau-related neurodegeneration (Teitsdottir et al., 2022).

5.2. Neuroinflammation and oxidative stress

Oxidative stress represents another significant factor associated with AChE deficiency. Studies on AD have demonstrated that exposure to oxidative agents alters AChE activity (Evans et al., 2024). Numerous studies have reported similar findings (Badal et al., 2026; Kashif et al., 2024). Based on these findings, AChE deficiency should be conceptualized not merely as a quantitative reduction in enzyme activity, but rather as a disruption of the enzyme's functional balance within this multifactorial pathological environment.

5.3. Synaptic dysfunction and cognitive decline

The complex interplay among amyloid-beta, tau pathology, neuroinflammation, oxidative stress, and cholinergic dysfunction contributes to synaptic impairment and cognitive decline in AD. Aβ accumulation has been associated with dysregulation of the cholinergic system, including increased

AChE activity and accelerated acetylcholine degradation, which may contribute to neurotransmitter deficiency and cognitive impairment (Iliyasu et al., 2023). In addition to its classical role in acetylcholine hydrolysis, AChE has been implicated in non-catalytic processes associated with neuronal development, cellular differentiation, and AD-related pathological mechanisms (Small et al., 1996). Although AChE activity showed a weak association with verbal episodic memory, age emerged as a substantially stronger predictor, suggesting that cholinergic enzyme activity alone is insufficient to explain cognitive performance (Teitsdottir et al., 2022). These findings indicate that alterations in AChE activity should be interpreted within the broader context of AD pathology rather than as an isolated determinant of cognitive decline. In conclusion, a multidimensional relationship exists between AChE and AD pathology. AChE is associated with cholinergic dysfunction and interacts with broader pathological processes involving Aβ, tau pathology, neuroinflammation, and oxidative stress. Therefore, future therapeutic strategies targeting AChE should consider both its catalytic and non-catalytic roles within this complex pathological network.

6. Can AChE Inhibitors Compensate for the Deficiency?

Current pharmacological approaches offer symptomatic relief; however, they cannot directly intervene in the pathological progression of the disease (Zuliani et al., 2024). Donepezil, galantamine, and rivastigmine remain the cornerstone of clinical management for AD, with these three acetylcholinesterase inhibitors continuing to occupy a central role in symptomatic treatment (Cacabelos et al., 2024). The mechanism of action of these drugs involves inhibiting AChE in the synaptic cleft, thereby slowing acetylcholine degradation

and preserving signal transmission between synapses (Cacabelos et al., 2024). However, the central question to be addressed in this section is: Can current AChE inhibitors truly compensate for the endogenous AChE deficiency observed in AD?

6.1. Pharmacodynamic rationale and limitations of clinical efficacy

The clinical efficacy of AChE inhibitors in patients has been extensively evaluated through numerous comprehensive studies and systematic reviews. A meta-analysis by Gao and colleagues (2024) demonstrated the positive effects of donepezil, galantamine, and rivastigmine on cognitive function compared to placebo. The findings revealed that all three drugs exhibited statistically significant success in improving cognitive performance (Gao et al., 2024). While these findings confirm that AChE inhibitors confer cognitive benefits, they also indicate that this effect remains quite modest in clinical practice. In other words, the drugs are effective, but the degree of improvement they produce is more limited than anticipated (Grabowska et al., 2025). The efficacy of AChE inhibitors depends on preserved neuronal capacity in the brain (Pozzi et al., 2022). Patients who exhibit symptoms such as hallucinations but have relatively preserved neural structures respond better to treatment. Consequently, these drugs do not restore the lost system; they only provide limited support by enhancing the efficiency of remaining intact mechanisms. Studies have revealed considerable interindividual variability in patient responses to inhibitor drugs (Pozzi et al., 2022). Given the considerable interindividual variability in drug response, the importance of personalized treatment approaches should be emphasized (Cacabelos et al., 2024).

6.2. Effects of AChE inhibitors on non-catalytic functions

While conventional drugs target only the catalytic site, the impact of AChE's amyloid-accelerating and structural roles—mediated through the PAS—on disease progression has been largely overlooked (Inestrosa et al., 1996). Currently used AChE inhibitors do not exert a sufficiently strong effect on these non-catalytic functions of the enzyme. Despite the testing of over ten thousand compounds, the failure of single-target approaches to achieve desired efficacy has directed research toward next-generation drugs that simultaneously target multiple mechanisms (Cacabelos et al., 2024). Studies conceptualizing AChE as a multifunctional target emphasize that dual-site inhibitors, by binding to both catalytic and peripheral sites, prevent AChE-mediated degradation while also mitigating amyloid-induced toxicity (Grabowska et al., 2025). These molecules may hold potential for use in the treatment of the disease.

6.3. BChE inhibitors: An alternative approach

The increase in BChE accompanying the decrease in AChE in the temporal lobe and hippocampus positions BChE as a compelling alternative therapeutic target in AD (Lane et al., 2006). In late-stage patients, where AChE levels are markedly reduced, targeting BChE to support cognitive function may yield more meaningful therapeutic outcomes (Ferjančič Benetik et al., 2024). According to survival data analysis, although the cognitive effects of these drugs appear limited, they have been shown to confer critical benefits in terms of long-term clinical outcomes and survival (Zuliani et al., 2024).

In conclusion, current AChE inhibitors do not fully compensate for the endogenous AChE deficiency observed in AD. These drugs exert limited and transient effects by acting through remaining intact neurons and enzyme activity, but they fail to arrest the loss of non-catalytic functions or the progressive nature of the

disease. Future therapeutic strategies are therefore evolving toward multi-target drugs, BChE inhibition, and molecules specifically designed to address non-catalytic functions.

7. Future Perspectives: Novel Strategies

Given the multifaceted nature of AChE deficiency, future therapeutic approaches are anticipated to achieve success only if they adopt a holistic and multi-targeted framework (Cacabelos et al., 2024). The limited clinical efficacy of classical AChE inhibitors, coupled with their inability to modulate the enzyme's structural (non-catalytic) functions, has driven the search for more comprehensive therapeutic strategies (Grabowska et al., 2025).

7.1. Modulation of AChE expression and isoform balance

Alterations in AChE expression and transcript composition may contribute to cholinergic dysregulation in AD. In particular, disease-associated changes in cholinesterase transcript variants suggest that AChE regulation involves not only overall enzyme activity but also isoform-specific alterations (Gok et al., 2022). This complexity is further underscored by the non-catalytic functions of AChE, particularly its ability to promote Aβ aggregation through interactions involving the peripheral anionic site (PAS) (Inestrosa et al., 1996; Grabowska et al., 2025). Therefore, therapeutic strategies targeting AChE should not simply aim to increase or decrease overall enzyme activity but should consider its distinct catalytic and non-catalytic functions. In this context, selective modulation of AChE isoforms or transcript variants may represent a potential avenue for future investigation (Gok et al., 2022). Moreover, emerging pharmacogenomic and epigenetic approaches may provide additional opportunities for individualized and multi-target therapeutic strategies in AD (Cacabelos et al., 2024).

7.2. Molecules targeting non-catalytic functions (PAS region)

Modulating the non-catalytic functions of AChE has emerged as a promising approach in modern research aimed at combating neurodegeneration (Grabowska et al., 2025). The association of the primary mechanism accelerating amyloid plaque formation with the PAS region renders this site a critical therapeutic target for arresting neurodegeneration (Inestrosa et al., 1996, 2008).

Dual-site inhibitors: These molecules simultaneously bind to both the CAS and PAS regions, thereby increasing neurotransmitter levels while halting AChE-induced amyloid accumulation—intervening in the pathology through two distinct mechanisms (Cacabelos et al., 2024).

Multi-target-directed ligands (MTDLs): Building upon AChE inhibition, these compounds simultaneously target interconnected pathological processes including oxidative stress, metal ion dyshomeostasis, and neuroinflammation (Da Conceição et al., 2026). This multidimensional strategy directly addresses the complexity of Alzheimer's pathology, offering a holistic and viable therapeutic model (Cacabelos et al., 2024). Research on novel AChE inhibitors has highlighted that unique scaffold structures derived from natural products are fundamental elements increasing chemical diversity in drug design (Arora et al., 2025).

Metal-chelating hybrid molecules: These are emerging as a rational treatment model (Grabowska et al., 2025). Metals such as copper, zinc, and iron not only accelerate Aβ fibrillization but also exacerbate neural tissue damage through the oxidative stress they generate (Iliyasu et al., 2023). AChE inhibition supported by this chelating capacity not only addresses the cholinergic deficit but also eliminates metal ion-catalyzed amyloid accumulation (Grabowska et al., 2025).

7.3. Biomarker Potential of AChE Alterations

Alterations in AChE activity and expression have been investigated as potential biomarkers reflecting cholinergic dysfunction and disease-related changes in AD (Teitsdottir et al., 2022; Valotassiou et al., 2018).

Imaging biomarkers: Changes in cerebral AChE activity can be evaluated using molecular imaging approaches, providing an *in vivo* measure of cholinergic dysfunction in AD (Valotassiou et al., 2018). The relationship between alterations in AChE activity and structural brain changes may further support the potential value of combining cholinergic and neuroimaging markers for monitoring disease-related changes (Richter et al., 2025).

Fluid biomarkers: CSF AChE activity and related cholinergic measures have been investigated in relation to cognitive performance and established AD biomarkers (Teitsdottir et al., 2022). However, because CSF collection requires an invasive lumbar puncture, the identification of more accessible peripheral biomarkers remains an important area of investigation (Lane et al., 2006).

Genetic biomarkers: Alterations in AChE expression and transcript variants have been reported in AD, indicating that regulation of the ACHE gene may contribute to disease-associated cholinergic changes (Gok et al., 2022). In this broader context, pharmacogenomic and genetic approaches may contribute to the development of more individualized therapeutic strategies for AD (Cacabelos et al., 2024; Pozzi et al., 2022).

8. Conclusion

In the treatment of AD, a fundamental paradigm shift is occurring—away from classical cholinesterase inhibitors that provide only symptomatic relief and toward multifunctional

molecules that simultaneously target the complex pathological mechanisms underlying the disease (Cacabelos et al., 2024). The impact of AChE on amyloid accumulation extends beyond classical enzyme inhibition, thereby paving the way for holistic therapeutic strategies that fundamentally target Alzheimer's pathophysiology (Dinamarca et al., 2010). Despite the symptomatic benefits provided by current AChE inhibitors, multifaceted strategies—such as those targeting non-catalytic domains like the PAS region or incorporating BChE inhibition—offer significantly greater potential for slowing disease progression (Grabowska et al., 2025). Traditionally considered an epiphenomenon resulting from neuronal loss, AChE deficiency is now being redefined—in light of current evidence—as a multilayered and independent pathophysiological mechanism that directly shapes Alzheimer's pathology (García-Ayllón et al., 2011; Gok et al., 2022). AChE deficiency plays a central role in the multifactorial architecture of AD, extending far beyond simple neurotransmitter loss. In this context, future research must transcend classical inhibition methods and prioritize disease-modifying next-generation therapeutic and diagnostic approaches that focus on the enzyme's molecular diversity (isoforms) and non-catalytic functions.

References

Apostolova, L. G. (2016). Alzheimer disease. *Continuum: Lifelong Learning in Neurology, 22*(2, Dementia), 419–434. https://doi.org/10.1212/CON.0000000000000307

Arora, P., Swati, Rani, S., Jha, S., Gupta, S., & Kumar, S. (2025). Innovative approaches in acetylcholinesterase inhibition: A pathway to effective Alzheimer's disease treatment. *Molecular Diversity, 29*(6), 5367–5396. https://doi.org/10.1007/s11030-025-11170-1

Badal, R., Kaur, B., Singh, H., Sharma, P., Yadav, P., Mahapatra, A., Ghildiyal, S., Patel, A. K., Kumar, L., Sharma, B., Prajapati, P. K., & Nesari, T. (2026). Saraswata Ghrita mitigates $AlCl_3$- and D-galactose-induced cognitive deficits in a murine Alzheimer's-like model: In silico and in vivo insights. *Brain Research Bulletin, 235*, 111742. https://doi.org/10.1016/j.brainresbull.2026.111742

Birks, J. (2006). Cholinesterase inhibitors for Alzheimer's disease. *Cochrane Database of Systematic Reviews, 2006*(1), CD005593. https://doi.org/10.1002/14651858.CD005593

Cacabelos, R., Martínez-Iglesias, O., Cacabelos, N., Carrera, I., Corzo, L., & Naidoo, V. (2024). Therapeutic options in Alzheimer's disease: From classic acetylcholinesterase inhibitors to multi-target drugs with pleiotropic activity. *Life, 14*(12), 1555. https://doi.org/10.3390/life14121555

Corey-Bloom, J. (2002). The ABC of Alzheimer's disease: Cognitive changes and their management in Alzheimer's disease and related dementias. *International Psychogeriatrics, 14*(S1), 51–75. https://doi.org/10.1017/S1041610203008664

da Conceição, R. A., Barbosa, M. L. D. C., & de Souza, A. M. T. (2026). Multitarget-directed ligands in Alzheimer's disease:

Identification of AChE and BACE1 inhibitors by in silico approaches. *Journal of Computer-Aided Molecular Design, 40*(1), 7. https://doi.org/10.1007/s10822-025-00712-2

Dinamarca, M. C., Sagal, J. P., Quintanilla, R. A., Godoy, J. A., Arrázola, M. S., & Inestrosa, N. C. (2010). Amyloid-β-acetylcholinesterase complexes potentiate neurodegenerative changes induced by the Aβ peptide: Implications for the pathogenesis of Alzheimer's disease. *Molecular Neurodegeneration, 5*, 4. https://doi.org/10.1186/1750-1326-5-4

Evans, N., Mahfooz, K., Garcia-Rates, S., & Greenfield, S. (2024). Oxidative stress triggers a pivotal peptide linked to Alzheimer's disease. *International Journal of Molecular Sciences, 25*(22), 12413. https://doi.org/10.3390/ijms252212413

Ferjančič Benetik, S., Knez, D., Obreza, A., Košak, U., & Gobec, S. (2024). Dual inhibition of butyrylcholinesterase and p38α mitogen-activated protein kinase: A new approach for the treatment of Alzheimer's disease. *Pharmacology & Therapeutics, 264*, 108748. https://doi.org/10.1016/j.pharmthera.2024.108748

Ferreira-Vieira, T. H., Guimaraes, I. M., Silva, F. R., & Ribeiro, F. M. (2016). Alzheimer's disease: Targeting the cholinergic system. *Current Neuropharmacology, 14*(1), 101–115. https://doi.org/10.2174/1570159X13666150716165726

Gao, Y., Liu, Y., & Li, Y. (2024). Safety and efficacy of acetylcholinesterase inhibitors for Alzheimer's disease: A systematic review and meta-analysis. *Advances in Clinical and Experimental Medicine, 33*(11), 1179–1187. https://doi.org/10.17219/acem/176051

García-Ayllón, M.-S., Small, D. H., Avila, J., & Sáez-Valero, J. (2011). Revisiting the role of acetylcholinesterase in Alzheimer's

disease: Cross-talk with P-tau and β-amyloid. *Frontiers in Molecular Neuroscience, 4*, 22. https://doi.org/10.3389/fnmol.2011.00022

Gok, M., Madrer, N., Zorbaz, T., Bennett, E. R., Greenberg, D., Bennett, D. A., & Soreq, H. (2022). Altered levels of variant cholinesterase transcripts contribute to the imbalanced cholinergic signaling in Alzheimer's and Parkinson's disease. *Frontiers in Molecular Neuroscience, 15*, 941467. https://doi.org/10.3389/fnmol.2022.941467

Grabowska, W., Bijak, M., Szelenberger, R., Gorniak, L., Podogrocki, M., Harmata, P., & Cichon, N. (2025). Acetylcholinesterase as a multifunctional target in amyloid-driven neurodegeneration: From dual-site inhibitors to anti-agregation strategies. *International Journal of Molecular Sciences, 26*(17), 8726. https://doi.org/10.3390/ijms26178726

Hampel, H., Mesulam, M.-M., Cuello, A. C., Farlow, M. R., Giacobini, E., Grossberg, G. T., Khachaturian, A. S., Vergallo, A., Cavedo, E., Snyder, P. J., & Khachaturian, Z. S. (2018). The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. *Brain, 141*(7), 1917–1933. https://doi.org/10.1093/brain/awy132

Hansen, R. A., Gartlehner, G., Webb, A. P., Morgan, L. C., Moore, C. G., & Jonas, D. E. (2008). Efficacy and safety of donepezil, galantamine, and rivastigmine for the treatment of Alzheimer's disease: A systematic review and meta-analysis. *Clinical Interventions in Aging, 3*(2), 211–225.

Hung, L.-W., Sanbonmatsu, K. Y., Williams, R. F., & Chen, J. C.-H. (2025). Acetylcholinesterase: Structure, dynamics, and interactions with organophosphorus compounds. *Protein Science, 34*(10), e70297. https://doi.org/10.1002/pro.70297

Iliyasu, M. O., Musa, S. A., Oladele, S. B., & Iliya, A. I. (2023). Amyloid-beta aggregation implicates multiple pathways in Alzheimer's disease: Understanding the mechanisms. *Frontiers in Neuroscience, 17*, 1081938. https://doi.org/10.3389/fnins.2023.1081938

Inestrosa, N. C., Alvarez, A., Pérez, C. A., Moreno, R. D., Vicente, M., Linker, C., Casanueva, O. I., Soto, C., & Garrido, J. (1996). Acetylcholinesterase accelerates assembly of amyloid-β-peptides into Alzheimer's fibrils: Possible role of the peripheral site of the enzyme. *Neuron, 16*(4), 881–891. https://doi.org/10.1016/S0896-6273(00)80108-7

Inestrosa, N. C., Dinamarca, M. C., & Alvarez, A. (2008). Amyloid–cholinesterase interactions: Implications for Alzheimer's disease. *The FEBS Journal, 275*(4), 625–632. https://doi.org/10.1111/j.1742-4658.2007.06238.x

Inestrosa, N. C., Sagal, J. P., & Colombres, M. (2005). Acetylcholinesterase interaction with Alzheimer amyloid β. In J. R. Harris & F. Fahrenholz (Eds.), *Alzheimer's disease: Cellular and molecular aspects of amyloid β* (pp. 299–317). Springer. https://doi.org/10.1007/0-387-23226-5_15

Kashif, M., Chandrabose, K., & Pandurangan, A. K. (2024). Plausible action of N-(3,4-dimethoxy-phenyl)-6,7-dimethoxyquinazoline-4-amine (TKM01) as an armor against Alzheimer's disease: In silico and in vivo insights. *Journal of Biochemical and Molecular Toxicology, 38*(12), e70048. https://doi.org/10.1002/jbt.70048

Kumar, A., Sidhu, J., Lui, F., & Tsao, J. W. (2025). Alzheimer disease. In *StatPearls*. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK499922/

Lane, R. M., Potkin, S. G., & Enz, A. (2006). Targeting acetylcholinesterase and butyrylcholinesterase in dementia. *International Journal of Neuropsychopharmacology, 9*(1), 101–124. https://doi.org/10.1017/S1461145705005833

Moreta, M. P.-G., Burgos-Alonso, N., Torrecilla, M., Marco-Contelles, J., & Bruzos-Cidón, C. (2021). Efficacy of acetylcholinesterase inhibitors on cognitive function in Alzheimer's disease: Review of reviews. *Biomedicines, 9*(11), 1689. https://doi.org/10.3390/biomedicines9111689

Pozzi, F. E., Conti, E., Appollonio, I., Ferrarese, C., & Tremolizzo, L. (2022). Predictors of response to acetylcholinesterase inhibitors in dementia: A systematic review. *Frontiers in Neuroscience, 16*, 998224. https://doi.org/10.3389/fnins.2022.998224

Rees, T. M., & Brimijoin, S. (2003). The role of acetylcholinesterase in the pathogenesis of Alzheimer's disease. *Drugs of Today, 39*(1), 75–83. https://doi.org/10.1358/dot.2003.39.1.740206

Richter, N., Breidenbach, L., Schmieschek, M. H. T., Heiss, W.-D., Fink, G. R., & Onur, O. A. (2025). Alzheimer-typical temporo-parietal atrophy and hypoperfusion are associated with a more significant cholinergic impairment in amnestic neurodegenerative syndromes. *Journal of Alzheimer's Disease, 104*(4), 1290–1300. https://doi.org/10.1177/13872877251324080

Safiri, S., Ghaffari Jolfayi, A., Fazlollahi, A., Morsali, S., Sarkesh, A., Daei Sorkhabi, A., Golabi, B., Aletaha, R., Motlagh Asghari, K., Hamidi, S., Mousavi, S. E., Jamalkhani, S., Karamzad, N., Shamekh, A., Mohammadinasab, R., Sullman, M. J. M., Şahin, F., & Kolahi, A.-A. (2024). Alzheimer's disease: A comprehensive review of epidemiology, risk factors, symptoms diagnosis, management, caregiving, advanced treatments and associated

challenges. *Frontiers in Medicine, 11*, 1474043. https://doi.org/10.3389/fmed.2024.1474043

Schwarthoff, S., Tischer, N., Sager, H., Schätz, B., Rohrbach, M. M., Raztsou, I., Robaa, D., Gaube, F., Arndt, H.-D., & Winckler, T. (2021). Evaluation of γ-carboline-phenothiazine conjugates as simultaneous NMDA receptor blockers and cholinesterase inhibitors. *Bioorganic & Medicinal Chemistry, 46*, 116355. https://doi.org/10.1016/j.bmc.2021.116355

Silman, I. (2021). The multiple biological roles of the cholinesterases. *Progress in Biophysics and Molecular Biology, 162*, 41–56. https://doi.org/10.1016/j.pbiomolbio.2020.12.001

Small, D. H., Michaelson, S., & Sberna, G. (1996). Non-classical actions of cholinesterases: Role in cellular differentiation, tumorigenesis and Alzheimer's disease. *Neurochemistry International, 28*(5–6), 453–483. https://doi.org/10.1016/0197-0186(95)00099-2

Teitsdottir, U. D., Darreh-Shori, T., Lund, S. H., Jonsdottir, M. K., Snaedal, J., & Petersen, P. H. (2022). Phenotypic displays of cholinergic enzymes associate with markers of inflammation, neurofibrillary tangles, and neurodegeneration in pre- and early symptomatic dementia subjects. *Frontiers in Aging Neuroscience, 14*, 876019. https://doi.org/10.3389/fnagi.2022.876019

Valotassiou, V., Malamitsi, J., Papatriantafyllou, J., Dardiotis, E., Tsougos, I., Psimadas, D., Alexiou, S., Hadjigeorgiou, G., & Georgoulias, P. (2018). SPECT and PET imaging in Alzheimer's disease. *Annals of Nuclear Medicine, 32*(9), 583–593. https://doi.org/10.1007/s12149-018-1292-6

Xie, W., Stribley, J. A., Chatonnet, A., Wilder, P. J., Rizzino, A., McComb, R. D., Taylor, P., Hinrichs, S. H., & Lockridge, O. (2000). Postnatal developmental delay and supersensitivity to

organophosphate in gene-targeted mice lacking acetylcholinesterase. *The Journal of Pharmacology and Experimental Therapeutics, 293*(3), 896–902.

Young, S., Chung, E., & Chen, M. A. (2021). Cardiovascular complications of acetylcholinesterase inhibitors in patients with Alzheimer's disease: A narrative review. *Annals of Geriatric Medicine and Research, 25*(3), 170–177. https://doi.org/10.4235/agmr.21.0079

Zuliani, G., Zuin, M., Romagnoli, T., Polastri, M., Cervellati, C., & Brombo, G. (2024). Acetyl-cholinesterase-inhibitors reconsidered: A narrative review of post-marketing studies on Alzheimer's disease. *Aging Clinical and Experimental Research, 36*(1), 23. https://doi.org/10.1007/s40520-023-02675-6

CHAPTER 8

SIGMA-1 RECEPTOR IN ALZHEIMER'S DISEASE: MOLECULAR MECHANISMS AND THERAPEUTIC POTENTIAL

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1. Introduction

Alzheimer's disease (AD) begins decades before clinical symptoms emerge. Neurodegenerative disorders such as Alzheimer's, Parkinson's, and Huntington's disease remain largely untreatable due to their multifaceted and complex nature, imposing substantial economic and humanitarian burdens on society. Therefore, the development of novel and innovative therapeutic strategies is of paramount importance. One particularly noteworthy target in this context is the chaperone protein sigma-1 receptor (S1R), which has garnered significant interest due to its neuroprotective properties (Ryskamp et al., 2019). Activation of S1R has demonstrated promising outcomes in the treatment of neurodegenerative diseases. Small-molecule modulators of sigma-1

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and sigma-2 receptors (σ1R and σ2R) have emerged as potential candidates for Alzheimer's therapy. σ1R agonists have demonstrated neuroprotective and anti-amnesic effects in experimental models of cognitive impairment and Alzheimer's disease, while generally showing little or no effect on learning performance under normal conditions (Jia et al., 2015; Maurice & Goguadze, 2017). Beyond Alzheimer's disease, S1R plays a role in various neurodegenerative disorders, including amyotrophic lateral sclerosis (ALS), Parkinson's and Huntington's diseases, stroke, and epilepsy. Several major pathogenic hypotheses have been proposed to explain the progression of Alzheimer's disease, aiming to elucidate the core molecular mechanisms involved in disease development (De Strooper & Karran, 2016). Different theoretical approaches have been developed to clarify the pathogenesis of Alzheimer's disease, enhancing understanding of its molecular and cellular foundations. The "amyloid hypothesis" suggests that the accumulation of amyloid-β (Aβ) initiates a cascade of pathological events, including the development and progression of tau pathology, neuroinflammation, and neuronal dysfunction, ultimately contributing to cognitive decline (Hardy & Higgins, 1992; Karran & De Strooper, 2022). The "MAM hypothesis" proposes that altered function of mitochondria-associated endoplasmic reticulum membranes (MAMs) may play a central role in AD pathogenesis (Area-Gomez & Schon, 2017; Li et al., 2023). The development of suitable PET radioligands for imaging σ1 and σ2 receptors in humans provides critical tools for monitoring Alzheimer's disease progression and evaluating therapeutic outcomes. This review highlights the role of sigma-1 (S1R) in Alzheimer's treatment, exploring its mechanisms of action and shedding light on its effects on neurodegeneration and neuroprotection.

2. General Overview of the Sigma-1 Receptor

Sigma receptors were initially considered a subtype of opioid receptors but were later identified as a distinct receptor family due to their unique pharmacological and molecular characteristics (Hayashi & Su, 2004). The sigma-1 receptor is a unique transmembrane protein composed of approximately 223 amino acids, exhibiting chaperone properties. It is predominantly located at contact sites between the endoplasmic reticulum (ER) and mitochondria, playing a critical role in intracellular signaling and calcium regulation (Penke et al., 2018; Kim & Bezprozvanny, 2023). Sigma-1 receptors interact with various endogenous neurosteroids and pharmacological compounds, modulating cellular stress responses and neuroprotective mechanisms. Owing to these ligand-binding properties, the sigma-1 receptor is considered a potential therapeutic target in neurodegenerative diseases such as Alzheimer's disease (Kim & Bezprozvanny, 2023).

2.1. Physiological functions of the sigma-1 receptor

The sigma-1 receptor interacts with endogenous neurosteroids and pharmacological compounds, modulating cellular stress responses and neuroprotective mechanisms. These ligand-binding properties render it a promising therapeutic target, particularly in neurodegenerative disorders such as Alzheimer's disease (Penke et al., 2018). The receptor regulates Ca^{2+} transfer from the ER to mitochondria via inositol 1,4,5-trisphosphate receptors (IP_3Rs), thereby contributing to the maintenance of cellular bioenergetics (Hayashi & Su, 2007). Its localization at mitochondria–ER contact sites directly modulates inter-organelle signaling and cellular adaptation (Resende et al., 2022). Experimental studies have demonstrated that S1R functions as a molecular chaperone involved in maintaining ER proteostasis and attenuating cellular damage associated with prolonged ER stress

(Penke et al., 2018). Through these mechanisms, the sigma-1 receptor suppresses neuronal apoptosis, exerts neuroprotective effects, and provides resilience against neurodegenerative processes (Maurice & Su, 2009).

3. Pathophysiology of AD

In the early stages of Alzheimer's disease, amyloid-β peptides generated from abnormal processing of amyloid precursor protein accumulate extracellularly, forming plaques that disrupt synaptic function (Selkoe & Hardy, 2016). Abnormal hyperphosphorylation of tau reduces its affinity for microtubules, resulting in microtubule destabilization and promoting tau aggregation into neurofibrillary tangles (Wang & Mandelkow, 2016). These pathological protein aggregates activate microglia and astrocytes, eliciting a chronic neuroinflammatory response in the brain (Heppner et al., 2015). Ongoing inflammation and mitochondrial dysfunction contribute to elevated reactive oxygen species, exacerbating oxidative stress-induced neuronal damage (Swerdlow et al., 2010). The combined effects of these pathological processes result in synaptic loss, neuronal integrity disruption, and progressive neuronal death, forming the basis of the clinical manifestations of Alzheimer's disease (DeKosky & Scheff, 1990).

3.1. The role of sigma-1 receptor in AD pathophysiology

Activation of the sigma-1 receptor (S1R) contributes to the preservation of neuronal communication by reducing synaptic dysfunction caused by amyloid-β accumulation (Ryskamp et al., 2019). S1R activation has been implicated in the modulation of signaling pathways associated with tau pathology, suggesting a potential protective role against tau-related neuronal dysfunction (Wang & Jia, 2023). Additionally, S1R signaling modulates microglial activity and neuroinflammatory responses, thereby contributing to the regulation of inflammation-associated neuronal

damage (Jia et al., 2018). The receptor also stabilizes mitochondrial functions and regulates intracellular calcium homeostasis, contributing to the reduction of oxidative stress-induced neuronal damage (Hayashi & Su, 2007). Taken together, these mechanisms suggest that S1R may slow neuronal loss, preserve synaptic integrity, and modulate the progression of Alzheimer's disease (Ryskamp et al., 2019).

3.2. Sigma-1 receptor in AD

S1R functions as a molecular chaperone at the ER–mitochondria interface and contributes to cellular adaptation to ER stress, thereby promoting cell survival (Hayashi & Su, 2007). Its localization at mitochondria–ER contact sites supports calcium transfer and energy metabolism, contributing to the maintenance of mitochondrial function (Hayashi & Su, 2007). In experimental Alzheimer's models, S1R activation has been shown to suppress apoptotic signaling pathways and enhance neuronal survival. Moreover, S1R-mediated signaling mechanisms regulate synaptic protein expression, supporting the preservation of synaptic integrity (Ryskamp et al., 2019). These multifaceted biological effects indicate that S1R may serve as a potential therapeutic target in Alzheimer's disease, promoting neuronal plasticity (Maurice & Su, 2009).

4. Sigma-1 Receptor and Amyloid-β Interaction

Activation of S1R reduces neuronal toxicity induced by amyloid-β peptides, contributing to the maintenance of cellular viability (Ryskamp et al., 2019). By regulating signaling between the ER and mitochondria, S1R limits amyloid-β-induced intracellular calcium dysregulation, thereby supporting neuronal function stability (Hayashi & Su, 2007). Experimental evidence suggests that S1R activation protects against amyloid-β-induced synaptic dysfunction and contributes to the preservation of synaptic stability

(Ryskamp et al., 2019). Furthermore, S1R activation stabilizes mitochondrial functions, decreasing reactive oxygen species production and mitigating oxidative stress (Mori et al., 2013). Collectively, these findings suggest that S1R represents a key molecular target that modulates amyloid-β-mediated neurodegenerative processes (Ryskamp et al., 2019).

4.1. Sigma-1 receptor and tau pathology

S1R contributes to the regulation of tau phosphorylation by promoting p35 turnover and thereby limiting aberrant CDK5/p25 signaling. Experimental evidence further indicates that S1R-mediated regulation of tau phosphorylation is associated with the maintenance of axonal integrity (Tsai et al., 2015). By regulating calcium transfer between mitochondria and the ER, S1R helps maintain the energy balance necessary for microtubule stability (Hayashi & Su, 2007). S1R activation has also been reported to facilitate axonal transport, enabling the proper trafficking of synaptic proteins along neuronal processes (Ryskamp et al., 2019). Together, these molecular effects suggest that S1R may exert neuroprotective effects by modulating tau phosphorylation, axonal integrity, and neuronal homeostasis (Tsai et al., 2015; Ryskamp et al., 2019).

4.2. Sigma-1 receptor and neuroinflammation

Activation of S1R suppresses excessive microglial activation, thereby attenuating the severity of neuroinflammatory responses (Jia et al., 2018). Experimental studies have demonstrated that S1R signaling regulates astrocyte reactivity, limiting uncontrolled release of inflammatory mediators (Guo et al., 2021). S1R activation also reduces the production of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1β, slowing the progression of inflammation in brain tissue (Eskandari et al., 2025). By modulating cellular stress signaling pathways, S1R contributes to the reduction of

inflammation-induced neuronal damage and apoptotic processes (Nguyen et al., 2015). Collectively, these findings highlight S1R as an important modulator of neuroinflammatory processes and a potential therapeutic target for limiting inflammation-associated neuronal damage (Jia et al., 2018).

4.3. Sigma-1 receptor-targeted therapeutic approaches

Sigma-1 receptor (S1R) agonists exert neuroprotective effects by regulating calcium homeostasis and cellular stress responses in neuronal cells, making them potential therapeutic agents for Alzheimer's disease. In experimental Alzheimer's models, S1R agonists have been shown to reduce synaptic dysfunction caused by amyloid-β accumulation and improve cognitive performance (Ryskamp et al., 2019). Preclinical studies suggest that S1R-targeted compounds can stabilize mitochondrial functions, thereby enhancing neuronal survival (Ryskamp et al., 2019). Several S1R-targeting compounds have entered clinical investigation for their potential effects on cognitive impairment and other manifestations of Alzheimer's disease (Wang & Jia, 2023). Taken together, these findings indicate that therapeutic strategies targeting S1R represent promising approaches capable of modulating neurodegenerative processes and potentially contributing to the development of disease-modifying treatments in the future (Shokr et al., 2025).

5. Molecular Imaging Techniques and Diagnostic Potential

Recent molecular imaging studies have highlighted the sigma-1 receptor (σ1R) as a significant biological target for understanding the pathophysiology of Alzheimer's disease (AD). Positron emission tomography (PET)-based imaging techniques allow for non-invasive evaluation of σ1R distribution and binding density in the brain. The development of ^18F-labeled radioligands with high brain penetration, suitable kinetic properties, and specific

binding capabilities enables detailed in vivo investigation of neurodegenerative processes (Jiang et al., 2026). In vivo PET imaging has demonstrated reduced σ1R binding in several brain regions of patients with early Alzheimer's disease compared with healthy elderly individuals, supporting the potential relevance of σ1R alterations to AD pathology (Mishina et al., 2008). The sigma-1 receptor is a chaperone protein localized at mitochondria-associated ER membranes, playing a critical role in regulating cellular stress responses. Through mechanisms such as calcium homeostasis maintenance, mitochondrial function preservation, and modulation of oxidative stress responses, σ1R exerts neuroprotective effects. Accordingly, σ1R PET imaging has been proposed as a potential approach for investigating receptor alterations associated with AD and may provide complementary information for disease assessment and therapeutic monitoring (Wang & Jia, 2023). Moreover, the metabolic stability and favorable safety profiles of σ1R-targeted radiopharmaceuticals provide important advantages for clinical translation. Preclinical evidence suggests that σ1R imaging could serve as a complementary biomarker to evaluate molecular changes associated with amyloid-β and tau pathologies. Additionally, σ1R PET imaging offers a valuable tool for monitoring the target engagement of novel pharmacological agents and assessing therapeutic responses (Lan et al., 2019). In summary, molecular imaging approaches targeting σ1R present a promising biomarker and research platform for early diagnosis of Alzheimer's disease, monitoring disease progression, and developing targeted therapeutic strategies. Considering σ1R's involvement in neuroprotective signaling networks, this receptor may play a crucial role not only in diagnostic evaluation but also in therapeutic target validation studies (Mastropasqua et al., 2025).

6. Conclusion

Recent studies indicate that the sigma-1 receptor (σ1R) is a key molecular target in Alzheimer's disease for regulating cellular stress responses. Its localization at ER–mitochondria contact sites contributes to maintaining critical processes such as calcium homeostasis and cellular energy balance (Wang & Jia, 2023). Activation of σ1R can promote cytoprotective signaling and support neuronal survival under pathological conditions (Maurice & Goguadze, 2017). In experimental models of Alzheimer's disease, σ1R activation has shown protective effects against amyloid-β-associated neurotoxicity and cognitive impairment (Maurice & Goguadze, 2017). Activation of σ1R has been reported to rescue social and contextual memory impairments induced by amyloid-β oligomers, highlighting its important role in reducing early cognitive deficits. Furthermore, σ1R modulates neuronal calcium signaling and neurotransmitter release, contributing to the maintenance of synaptic communication (Djebari et al., 2025). The development of novel σ1R radiopharmaceuticals for imaging studies has enabled the visualization of receptor density changes in Alzheimer's models, providing significant progress for early diagnosis and understanding disease pathophysiology (Mastropasqua et al., 2025). Recent evidence suggests that σ1R interaction with mitochondria-associated ER membranes can regulate cellular stress responses that contribute to amyloid and tau pathology (Resende et al., 2022). The receptor's interaction with multiple cellular signaling networks allows it to simultaneously influence key processes of Alzheimer's pathology, including inflammation, protein misfolding, and synaptic dysfunction. In addition, pharmacological agents targeting σ1R have been reported to exert neuroprotective effects in early-stage Alzheimer's pathology, contributing to the preservation of cognitive functions (Voronin et al., 2023). Overall, the regulatory effects of σ1R on neuroprotection, synaptic plasticity, and protein homeostasis

underscore its significant potential as both a diagnostic biomarker and a therapeutic target in Alzheimer's disease (Drewes et al., 2025).

References

Area-Gomez, E., & Schon, E. A. (2017). On the pathogenesis of Alzheimer's disease: The MAM hypothesis. *FASEB Journal, 31*(3), 864–867. https://doi.org/10.1096/fj.201601309

DeKosky, S. T., & Scheff, S. W. (1990). Synapse loss in frontal cortex biopsies in Alzheimer's disease: Correlation with cognitive severity. *Annals of Neurology, 27*(5), 457–464. https://doi.org/10.1002/ana.410270502

Djebari, S., Jiménez-Herrera, R., Iborra-Lázaro, G., Jiménez-Díaz, L., & Navarro-López, J. D. (2025). Social and contextual memory impairments induced by amyloid-β oligomers are rescued by sigma-1 receptor activation. *Biomedicine & Pharmacotherapy, 184*, 117914. https://doi.org/10.1016/j.biopha.2025.117914

dos Santos Maia, M., Rodrigues, G. C. S., de Sousa, N. F., Scotti, M. T., Scotti, L., & Mendonça-Junior, F. J. B. (2020). Identification of new targets and the virtual screening of lignans against Alzheimer's disease. *Oxidative Medicine and Cellular Longevity, 2020*, 3098673. https://doi.org/10.1155/2020/3098673

Drewes, N., Fang, X., Gupta, N., & Nie, D. (2025). Pharmacological and pathological implications of sigma-1 receptor in neurodegenerative diseases. *Biomedicines, 13*(6), 1409. https://doi.org/10.3390/biomedicines13061409

Eskandari, K., Bélanger, S.-M., Lachance, V., & Kourrich, S. (2025). Repurposing sigma-1 receptor-targeting drugs for therapeutic advances in neurodegenerative disorders. *Pharmaceuticals, 18*(5), 700. https://doi.org/10.3390/ph18050700

Guo, L., Gao, T., Gao, C., Jia, X., Ni, J., Han, C., & Wang, Y. (2021). Stimulation of astrocytic sigma-1 receptor is sufficient to ameliorate inflammation-induced depression. *Behavioural Brain Research, 410*, 113344. https://doi.org/10.1016/j.bbr.2021.113344

Hardy, J. A., & Higgins, G. A. (1992). Alzheimer's disease: The amyloid cascade hypothesis. *Science, 256*(5054), 184–185. https://doi.org/10.1126/science.1566067

Hayashi, T., & Su, T.-P. (2004). Sigma-1 receptor ligands: Potential in the treatment of neuropsychiatric disorders. *CNS Drugs, 18*(5), 269–284. https://doi.org/10.2165/00023210-200418050-00001

Hayashi, T., & Su, T.-P. (2007). Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate Ca^{2+} signaling and cell survival. *Cell, 131*(3), 596–610. https://doi.org/10.1016/j.cell.2007.08.036

Heppner, F. L., Ransohoff, R. M., & Becher, B. (2015). Immune attack: The role of inflammation in Alzheimer disease. *Nature Reviews Neuroscience, 16*(6), 358–372. https://doi.org/10.1038/nrn3880

Jack, C. R., Knopman, D. S., Jagust, W. J., Petersen, R. C., Weiner, M. W., Aisen, P. S., Shaw, L. M., Vemuri, P., Wiste, H. J., Weigand, S. D., Lesnick, T. G., Pankratz, V. S., Donohue, M. C., & Trojanowski, J. Q. (2013). Tracking pathophysiological processes in Alzheimer's disease: An updated hypothetical model of dynamic biomarkers. *The Lancet Neurology, 12*(2), 207–216. https://doi.org/10.1016/S1474-4422(12)70291-0

Jia, J., Cheng, J., Wang, C., & Zhen, X. (2015). Roles of sigma-1 receptors in Alzheimer's disease. *Acta Pharmacologica Sinica, 36*(8), 967–974. https://doi.org/10.1038/aps.2015.20

Jia, J., Cheng, J., Wang, C., & Zhen, X. (2018). Sigma-1 receptor-modulated neuroinflammation in neurological diseases. *Frontiers in Cellular Neuroscience, 12*, 314. https://doi.org/10.3389/fncel.2018.00314

Jiang, H., Jadhav, S. B., Soda, A. K., Zhou, W., Chen, H., Xu, S., Qiu, L., Huang, T., Xing, Z., Zhao, L., Lee, J. J., Ni, R., Wong, D. F., Peng, G., Perlmutter, J. S., Benzinger, T. L. S., & Tu, Z. (2026). Design, synthesis, and characterization of F-18 sigma-1 receptor radiotracers for Alzheimer disease. *European Journal of Medicinal Chemistry, 307*, 118647. https://doi.org/10.1016/j.ejmech.2026.118647

Karran, E., & De Strooper, B. (2022). The amyloid hypothesis in Alzheimer disease: New insights from new therapeutics. *Nature Reviews Drug Discovery, 21*(4), 306–318. https://doi.org/10.1038/s41573-022-00391-w

Kim, M., & Bezprozvanny, I. (2023). Structure-based modeling of sigma 1 receptor interactions with ligands and cholesterol and implications for its biological function. *International Journal of Molecular Sciences, 24*(16), 12980. https://doi.org/10.3390/ijms241612980

Koshenov, Z., Oflaz, F. E., Hirtl, M., Pilic, J., Bachkoenig, O. A., Gottschalk, B., Madreiter-Sokolowski, C. T., Rost, R., Malli, R., & Graier, W. F. (2021). Sigma-1 receptor promotes mitochondrial bioenergetics by orchestrating ER Ca^{2+} leak during early ER stress. *Metabolites, 11*(7), 422. https://doi.org/10.3390/metabo11070422

Lan, Y., Bai, P., Chen, Z., Neelamegam, R., Placzek, M. S., Wang, H., Fiedler, S. A., Yang, J., Yuan, G., Qu, X., Schmidt, H. R., Song, J., Normandin, M. D., Ran, C., & Wang, C. (2019). Novel radioligands for imaging sigma-1 receptor in brain using positron emission tomography (PET). *Acta Pharmaceutica Sinica B, 9*(6), 1204–1215. https://doi.org/10.1016/j.apsb.2019.07.002

Li, Z., Cao, Y., Pei, H., Ma, L., Yang, Y., & Li, H. (2023). The contribution of mitochondria-associated endoplasmic reticulum membranes (MAMs) dysfunction in Alzheimer's disease and the

potential countermeasure. *Frontiers in Neuroscience, 17*, 1158204. https://doi.org/10.3389/fnins.2023.1158204

Mastropasqua, F., Ludwig, F.-A., & Abate, C. (2025). A full-spectrum evaluation of sigma-1 receptor (S1R) positron emission tomography (PET) radioligands from binding affinity to clinical imaging. *Molecules, 30*(21), 4296. https://doi.org/10.3390/molecules30214296

Maurice, T., & Goguadze, N. (2017). Sigma-1 (σ1) receptor in memory and neurodegenerative diseases. *Handbook of Experimental Pharmacology, 244*, 81–108. https://doi.org/10.1007/164_2017_15

Maurice, T., & Su, T.-P. (2009). The pharmacology of sigma-1 receptors. *Pharmacology & Therapeutics, 124*(2), 195–206. https://doi.org/10.1016/j.pharmthera.2009.07.001

Mishina, M., Ohyama, M., Ishii, K., Kitamura, S., Kimura, Y., Oda, K., Kawamura, K., Sasaki, T., Kobayashi, S., Katayama, Y., & Ishiwata, K. (2008). Low density of sigma1 receptors in early Alzheimer's disease. *Annals of Nuclear Medicine, 22*(3), 151–156. https://doi.org/10.1007/s12149-007-0094-z

Mori, T., Hayashi, T., Hayashi, E., & Su, T.-P. (2013). Sigma-1 receptor chaperone at the ER-mitochondrion interface mediates the mitochondrion-ER-nucleus signaling for cellular survival. *PLOS ONE, 8*(10), e76941. https://doi.org/10.1371/journal.pone.0076941

Nguyen, L., Lucke-Wold, B. P., Mookerjee, S. A., Cavendish, J. Z., Robson, M. J., Scandinaro, A. L., & Matsumoto, R. R. (2015). Role of sigma-1 receptors in neurodegenerative diseases. *Journal of Pharmacological Sciences, 127*(1), 17–29. https://doi.org/10.1016/j.jphs.2014.12.005

Penke, B., Fulop, L., Szucs, M., & Frecska, E. (2018). The role of sigma-1 receptor, an intracellular chaperone in

neurodegenerative diseases. *Current Neuropharmacology, 16*(1), 97–116. https://doi.org/10.2174/1570159X15666170529104323

Resende, R., Fernandes, T., Pereira, A. C., Marques, A. P., & Pereira, C. F. (2022). Endoplasmic reticulum-mitochondria contacts modulate reactive oxygen species-mediated signaling and oxidative stress in brain disorders: The key role of sigma-1 receptor. *Antioxidants & Redox Signaling, 37*(10–12), 758–780. https://doi.org/10.1089/ars.2020.8231

Ryskamp, D. A., Korban, S., Zhemkov, V., Kraskovskaya, N., & Bezprozvanny, I. (2019). Neuronal sigma-1 receptors: Signaling functions and protective roles in neurodegenerative diseases. *Frontiers in Neuroscience, 13*, 862. https://doi.org/10.3389/fnins.2019.00862

Selkoe, D. J., & Hardy, J. (2016). The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Molecular Medicine, 8*(6), 595–608. https://doi.org/10.15252/emmm.201606210

Shokr, M. M., Badawi, G. A., Elshazly, S. M., Zaki, H. F., & Mohamed, A. F. (2025). Sigma 1 receptor and its pivotal role in neurological disorders. *ACS Pharmacology & Translational Science, 8*(1), 47–65. https://doi.org/10.1021/acsptsci.4c00564

Swerdlow, R. H., Burns, J. M., & Khan, S. M. (2010). The Alzheimer's disease mitochondrial cascade hypothesis. *Journal of Alzheimer's Disease, 20*(Suppl. 2), S265–S279. https://doi.org/10.3233/JAD-2010-100339

Tsai, S.-Y. A., Pokrass, M. J., Klauer, N. R., Nohara, H., & Su, T.-P. (2015). Sigma-1 receptor regulates tau phosphorylation and axon extension by shaping p35 turnover via myristic acid. *Proceedings of the National Academy of Sciences of the United States of America, 112*(21), 6742–6747. https://doi.org/10.1073/pnas.1422001112

Voronin, M. V., Abramova, E. V., Verbovaya, E. R., Vakhitova, Y. V., & Seredenin, S. B. (2023). Chaperone-dependent mechanisms as a pharmacological target for neuroprotection. *International Journal of Molecular Sciences, 24*(1), 823. https://doi.org/10.3390/ijms24010823

Wang, T., & Jia, H. (2023). The sigma receptors in Alzheimer's disease: New potential targets for diagnosis and therapy. *International Journal of Molecular Sciences, 24*(15), 12025. https://doi.org/10.3390/ijms241512025

Wang, Y., & Mandelkow, E. (2016). Tau in physiology and pathology. *Nature Reviews Neuroscience, 17*(1), 5–21. https://doi.org/10.1038/nrn.2015.1

CHAPTER 9

NEUROPROTECTIVE EFFECTS OF PHENOLIC COMPOUNDS AND FLAVONOIDS IN ALZHEIMER'S DISEASE

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1. Introduction

Alzheimer's disease (AD) represents one of the most pressing public health challenges of the 21st century. AD prevalence surged by 148% between 1990 and 2019, currently affecting an estimated 55 million individuals worldwide, with projections indicating more than 150 million cases by 2050 (Twiss et al., 2025). Global burden of disease projections further indicates a 50.1% increase in age-standardized prevalence by 2050, corresponding to roughly 191 million cases and 6.8 million deaths annually (Liu et al., 2026); age-period-cohort modeling separately projects annual incident AD cases to reach 19.12 million by 2036 (Xu et al., 2025). Beyond its epidemiological scale, AD imposes a profound socioeconomic

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burden through caregiving needs, lost productivity, and escalating healthcare costs (Twiss et al., 2025). Underlying this burden, AD pathophysiology is understood as multifactorial. The amyloid cascade hypothesis holds that misfolded amyloid-β (Aβ) accumulation initiates neuronal dysfunction, while the tauopathy hypothesis emphasizes hyperphosphorylated tau and neurofibrillary tangles, which correlate more closely with cognitive decline than amyloid burden alone (Hein et al., 2025). The cholinergic hypothesis attributes memory impairment to loss of basal forebrain cholinergic neurons, while systems-level views converge on neuroinflammation as a hub linking amyloid, tau, vascular, and mitochondrial dysfunction (Hein et al., 2025). Oxidative stress is now recognized as an early, self amplifying event that promotes tau hyperphosphorylation and is worsened by Aβ induced mitochondrial toxicity (Arnsten et al., 2025). Together, amyloidogenesis, tauopathy, cholinergic deficit, oxidative stress, and neuroinflammation constitute the principal molecular targets explored in this chapter. Current pharmacological approaches remain largely symptomatic. Acetylcholinesterase inhibitors and memantine offer modest, temporary benefit without altering disease trajectory (Goswami et al., 2026). The antiamyloid antibodies lecanemab and donanemab produce statistically significant but clinically modest slowing of decline, while carrying substantial ARIA-related safety concerns requiring ongoing MRI monitoring, and their benefit risk balance remains debated, as reflected in the EMA's initial refusal and subsequent conditional approval of both agents (Goswami et al., 2026) underscoring the need for safer, multitarget strategies such as phenolic compounds and flavonoids. These plant-derived secondary metabolites, with more than 6,000 distinct flavonoid structures identified, are characterized by a multi target mechanism modulating cholinesterase activity, β secretase mediated amyloidogenesis, tau aggregation, oxidative stress, and inflammatory signaling (NF-κB, MAPK, PI3K/Akt) simultaneously, and certain flavonoids cross the

blood brain barrier, positioning them for direct CNS action (Calderaro et al., 2022). This chapter provides an overview of the neuroprotective potential of phenolic compounds and flavonoids in AD: their chemical classification, principal molecular targets, mechanisms of neuroprotection, key representative compounds, bioavailability limitations, and clinical trial perspectives.

2. Classification and Chemical Structure of Phenolic Compounds and Flavonoids

2.1. General classification

Phenolic compounds constitute the most abundant class of plant secondary metabolites, with more than 8,000 distinct molecules identified, all sharing an aromatic ring bearing hydroxyl substituents (Zhang et al., 2022; Xu & Wang, 2025). They are broadly classified into flavonoids and non-flavonoid polyphenols the latter encompassing phenolic acids, stilbenes, lignans, and tannins (Zhang et al., 2022) deriving from two basic carbon skeletons: The C6-C3-C6 configuration typical of flavonoids, and the simpler C6-C1 configuration typical of hydroxybenzoic acids. Phenolic acids divide into hydroxybenzoic (e.g., gallic acid) and hydroxycinnamic (e.g., caffeic, ferulic, chlorogenic acid) derivatives, which typically occur conjugated rather than free. Stilbenes (e.g., resveratrol) and lignans represent structurally distinct non-flavonoid classes sharing overlapping antioxidant and neuroprotective properties with flavonoids.

2.2. Flavonoid subclasses

Flavonoids share a common C6-C3-C6 diphenylpropane skeleton with two aromatic rings (A, B) connected by a heterocyclic pyran ring (C) (Chen et al., 2023). Based on C-ring oxidation state, they are classified into seven subclasses: flavones (apigenin, luteolin), flavonols (quercetin, kaempferol, myricetin), flavanones

(naringenin, hesperetin), flavan-3-ols (catechins, EGCG), isoflavones (genistein, daidzein), anthocyanins, and chalcones (Chen et al., 2023). Structural differences within each subclass arise from hydroxylation, glycosylation, and methylation patterns that, as discussed in Section 2.3, determine antioxidant capacity, blood brain barrier permeability, and neuroprotective potential.

2.3. Structure activity relationship (SAR)

Neuroprotective and antioxidant potency is governed by specific structural features. The number and position of hydroxyl (-OH) groups are important determinants of antioxidant activity, while structural features such as catechol substitution in the B-ring and conjugation within the C-ring can enhance radical-scavenging capacity. Hydroxyl substitution at C3′/C5′ of ring B and methoxy substitution at C7 of ring A have also been associated with neuroprotective, antioxidant, and anti-inflammatory activity (Preet et al., 2023). Glycosylation can modify flavonoid activity by altering physicochemical properties and the availability of free hydroxyl groups. In terms of CNS availability, certain flavonoids are capable of crossing the blood–brain barrier, although their brain penetration and bioavailability vary considerably depending on their individual physicochemical and pharmacokinetic properties (Hasan et al., 2023). These structure-dependent differences in biological activity, solubility, bioavailability, and CNS penetration are important considerations in the development of flavonoid-based neuroprotective strategies.

3. Molecular Targets in Alzheimer's Pathogenesis

3.1. Amyloid-β aggregation and tau hyperphosphorylation

Aβ is generated through sequential cleavage of APP by β-secretase (BACE1) and γ-secretase, yielding the neurotoxic Aβ42 isoform (Cervellati et al., 2021); these secretases collectively

account for nearly half of the amyloid targeted AD drug pipeline (Maia & Sousa, 2019). Aβ42 aggregates into oligomers, protofibrils, and insoluble fibrils, with oligomers considered the most synaptotoxic species. Aβ accumulation triggers tau hyperphosphorylation via GSK-3β and CDK5, causing tau to detach from microtubules and self aggregate into neurofibrillary tangles. GSK-3β additionally regulates APP cleavage and neuroinflammation, functioning as a convergence node that positions secretase inhibition and GSK-3β/CDK5 modulation as key targets for the antiamyloidogenic and anti tau activities of flavonoids discussed in Section 4.

3.2. Cholinergic dysfunction (AChE/BChE)

The cholinergic hypothesis attributes memory deficits to degeneration of basal forebrain cholinergic neurons projecting to the hippocampus and neocortex, with autopsy studies documenting 70-80% neuronal loss (Liu et al., 2022; Chen & Mobley, 2019). Cholinergic signaling is terminated by acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), whose relative contribution increases as AD progresses a shift underlying the dual inhibition mechanism of donepezil, rivastigmine, and galantamine. Cholinergic neurons also exert antiinflammatory effects via microglial nicotinic receptors, so their degeneration compounds cognitive impairment with loss of this protective tone, making AChE/BChE inhibition a key focus for evaluating flavonoid activity in Section 4.

3.3. Oxidative stress and mitochondrial dysfunction

Oxidative stress arises when reactive oxygen species (ROS) production exceeds antioxidant defenses a condition to which the brain is particularly vulnerable. Mitochondrial dysfunction increases ROS output while ROS damage further impairs mitochondrial function, creating a self amplifying cycle that promotes both Aβ

aggregation and tau hyperphosphorylation (Simakov et al., 2025). The principal endogenous defense is orchestrated by Nrf2, which upregulates cytoprotective genes via the antioxidant response element (ARE) (George & Brown, 2020); Nrf2 function is impaired in AD, making pharmacological or phytochemical Nrf2 activation achieved by numerous flavonoids one of the most pursued therapeutic strategies.

3.4. Neuroinflammation (microglial activation, cytokines)

AD involves innate immune dysregulation in which microglia shift toward a chronically proinflammatory phenotype in response to Aβ and tau aggregates (Wang et al., 2026; Chen et al., 2024). TREM2 variants shape the balance between protective phagocytosis and pathological inflammation, while the NLRP3 inflammasome activated via NF-κB priming drives caspase-1-dependent release of IL-1β and IL-18 (Wang et al., 2026). Sustained NLRP3 activation and elevated TNF-α/IL-6 perpetuate chronic inflammation that reciprocally accelerates amyloid and tau pathology (Chen et al., 2024), positioning the NF-κB/NLRP3/cytokine axis as a major target for the antiinflammatory activity of flavonoids discussed in Section 4.

4. Neuroprotective Mechanisms

4.1. Antioxidant activity (ROS scavenging, Nrf2/ARE pathway)

Flavonoids act through direct radical scavenging and metal chelation, governed by hydroxyl group positioning (Section 2.3). Quercetin reduces intracellular ROS and preserves mitochondrial membrane potential in Aβ exposed neuroblastoma cells (Adnan et al., 2025), and in APP/PS1 mice activates the Keap1/Nrf2/HO-1 pathway, elevating antioxidant enzymes and improving cognitive performance. This Nrf2-inductive capacity extends to other flavonoids hesperetin, for instance, concurrently activates Nrf2

while suppressing NF-κB-driven inflammation, illustrating the mechanistic overlap with antiinflammatory action (Section 4.3).

4.2. Anti amyloidogenic effects

Flavonoids act both enzymatically and through direct peptide interaction. Genistein, syringetin, and tamarixetin inhibit BACE1, while luteolin combines this with upregulation of insulin degrading enzyme, yielding a dual reduction and clearance effect. Biflavonoids such as amentoflavone bind the N-terminal region of Aβ fibrils via π-π stacking, disrupting hydrogen bonds and actively promoting fibril disaggregation (Windsor et al., 2021), with hydroxyl positioning again critical (Section 2.3). Quercetin shows additive BACE1 inhibitory activity when combined with donepezil, suggesting flavonoids may complement rather than replace existing pharmacotherapy (Section 7).

4.3. Anti inflammatory effects (NF-κB inhibition)

Flavonoids suppress neuroinflammation primarily via NF-κB inhibition. Hesperetin reduces nitric oxide and cytokine release in activated microglia while inhibiting NF-κB (Evans et al., 2023); luteolin blocks IκBα degradation and NF-κB p65 nuclear translocation in a human blood brain barrier model exposed to Aβ, reducing COX-2, TNF-α, IL-1β, IL-6, and IL-8 (Zhang et al., 2017). Luteolin and apigenin also inhibit MAPK/AP-1 and STAT1 dependent pathways, directly targeting the NF-κB-NLRP3-cytokine axis identified in Section 3.4.

4.4. Signaling pathway modulation (PI3K/Akt, MAPK, Sirtuins)

Flavonoids modulate intracellular signaling linking survival, apoptosis, and tau phosphorylation. Several activate PI3K/Akt to inhibit downstream GSK-3β, reducing tau hyperphosphorylation while suppressing caspase-3-mediated apoptosis (Calderaro et al.,

2022). Naringenin activates PI3K/Akt/GSK-3β while raising hippocampal SIRT1 and reducing NF-κB translocation; eriodictyol similarly inhibits MAPK/PI3K/Akt while activating SIRT1 demonstrating that these pathways rarely act in isolation and that MAPK-family inhibition offers an additional route to reducing tauopathy (Section 3.1).

4.5. Effects on neurogenesis and synaptic plasticity

Flavonoids promote hippocampal neurogenesis and plasticity via the ERK/CREB/BDNF axis alongside PI3K/Akt signaling (Bakoyiannis et al., 2019). BDNF/TrkB signaling, reduced in the AD hippocampus, supports neurogenesis and synaptic plasticity; flavonoids enhance hippocampal long term potentiation and increase BDNF, synaptotagmin-1, and phosphorylated CREB alongside improved learning and memory performance complementing the antioxidant, anti amyloidogenic, and antiinflammatory mechanisms described above.

5. Key Compounds and Evidence

Quercetin remains the most extensively studied flavonoid, though robust human trial data are limited. Resveratrol acts principally through SIRT1 activation, inhibiting Aβ aggregation and NF-κB signaling (Puranik et al., 2025); a 52-week phase 2 RCT found it safe and well tolerated, measurably altering CSF/plasma Aβ biomarker trajectories, though cognitive effects were inconclusive (Turner et al., 2015). Curcumin disrupts Aβ aggregation and suppresses NF-κB/MAPK signaling; clinical evidence is mixed one 2025 meta analysis (9 RCTs, n = 501) found significant global cognitive improvement at 0.8 g/day (Wang et al., 2025), while another found consistent rodent benefits but no significant human effect (Yu et al., 2025), a discrepancy attributable to poor oral bioavailability (Section 6). EGCG shows strong anti amyloidogenic and antioxidant activity in vitro but similarly limited *in vivo* stability.

This pattern strong preclinical efficacy contrasted with modest clinical benefit is addressed directly in Section 6.

6. Bioavailability and Pharmacokinetic Limitations

Clinical translation is constrained by poor intestinal absorption, extensive first pass metabolism, and limited blood brain barrier penetration (Kumbhar et al., 2025). Curcumin and resveratrol undergo rapid glucuronidation/sulfation, yielding low bioavailability and short half lives that likely explain their inconsistent clinical outcomes (Section 5); the BBB itself further restricts hydrophilic glycosides more than lipophilic aglycones (Section 2.3). Nanotechnology based delivery liposomes, solid lipid nanoparticles, PLGA nanoparticles, inorganic nanosystems, and biomimetic carriers has become the dominant strategy for overcoming these barriers (Kumbhar et al., 2025); PLGA encapsulated ellagic acid, for example, achieved a 3.7-fold increase in brain concentration, improving antioxidant activity and cognitive outcomes in an AD rat model. Self nanoemulsifying systems (SNEDDS) and intranasal formulations offer additional non invasive routes bypassing first pass metabolism, widely regarded as prerequisites for realizing the clinical potential of the mechanisms reviewed in Sections 4-5.

7. Clinical Trials and Future Perspectives

Phenolic compounds and flavonoids remain underrepresented in registered clinical trials relative to their mechanistic promise, with existing studies limited by small samples, short duration, and heterogeneous outcomes (Kruszka et al., 2025). A phase II RCT of a Flos Gossypii flavonoid tablet in mild to moderate AD was completed in 2024 (ClinicalTrials.gov, NCT05269173), and a polyphenol rich nutraceutical containing over 119 compounds showed cognitive benefits over two 16 week periods (Chen et al., 2025) suggesting complex mixtures may complement single compound trials. Flavonoids also show synergistic potential

combined with cholinesterase inhibitors, pointing toward adjunctive rather than replacement positioning (Kruszka et al., 2025). Future priorities include longer disease modifying trials, biomarker guided (e.g., APOE genotype) stratification, dosing standardization, and continued nanoformulation development (Section 6) to bridge the gap between preclinical efficacy and clinical benefit.

8. Conclusion

AD's amyloidogenic, tauopathic, cholinergic, oxidative, and neuroinflammatory pathways are increasingly understood as interconnected. Against existing symptomatic or high risk pharmacotherapies, phenolic compounds and flavonoids offer a distinctive multitarget profile spanning antioxidant, antiamyloidogenic, antiinflammatory, and proneurogenic mechanisms. Compounds such as quercetin, resveratrol, curcumin, and EGCG show robust preclinical efficacy, but clinical translation remains constrained by poor bioavailability and limited BBB penetration a gap that emerging nanoformulation strategies are working to close. Realizing their therapeutic potential will require larger, longer, and more rigorously designed trials, biomarker guided patient selection, and standardized formulations. Positioned as multi target adjuncts rather than replacements for existing pharmacotherapy, phenolic compounds and flavonoids represent a scientifically compelling avenue for future Alzheimer's disease research and care.

References

Adnan, M., Siddiqui, A. J., Bardakci, F., Surti, M., Badraoui, R., & Patel, M. (2025). Neuroprotective potential of quercetin in Alzheimer's disease: Targeting oxidative stress, mitochondrial dysfunction, and amyloid-β aggregation. *Frontiers in Pharmacology, 16*, 1593264. https://doi.org/10.3389/fphar.2025.1593264

Arnsten, A. F. T., Del Tredici, K., Barthélemy, N. R., Gabitto, M., van Dyck, C. H., Lein, E., Braak, H., & Datta, D. (2025). An integrated view of the relationships between amyloid, tau, and inflammatory pathophysiology in Alzheimer's disease. *Alzheimer's & Dementia, 21*(8), e70404. https://doi.org/10.1002/alz.70404

Bakoyiannis, I., Daskalopoulou, A., Pergialiotis, V., & Perrea, D. (2019). Phytochemicals and cognitive health: Are flavonoids doing the trick? *Biomedicine & Pharmacotherapy, 111*, 646–654. https://doi.org/10.1016/j.biopha.2018.12.105

Calderaro, A., Patanè, G. T., Tellone, E., Barreca, D., Ficarra, S., Misiti, F., & Laganà, G. (2022). The neuroprotective potentiality of flavonoids on Alzheimer's disease. *International Journal of Molecular Sciences, 23*(23), 14835. https://doi.org/10.3390/ijms232314835

Cervellati, C., Valacchi, G., & Zuliani, G. (2021). BACE1: From biomarker to Alzheimer's disease therapeutical target. *Aging, 13*(9), 12299–12300. https://doi.org/10.18632/aging.203064

Chen, G., Su, Y., Chen, S., Lin, T., & Lin, X. (2025). Polyphenols and Alzheimer's disease: A review on molecular and therapeutic insights with in silico support. *Food Science & Nutrition, 13*(9), e70496. https://doi.org/10.1002/fsn3.70496

Chen, H., Zeng, Y., Wang, D., Li, Y., Xing, J., Zeng, Y., Liu, Z., Zhou, X., & Fan, H. (2024). Neuroinflammation of microglial

regulation in Alzheimer's disease: Therapeutic approaches. *Molecules, 29*(7), 1478. https://doi.org/10.3390/molecules29071478

Chen, S., Wang, X., Cheng, Y., Gao, H., & Chen, X. (2023). A review of classification, biosynthesis, biological activities and potential applications of flavonoids. *Molecules, 28*(13), 4982. https://doi.org/10.3390/molecules28134982

Chen, X.-Q., & Mobley, W. C. (2019). Exploring the pathogenesis of Alzheimer disease in basal forebrain cholinergic neurons: Converging insights from alternative hypotheses. *Frontiers in Neuroscience, 13*, 446. https://doi.org/10.3389/fnins.2019.00446

ClinicalTrials.gov. (2024). *Efficacy and safety of Flos Gossypii flavonoids tablet in the treatment of Alzheimer's disease (NCT05269173).* U.S. National Library of Medicine. https://clinicaltrials.gov/study/NCT05269173

Evans, J. A., Mendonca, P., & Soliman, K. F. A. (2023). Involvement of Nrf2 activation and NF-κB pathway inhibition in the antioxidant and anti-inflammatory effects of hesperetin in activated BV-2 microglial cells. *Brain Sciences, 13*(8), 1144. https://doi.org/10.3390/brainsci13081144

George, S., & Brown, D. R. (2020). NRF2 as a therapeutic target in neurodegenerative diseases. *ASN Neuro, 12*, 1759091419899782. https://doi.org/10.1177/1759091419899782

Goswami, S., Masurkar, P. P., & Sherer, J. T. (2026). Donanemab and lecanemab in Alzheimer's disease treatment: A narrative review of clinical trials and discussion of implications for patient access. *Equity Neuroscience, 2*(2), 100057. https://doi.org/10.1016/j.neuros.2026.100057

Hasan, S., Khatri, N., Rahman, Z. N., Menezes, A. A., Martini, J., Shehjar, F., Mujeeb, N., & Shah, Z. A. (2023).

Neuroprotective potential of flavonoids in brain disorders. *Brain Sciences, 13*(9), 1258. https://doi.org/10.3390/brainsci13091258

Hein, Z. M., Karikalan, B., Gopalakrishna, P. K., Dhevi, K., Alkatiri, A., Hussan, F., Mohd Moklas, M. A., Jagadeesan, S., Che Ramli, M. D., Che Mohd Nassir, C. M. N., & Vishnumukkala, T. (2026). Toward a unified framework in molecular neurobiology of Alzheimer's disease: Revisiting the pathophysiological hypotheses. *Molecular Neurobiology, 63*, 282. https://doi.org/10.1007/s12035-025-05602-0

Kruszka, J., Martyński, J., Szewczyk-Golec, K., Woźniak, A., & Nuszkiewicz, J. (2025). The role of selected flavonoids in modulating neuroinflammation in Alzheimer's disease: Mechanisms and therapeutic potential. *Brain Sciences, 15*(5), 485. https://doi.org/10.3390/brainsci15050485

Kumbhar, P. S., Kolekar, K. A., Vishwas, S., Kamble, V., Bashir, B., Patil, K. S., Bhagwat, D., Gupta, G., Prasher, P., Singh, M., Singh, S. K., Disouza, J., & Patravale, V. B. (2025). Plant-based flavonoids and their nanoparticles: Latest arsenal against Alzheimer's disease. *Drug Delivery and Translational Research, 15*(12), 4411–4451. https://doi.org/10.1007/s13346-025-01906-9

Liu, S., Wu, G., Ma, X., Xu, K., Lan, X., Wang, L., Li, H., Gu, D., Wang, M., & Liu, J. (2026). Trends and cross-country inequalities in the global burden of Alzheimer's disease and other dementias among adults aged 65+ years, 1990–2021: A population-based study with projections into 2050. *Frontiers in Aging Neuroscience, 17*, 1683158. https://doi.org/10.3389/fnagi.2025.1683158

Liu, W., Li, J., Yang, M., Ke, X., Dai, Y., Lin, H., Wang, S., Chen, L., & Tao, J. (2022). Chemical genetic activation of the cholinergic basal forebrain hippocampal circuit rescues memory loss

in Alzheimer's disease. *Alzheimer's Research & Therapy, 14*(1), 53. https://doi.org/10.1186/s13195-022-00994-w

Maia, M. A., & Sousa, E. (2019). BACE-1 and γ-secretase as therapeutic targets for Alzheimer's disease. *Pharmaceuticals, 12*(1), 41. https://doi.org/10.3390/ph12010041

Preet, G., Haj Hasan, A. W., Jaspars, M., Ramlagan, P., Fawdar, S., & Boulle, F. (2023). Anti-neurodegenerating activity: Structure–activity relationship analysis of flavonoids. *Molecules, 28*(20), 7188. https://doi.org/10.3390/molecules28207188

Puranik, N., Kumari, M., Tiwari, S., Dhakal, T., & Song, M. (2025). Resveratrol as a therapeutic agent in Alzheimer's disease: Evidence from clinical studies. *Nutrients, 17*(15), 2557. https://doi.org/10.3390/nu17152557

Simakov, A., Chhor, S., Ismaili, L., & Martin, H. (2025). Nrf2 activation and antioxidant properties of chromone-containing MTDLs for Alzheimer's disease treatment. *Molecules, 30*(9), 2048. https://doi.org/10.3390/molecules30092048

Turner, R. S., Thomas, R. G., Craft, S., van Dyck, C. H., Mintzer, J., Reynolds, B. A., Brewer, J. B., Rissman, R. A., Raman, R., Aisen, P. S., & Alzheimer's Disease Cooperative Study. (2015). A randomized, double-blind, placebo-controlled trial of resveratrol for Alzheimer disease. *Neurology, 85*(16), 1383–1391. https://doi.org/10.1212/WNL.0000000000002035

Twiss, E., McPherson, C., & Weaver, D. F. (2025). Global diseases deserve global solutions: Alzheimer's disease. *Neurology International, 17*(6), 92. https://doi.org/10.3390/neurolint17060092

Wang, T., Liu, X., Wang, X., Hua, F., & Yan, L. (2026). TREM2 and microglial immunity in Alzheimer's disease: Mechanisms, genetics, and therapeutic opportunities. *Frontiers in*

Immunology, 17, 1739875. https://doi.org/10.3389/fimmu.2026.1739875

Wang, W., Zhao, R., Liu, B., & Li, K. (2025). The effect of curcumin supplementation on cognitive function: An updated systematic review and meta-analysis. *Frontiers in Nutrition, 12*, 1549509. https://doi.org/10.3389/fnut.2025.1549509

Windsor, P. K., Plassmeyer, S. P., Mattock, D. S., Bradfield, J. C., Choi, E. Y., Miller, B. R., & Han, B. H. (2021). Biflavonoid-induced disruption of hydrogen bonds leads to amyloid-β disaggregation. *International Journal of Molecular Sciences, 22*(6), 2888. https://doi.org/10.3390/ijms22062888

Xu, L., & Wang, X. (2025). A comprehensive review of phenolic compounds in horticultural plants. *International Journal of Molecular Sciences, 26*(12), 5767. https://doi.org/10.3390/ijms26125767

Xu, L., Wang, Z., Li, M., & Li, Q. (2025). Global incidence trends and projections of Alzheimer disease and other dementias: An age-period-cohort analysis 2021. *Journal of Global Health, 15*, 04156. https://doi.org/10.7189/jogh.15.04156

Yu, L., Li, N., Li, B., Ye, K. X., Guo, J., Shan, J., Cao, L., Song, M., Wang, Y., Lee, T.-S., Maier, A. B., & Feng, L. (2025). Targeting cognitive aging with curcumin supplementation: A systematic review and meta-analysis. *The Journal of Prevention of Alzheimer's Disease, 12*(8), 100248. https://doi.org/10.1016/j.tjpad.2025.100248

Zhang, J.-X., Xing, J.-G., Wang, L.-L., Jiang, H.-L., Guo, S.-L., & Liu, R. (2017). Luteolin inhibits fibrillary β-amyloid1-40-induced inflammation in a human blood-brain barrier model by suppressing the p38 MAPK-mediated NF-κB signaling pathways. *Molecules, 22*(3), 334. https://doi.org/10.3390/molecules22030334

Zhang, Y., Cai, P., Cheng, G., & Zhang, Y. (2022). A brief review of phenolic compounds identified from plants: Their extraction, analysis, and biological activity. *Natural Product Communications, 17*(1), 1–14. https://doi.org/10.1177/1934578X211069721

CHAPTER 10

CURRENT THERAPEUTIC APPROACHES AND EMERGING STRATEGIES FOR ALZHEIMER'S DISEASE

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1. Introduction

Alzheimer's disease (AD) represents a major and growing global health challenge, currently affecting an estimated 55 to 57 million individuals worldwide (Bhardwaj, 2025; Dib et al., 2022; Johnston et al., 2023; Ma et al., 2022; Patel et al., 2023), with epidemiological projections suggesting this prevalence could exceed 139 million by 2050 (Bhardwaj, 2025; Gudla et al., 2025; Johnston et al., 2023). In addition to its severe clinical impact, the economic burden is substantial, generating global healthcare and caregiving costs that reached approximately US$1 trillion annually and are forecast to double by 2030 (Dib et al., 2022; A. M. Saunders et al., 2021; S. Saunders et al., 2021). Despite this growing societal and economic challenge, conventional FDA-approved pharmacological interventions continue to provide primarily symptomatic management rather than halting underlying neurodegeneration.

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Clinical literature repeatedly highlights that acetylcholinesterase inhibitors and memantine are constrained by modest therapeutic efficacy, common adverse effects, and an inability to stop disease progression (Chen et al., 2025; Mumtaz et al., 2025; Passeri et al., 2022; Tutubala et al., 2025). Furthermore, targeting a single biological pathway is increasingly viewed as inadequate given the multifactorial biology of AD (Chen et al., 2025; Hossain & Hussain, 2025). Although newer anti-amyloid therapies mark progress in clinical management, their modest effect sizes have accelerated interest in complementary targets (B.-F. Li et al., 2026; Zhang et al., 2024).

Consequently, current therapeutic strategies are expanding from single-target or amyloid-exclusive frameworks toward multi-target interventions that address neuroinflammation, tau pathology, synaptic failure, and epigenetic dysregulation (Hossain & Hussain, 2025; B.-F. Li et al., 2026; Tutubala et al., 2025; Zhang et al., 2024). Because AD operates through complex pathological networks, single-drug strategies are being replaced by multi-target drug design, polypharmacology, and combination approaches (Guo et al., 2023; Hossain & Hussain, 2025; B.-F. Li et al., 2026). Within this expanded scope, neuroinflammation is a central focus, particularly pathways involving microglia, TREM2-APOE, CD33, and the NLRP3 inflammasome (B.-F. Li et al., 2026; Wang et al., 2025). Additionally, neuroepigenetic strategies, including microRNA regulation of inflammasome pathways and BDNF-focused epigenetic editing, offer novel mechanisms to alter disease mechanisms (Saad et al., 2026; Toader et al., 2025).

A primary barrier to implementing these advanced molecular interventions is the limited permeability of the blood-brain barrier. To overcome these delivery limitations, engineered nanoplatforms such as functionalized nanoparticles, exosomes, liposomes, polymeric carriers, and biomimetic systems are developed to protect

therapeutic cargo and target brain tissue selectively (Le et al., 2026; B.-F. Li et al., 2026; Wang et al., 2025; Zhang et al., 2025). Preclinical evaluations indicate that targeted nanocarriers, including co-delivery platforms and nucleic acid delivery systems, enhance brain uptake, reduce amyloid and tau pathology, and suppress neuroinflammation in animal models (Bian et al., 2025; Chen et al., 2025; Gao et al., 2025; Guo et al., 2023; Yang et al., 2022). Despite strong preclinical evidence, significant translation bottlenecks persist, including long-term safety concerns, complex manufacturing standards, and limited clinical trial validation (Kochman et al., 2025; Le et al., 2026; Tutubala et al., 2025; Zhang et al., 2025).

2. Current FDA-Approved Therapies

Current FDA-approved pharmacological treatments for AD are broadly categorized into symptomatic therapies and disease-modifying therapies. Symptomatic treatments are primarily utilized to mitigate cognitive decline and improve daily functioning by addressing neurotransmitter imbalances (Khaled & Hazar-Yavuz, 2025; Khalefa et al., 2024; Varadharajan et al., 2023). These symptomatic drugs are categorized into two main classes: acetylcholinesterase inhibitors and NMDA receptor antagonists (Jiang, 2023; Khaled & Hazar-Yavuz, 2025; Vishwas et al., 2023). In contrast, disease-modifying therapies, which are primarily monoclonal antibodies, target the underlying biological pathology, specifically the accumulation of amyloid-beta plaques. These targeted therapies aim to alter the progression of the disease rather than solely alleviating symptoms (Geldmacher, 2024; Khaled & Hazar-Yavuz, 2025; Thawabteh et al., 2024). Additionally, beyond cognitive decline, the FDA has approved specific pharmacological agents to manage the behavioral and psychological symptoms of dementia that often accompany the progression of the disease (Geldmacher, 2024; Thangwaritorn et al., 2024; Varadharajan et al.,

2023). The subsequent subsections explore these primary therapeutic categories in detail.

2.1. Symptomatic treatments

Currently, FDA-approved symptomatic treatments for AD primarily address cognitive and functional decline without altering the underlying disease pathology (Buccellato et al., 2023; Morató et al., 2022; Qi et al., 2024; Zhang et al., 2024). These pharmacological interventions predominantly include acetylcholinesterase (AChE) inhibitors and the N-methyl-D-aspartate (NMDA) receptor antagonist, memantine, which provide small-to-moderate, stage-dependent symptomatic benefits (Qi et al., 2024; Zhang et al., 2024).

AChE inhibitors, such as donepezil, galantamine, and rivastigmine, demonstrate modest efficacy in improving cognitive function, particularly in mild-to-moderate AD (Gao et al., 2024; Moreta et al., 2021). Among these, donepezil frequently exhibits the clearest cognitive signal (Veroniki et al., 2022), whereas transdermal rivastigmine is often favored for mitigating gastrointestinal adverse events commonly associated with oral administration (Zhang et al., 2024). Despite their clinical utility, the symptomatic benefits of AChE inhibitors are inherently temporary; their efficacy diminishes progressively as cholinergic neurons are lost during disease advancement. Furthermore, their sustained use is often limited by cholinergic adverse effects, including nausea, vomiting, bradycardia, and syncope (Morató et al., 2022; Moreta et al., 2021).

Conversely, memantine provides symptomatic relief primarily in moderate-to-severe AD, showing minimal clinical benefit in mildly affected patients (Nguyen et al., 2021; Veroniki et al., 2022; Zhang et al., 2024). For advanced disease stages, combination therapy (e.g., donepezil plus memantine) tends to outperform monotherapy in managing cognitive and global functional decline without significantly increasing the side-effect

burden (Kim et al., 2024; Morató et al., 2022). However, the long-term optimal sequencing and cessation rules for these combinations remain underexplored (Lista et al., 2022; Morató et al., 2022).

Ultimately, the defining limitation of both AChE inhibitors and memantine is their inability to modify the disease course or biological markers of AD (Peng et al., 2023; Qi et al., 2024; Zhang et al., 2024). They offer temporary stabilization of symptoms rather than a reversal of neurodegeneration, underscoring the critical necessity for disease-modifying strategies (Peng et al., 2023).

2.2. Disease-modifying therapies (DMTs)

In recent years, the therapeutic landscape of AD has shifted from purely symptomatic management to disease-modifying therapies (DMTs) targeting core neuropathological hallmarks. Among these, anti-amyloid-beta monoclonal antibodies have emerged as the primary breakthrough class, achieving accelerated and full regulatory approvals based on their ability to clear cerebral amyloid plaques and modestly decelerate clinical cognitive decline.

Lecanemab: Lecanemab was FDA-approved in 2023 for AD. The pivotal trial enrolled individuals with mild cognitive impairment or mild dementia due to AD with confirmed amyloid pathology (U.S. Food and Drug Administration [FDA], 2025; van Dyck et al., 2023). At 18 months, clinical outcomes indicated that lecanemab modestly slowed clinical decline. The Clinical Dementia Rating-Sum of Boxes (CDR-SB) worsened by 1.21 points on lecanemab versus 1.66 on placebo, demonstrating a difference of 0.45 points in favor of the treatment. Furthermore, other cognitive and functional endpoints, as well as amyloid PET, favored lecanemab (van Dyck et al., 2023). Regarding adverse effects, the main safety issue is amyloid-related imaging abnormalities (ARIA). Lecanemab carries a boxed warning and requires MRI monitoring before treatment and during early infusions. Common adverse

reactions include infusion reactions, ARIA-H (microhemorrhages), ARIA-E (effusion), and headache. Specifically, in the CLARITY-AD trial, infusion reactions occurred in 26.4% of patients, and ARIA-E occurred in 12.6% (U.S. Food and Drug Administration [FDA], 2025).

Donanemab: In 2024, donanemab received FDA approval for AD. The TRAILBLAZER-ALZ 2 trial enrolled patients with early symptomatic AD who had both amyloid and tau pathology (Sims et al., 2023; U.S. Food and Drug Administration [FDA], 2025). Efficacy results showed that donanemab also slowed clinical decline modestly. In the subgroup with low/medium tau, the effect was larger, with the iADRS decline differing by 3.25 points and the CDR-SB differing by 0.67 points versus placebo. Additionally, plaque clearance was substantial, and 23 of 24 gated outcomes were statistically significant (Sims et al., 2023). The safety profile of donanemab also includes a boxed warning for ARIA, necessitating MRI monitoring early in the treatment. In the TRAILBLAZER-ALZ 2 trial, ARIA-E occurred in 24.0% of treated patients, ARIA-H was observed in 31.4%, and infusion-related reactions occurred in 8.7%. Furthermore, three deaths in the trial were considered treatment-related (Sims et al., 2023; U.S. Food and Drug Administration [FDA], 2025).

Aducanumab: While agents such as lecanemab and donanemab show consistent efficacy signals, the efficacy of aducanumab remains mixed and controversial (Alkhalifa et al., 2025; Avgerinos et al., 2024; Mostafa et al., 2025). Although aducanumab demonstrated the ability to reduce amyloid plaques, its cognitive results were inconsistent across clinical trials. This clinical inconsistency drove major regulatory controversy, which ultimately led to the drug's eventual withdrawal and discontinuation (Avgerinos et al., 2024; Chundu et al., 2025; Mostafa et al., 2025).

2.3. Overall Biomarker Responses and Safety Limitations

Overall, while FDA-approved anti-amyloid monoclonal antibodies reliably clear amyloid plaques and demonstrate strong target engagement, their clinical benefits are often modest and appear most effective primarily during early disease stages (Alkhalifa et al., 2025; Avgerinos et al., 2021; Cummings, 2025; Tonegawa-Kuji et al., 2025; Wei & Li, 2025; Wu et al., 2023). Furthermore, the benefit-harm balance of these therapies remains significantly constrained by limited clinical effect sizes, uncertainty regarding long-term meaningful benefits, and nontrivial ARIA toxicity, which is particularly elevated in APOE ε4 carriers (Alkhalifa et al., 2025; Shim et al., 2025). These persistent challenges, alongside the requirement for rigorous MRI monitoring and careful risk management to reduce serious adverse events, underscore the urgent need for novel therapeutic paradigms and advanced delivery mechanisms capable of enhancing clinical efficacy while mitigating severe toxicities (Alkhalifa et al., 2025; Cummings, 2025; Cummings et al., 2023).

3. Emerging Strategies and Novel Target Interventions

Current literature indicates a fundamental paradigm shift in AD pharmacotherapy, moving beyond the traditional amyloid-centric model. Although recent anti-amyloid agents, such as lecanemab and donanemab, improve plaque clearance, their overall clinical utility remains constrained by amyloid-related imaging abnormalities (ARIA). Consequently, the field is transitioning from monotherapies toward multi-target cocktails and precision neuropharmacology, recognizing AD as a complex network pathology (Hafez et al., 2026).

A prominent non-amyloid therapeutic axis is neuroinflammation, with microglial and astrocytic dysfunction recognized as key drivers of pathogenesis (Kamila et al., 2025).

Interventions increasingly target specific neuroimmune nodes, including TREM2, CD33, NLRP3, IL-1β, TNF-α, and CB2 pathways (Kamila et al., 2025). Among these, TREM2 is particularly significant as it links innate immunity to genetic risk and microglial phagocytosis (Wang et al., 2026). Additionally, the NLRP3 inflammasome represents a convergent target associated with amyloid-beta, tau pathology, reactive oxygen species, and pyroptosis (Li & Gong, 2025).

Parallel to neuroimmune modulation, neuroepigenetics represents a major emerging therapeutic avenue. Reversible regulators of Alzheimer's-relevant gene expression (such as DNA methylation, histone modifications, histone deacetylases [HDACs], BET proteins, and microRNAs) are critical mechanisms of disease progression and potential therapeutic targets. Within this domain, HDAC inhibition possesses the most developed mechanistic rationale. Preclinical models demonstrate that HDAC inhibitors reduce amyloid and tau pathologies while attenuating neuroinflammation; however, clinical translation remains limited by challenges related to target selectivity and blood-brain barrier penetrance (Ma et al., 2023).

To address the multifactorial nature of the disease, polypharmacology has emerged as a central design philosophy. Recent drug development strategies emphasize multi-target-directed ligands (MTDLs) and hybrid scaffolds that combine cholinesterase inhibition, anti-amyloid activity, antioxidant effects, metal chelation, and inflammatory-pathway modulation within a single molecule. Prominent examples of these structurally integrated agents include donepezil-based hybrids, coumarin-based hybrids, and chalcone-rivastigmine scaffolds (Angelova et al., 2024). Ultimately, the therapeutic landscape is evolving into a tripartite strategy: reprogramming microglial biology, modulating epigenetic control of inflammatory and synaptic programs and developing chemically

tractable multi-target agents. However, the clinical success of these sophisticated targets ultimately depends on their capacity to cross the blood-brain barrier, making advanced delivery platforms an essential pillar of this evolving therapeutic landscape.

3.1. Advanced drug delivery systems

The blood-brain barrier remains the most formidable anatomical and physiological obstacle in AD pharmacotherapy, preventing the central nervous system entry of most conventional drugs and nearly all large-molecule biologics. To overcome this restriction, current research focuses on three primary advanced delivery strategies: nanoparticle-based transport, anatomical bypass via intranasal administration, and physical barrier permeabilization.

3.1.1. Nanocarrier-based transport systems

Nanotechnology provides highly versatile platforms designed to encapsulate, protect, and transport therapeutic agents across the blood-brain barrier. Current nanocarrier systems are broadly classified into lipid-based architectures including liposomes and solid lipid nanoparticles, polymeric matrices, dendrimers, exosomes, and inorganic platforms (J. Li et al., 2026; Roamcharern & Yubolphan, 2026; Satyanarayana et al., 2026). These systems facilitate brain entry primarily through receptor-mediated transcytosis by functionalizing the nanoparticle surface with specific ligands that bind to endogenous endothelial receptors.

The transferrin receptor 1 (TfR1) and the low-density lipoprotein receptor-related protein 1 (LRP1) are among the most validated targets for this transport mechanism (Shen et al., 2025). For instance, LRP1-targeted nanoparticles utilizing Angiopep-2 and lactoferrin-functionalized lipid carriers have demonstrated superior brain targeting and significant reduction of amyloid pathology in preclinical models (Pan et al., 2025; Xu et al., 2025). By utilizing these pathways, nanocarriers protect fragile therapeutic cargos, such

as peptides, small interfering RNA, and bispecific antibodies, from premature degradation in systemic circulation (Sumbria & Boado, 2026). Furthermore, recent carrier designs incorporate stimuli-responsive elements that trigger drug release selectively in the presence of pathological cues, such as altered pH levels, reactive oxygen species, or specific enzymes present in the neurodegenerative microenvironment (J. Li et al., 2026). Despite strong preclinical efficacy, clinical translation remains constrained by challenges regarding scalable manufacturing, receptor saturation, endogenous ligand competition, and intracellular trafficking (J. Li et al., 2026; Shen et al., 2025).

3.1.2. Anatomical bypass: ıntranasal delivery

Beyond systemic administration, intranasal delivery provides a direct anatomical route to bypass the blood-brain barrier. Rather than relying on systemic circulation, this approach utilizes the unique structural connections of the olfactory and trigeminal neural pathways, which link the nasal mucosa directly to the central nervous system (Hanson & Frey, 2008; Sood et al., 2014; C. Y. J. Wong et al., 2024). Mechanistically, drugs deposited on the nasal epithelium migrate along extracellular and perineural corridors surrounding these nerves, allowing therapeutic agents to reach central targets while minimizing systemic exposure and hepatic metabolism (C. Y. J. Wong et al., 2024; Xiao et al., 2013). This direct neural routing is particularly advantageous for AD because the olfactory system connects directly to memory-related brain regions, such as the entorhinal cortex and hippocampus. Preclinical and clinical studies demonstrate that biotherapeutics, including intranasal insulin and targeted anti-amyloid-beta antibodies, can enter the brain through these neural pathways to preserve cognitive function and attenuate regional metabolic deficits (C. Y. J. Wong et al., 2024; Xiao et al., 2013). However, the therapeutic translational efficiency of intranasal administration is constrained by biological

and anatomical hurdles, including rapid mucociliary clearance, local enzymatic degradation, tight epithelial junctions, and variable nasal cavity geometry (C. Y. J. Wong et al., 2024).

3.1.3. Physical permeabilization: Focused ultrasound

A more recent structural advancement in overcoming the blood-brain barrier involves physical permeabilization using low-intensity focused ultrasound combined with intravenous microbubbles. When targeted with precise acoustic pressure, the injected microbubbles undergo stable, non-linear oscillation within the cerebral microvasculature. This mechanical stress temporarily and locally disrupts the barrier through a combination of tight-junction loosening and enhanced caveolae-mediated transcellular transport (Deng et al., 2011; Tung et al., 2011). The critical safety parameter in this mechanism involves maintaining acoustic pressures strictly below the threshold for inertial cavitation to prevent vascular and tissue injury. Therefore, continuous cavitation monitoring and precise control over microbubble size relative to capillary diameter are essential to ensure the procedure remains in the safer oscillatory regime (Tung et al., 2011).

In the context of AD, this physical permeabilization approach has successfully transitioned to early-stage clinical evaluation. First-in-human and subsequent Phase I trials have demonstrated that MR-guided focused ultrasound can safely, reversibly, and repeatedly open the blood-brain barrier in targeted regions, with the barrier typically closing within 24 to 48 hours without serious procedure-related adverse events. While current clinical data strongly establish the safety and feasibility of this permeabilization strategy, its ultimate therapeutic impact on cognitive decline or amyloid clearance remains exploratory and requires validation in larger efficacy trials (Lipsman et al., 2018).

3.2. Epigenetic modulations and gene therapy

While preclinical research continues to identify novel molecular targets, the clinical landscape for epigenetic and gene therapy interventions in AD remains in early developmental stages. Current literature indicates that while these approaches demonstrate significant biological activity in experimental models, compelling evidence of human disease modification has not yet been established.

Epigenetic modulation represents the broadest, yet least mature, therapeutic umbrella. Although emerging literature frames epigenetic reprogramming as a highly promising strategy to influence AD biology, translating these interventions from the bench to the bedside requires overcoming substantial pharmacological hurdles (Liu et al., 2018). Within this category, histone deacetylase (HDAC) inhibitors constitute the most well-defined drug class. In various animal models, HDAC inhibitors successfully improve cognitive function and attenuate amyloid and tau-related pathologies. However, successful outcomes in classical transgenic models have rarely translated into clinical efficacy, with recent reviews consistently highlighting target selectivity and blood-brain barrier penetrance as the primary limiting factors for this drug class (Yang et al., 2017).

In parallel to epigenetic modulation, precision gene editing and gene therapies are advancing rapidly in preclinical settings. The CRISPR-Cas9 system has demonstrated powerful proof-of-concept efficacy in modifying disease progression in laboratory models. For instance, an APOE4 allele-specific knockout study achieved approximately a 70% reduction in APOE4 protein levels without altering the neuroprotective APOE3 isoform (Lu et al., 2021). Systematic reviews further summarize that these CRISPR-mediated interventions can significantly reduce amyloid-beta deposition, tau phosphorylation, and neuronal loss; nevertheless, effective delivery

across the blood-brain barrier remains the principal obstacle for clinical application (Gupta & Chaudhuri, 2026).

Among these novel interventions, APOE4-targeted gene therapy is currently the most clinically advanced approach, albeit exclusively concerning early safety and feasibility profiles (Safieh et al., 2019). For example, LX1001, an intrathecal AAVrh.10-based hAPOE2 gene therapy, is currently undergoing Phase I/II evaluation in APOE4 homozygote patients. These initial trials (NCT03634007 and NCT05400330) are primarily designed to assess procedural safety and determine whether the cerebrospinal fluid isoform profile shifts from APOE4 toward an APOE2/APOE4 composition, with long-term follow-up focused on establishing baseline safety rather than definitive cognitive efficacy (U.S. National Library of Medicine, 2018, 2022).

In summary, if therapeutic efficacy is defined by the attenuation of cognitive decline or the modification of clinical biomarker progression, none of these four strategies have established clinical efficacy. Mechanistically, CRISPR and APOE4-directed strategies exhibit the most potent biological activity in current models, whereas HDAC inhibitors possess the longest experimental track record but currently face the weakest prospects for immediate clinical translation.

3.3. Neuroinflammation and ımmune system modulation

In recent years, the therapeutic paradigm regarding neuroinflammation has shifted from generalized immunosuppression toward the targeted reprogramming of immune cells. The objective is no longer to suppress microglia wholesale, but rather to shift their phenotype into a more phagocytic, metabolically competent state capable of engulfing amyloid plaques and mitigating neurotoxicity.

Among current neuroimmune interventions, TREM2 agonism exhibits the highest degree of translational maturity. First-in-human studies of the TREM2 agonistic antibody AL002 demonstrated central nervous system target engagement, characterized by a dose-dependent reduction of cerebrospinal fluid sTREM2 alongside parallel increases in TREM2 signaling and microglial recruitment, which subsequently led to a Phase II trial involving early AD patients (Long et al., 2024). Furthermore, small-molecule interventions, such as MTX46943, have shown that chronic administration induces microglial reprogramming and reduces neurotoxic amyloid-beta species in pathological models (Polydoro et al., 2025).

Parallel to TREM2 agonism, targeting CD33 represents a compelling "brake-release" strategy, as CD33 functions as an inhibitory checkpoint on microglial activation. Preclinical evidence demonstrates that downregulating CD33 via viral vectors (e.g., AAV-miRCD33) lowers soluble amyloid-beta levels and reduces the expression of pro-inflammatory genes, including TNF-α and Ccl2. Similarly, utilizing antibodies such as HuM195 or its single-chain variable fragment in human microglia and monocytes promotes CD33 degradation, increases amyloid-beta phagocytosis, and stimulates IL-33 secretion, thereby facilitating microglial reprogramming (E. Wong et al., 2024). Despite its mechanistic attractiveness, CD33-targeted therapies remain largely confined to the preclinical stage.

To interrupt the pathogenic feed-forward cycle of the inflammasome and pyroptosis, NLRP3 inhibition has emerged as a robust anti-inflammatory axis. In transgenic models, specific NLRP3 inhibitors, such as JC-124, have successfully attenuated microglial activation, reduced amyloid-beta deposition, and diminished overall inflammasome activation (Yin et al., 2018). Subsequent research underscores that chronic inhibition of NLRP3

enhances microglial metabolism and amyloid clearance (McManus et al., 2025). While current literature highlights small molecules, natural compounds, and IL-1β inhibitors as primary candidates for modulating this pathway, translating these approaches requires overcoming significant challenges regarding blood-brain barrier penetrance and long-term efficacy (Li & Gong, 2025).

Beyond microglia, astrocyte-directed therapies increasingly focus on state-shifting rather than broad functional suppression. Astrocytes exhibit both neuroprotective and neurotoxic phenotypes depending on the disease stage and local microenvironment, necessitating precision reprogramming. Preclinical studies demonstrate that compounds such as NLY01 can block the microglia-mediated conversion of astrocytes into reactive states, consequently improving spatial learning and memory. Similarly, activating Nrf2 signaling pathways (e.g., ALGERNON2) suppresses reactive astrogliosis and rescues cognitive deficits in classical transgenic models (Nakano-Kobayashi et al., 2023).

Ultimately, astrocyte-directed reprogramming offers a critical supplementary strategy to disrupt the pathogenic amplification loops between microglia and astrocytes. While TREM2 agonism currently leads in translational progress, and CD33 and NLRP3 modulation represent highly plausible preclinical mechanisms, together they reinforce the necessity of targeted immune modulation in AD therapeutics.

4. Challenges in Clinical Trials and Future Perspectives

The high attrition rate in AD drug development reflects a layered translational problem rather than the failure of isolated molecules or single targets. A systematic review of failed clinical compounds identified 98 unique Phase II and Phase III failures, estimating a success rate of merely 2.0% since 2003 (Kim et al., 2022).

Across the literature, several persistent failure modes emerge. AD is a biologically heterogeneous condition and is unlikely to be effectively treated by monotherapy. Furthermore, many therapeutic interventions are initiated too late in the disease continuum, often utilizing suboptimal dosages or targeting inappropriate mechanisms. Consequently, long-term toxicity or the absence of durable disease modification frequently halts clinical development (Elmaleh et al., 2019; Yiannopoulou et al., 2019). These biological challenges are compounded by persistent methodological limitations, including inadequate target engagement, the enrollment of participants lacking the intended underlying pathology, difficulties in staging the disease during its earliest phases, and the reliance on clinical outcome measures that lack the sensitivity required to detect subtle changes in a slowly progressing disorder (Beach, 2017; Yaari & Hake, 2015).

In this context, biomarkers are primarily utilized to enrich appropriate patient populations and improve statistical power; however, their validation as definitive surrogate endpoints remains a continuous challenge (Wu et al., 2011). Delivery mechanisms represent another critical bottleneck. It is estimated that 98% of small-molecule therapeutics and virtually 100% of large-molecule biologics fail to cross the blood-brain barrier. Thus, a compound may exhibit strong theoretical and in vitro efficacy but ultimately fail in human trials due to insufficient central nervous system penetrance (Pardridge, 2020).

Fundamentally, the most profound translational mismatch lies in the gap between preclinical models and clinical reality. The discrepancy between expectations derived from animal models and actual clinical trial outcomes questions the validity of every stage of the translational process, providing a comprehensive explanation for the stalled progress of numerous novel molecular therapies (Savonenko et al., 2012). Moving forward, overcoming these

challenges will require a shift toward polypharmacology, advanced delivery platforms, and earlier intervention windows guided by robust fluid and imaging biomarkers.

5. Conclusion

AD pharmacotherapy is actively transitioning away from traditional monotherapy models. For decades, treatments were limited to symptomatic management using cholinergic and glutamatergic modulators, which provided cognitive relief but did not alter disease progression. Recent anti-amyloid monoclonal antibodies demonstrate that biological disease modification is possible. However, their modest clinical benefits and safety risks indicate that targeting a single protein is not enough to treat a highly heterogeneous condition.

The research focus is moving toward a network-disease model that requires interventions aimed at multiple pathological nodes. Neuroinflammation is now established as a primary therapeutic axis. The goal has shifted from general immunosuppression to specific immune reprogramming using targets like TREM2 and the NLRP3 inflammasome. At the same time, epigenetic modulation and gene-editing technologies like CRISPR and APOE4-directed therapies provide new ways to alter disease mechanisms at the transcriptional level, even though translational bottlenecks still limit their clinical use.

The viability of these novel targets depends entirely on improved drug delivery. The blood-brain barrier is the main physical limitation in central nervous system pharmacology. Integrating advanced delivery methods, such as bioengineered nanocarriers, intranasal transport, and focused ultrasound, is necessary for clinical success. Even a highly selective multi-target ligand will fail if it cannot reach adequate therapeutic concentrations in the brain.

The high failure rate of past clinical trials points to structural problems in translational research. Two decades of stalled progress show that future trials need earlier intervention windows, better biomarkers for patient selection, and a practical shift toward polypharmacology. Effective AD treatment will likely depend on a combinatorial strategy. This means delivering rationally designed, multi-target agents through specialized delivery platforms to the right patient populations long before severe cognitive decline begins.

References

Alkhalifa, A. E., Al Mokhlf, A., Ali, H., Al-Ghraiybah, N. F., & Syropoulou, V. (2025). Anti-amyloid monoclonal antibodies for Alzheimer's disease: Evidence, ARIA risk, and precision patient selection. *Journal of Personalized Medicine, 15*(9), 437. https://doi.org/ 10.3390/jpm15090437

Angelova, V. T., Stoyanov, B. P., & Simeonova, R. (2024). New insights into the development of donepezil-based hybrid and natural molecules as multi-target drug agents for Alzheimer's disease treatment. *Molecules, 29*(22), 5314. https://doi.org/10.3390/molecules29225314

Avgerinos, K., Ferrucci, L., & Kapogiannis, D. (2021). Effects of monoclonal antibodies against amyloid-β on clinical and biomarker outcomes and adverse event risks: A systematic review and meta-analysis of phase III RCTs in Alzheimer's disease. *Ageing Research Reviews, 68*, 101339. https://doi.org/10.1016/j.arr.2021.101339

Avgerinos, K., Manolopoulos, A., Ferrucci, L., & Kapogiannis, D. (2024). Critical assessment of anti-amyloid-β monoclonal antibodies effects in Alzheimer's disease: A systematic review and meta-analysis highlighting target engagement and clinical meaningfulness. *Scientific Reports, 14*(1), 25741. https://doi.org/10.1038/s41598-024-75204-8

Beach, T. G. (2017). A review of biomarkers for neurodegenerative disease: Will they swing us across the valley? *Neurology and Therapy, 6*, 5–13. https://doi.org/10.1007/s40120-017-0072-x

Bhardwaj, S. (2025). Targeted and surface-engineered nanocarriers: Precision therapy in Alzheimer's disease. In R. Pal, S. Shukla, A. Rai, & A. Sharma (Eds.), *Nano-brain revolution in*

Alzheimer's disease: A new era of brain drug delivery (pp. 152–177). Deep Science Publishing. https://doi.org/10.70593/978-93-7185-149-7_7

Bian, X., Guo, Q., Yau, L.-F., Yang, L., Wang, X., Zhao, S., Wu, S., Qin, X., Jiang, Z.-H., & Li, C. (2025). Berberine-inspired ionizable lipid for self-structure stabilization and brain targeting delivery of nucleic acid therapeutics. *Nature Communications, 16*(1), 1820. https://doi.org/ 10.1038/s41467-025-57488-0

Buccellato, F. R., D'Anca, M., Tartaglia, G. M., Del Fabbro, M., Scarpini, E., & Galimberti, D. (2023). Treatment of Alzheimer's disease: Beyond symptomatic therapies. *International Journal of Molecular Sciences, 24*(18), 13900. https://doi.org/10.3390/ijms241813900

Chen, L., Guan, Y., Wang, S., Han, X., Guo, F., & Wang, Y. (2025). Engineered nanoplatforms for brain-targeted co-delivery of phytochemicals in Alzheimer's disease: Rational design, blood-brain barrier penetration, and multi-target therapeutic synergy. *Neurotherapeutics, 22*(2), e00722. https://doi.org/10.1016/j.neurot.2025.e00722

Chundu, U. C., Thiriveedhi, S., Bhatti, C., Mupparaju, J. S., Otinashvili, N., & Gelishvili, E. (2025). A systematic review of the efficacy and safety of anti-amyloid monoclonal antibodies in Alzheimer's disease. *Cureus, 17*, e85377. https://doi.org/10.7759/cureus.85377

Cummings, J. L. (2025). Maximizing the benefit and managing the risk of anti-amyloid monoclonal antibody therapy for Alzheimer's disease: Strategies and research directions. *Neurotherapeutics, 22*(3), e00570. https://doi.org/10.1016/j.neurot.2025.e00570

Cummings, J. L., Leisgang Osse, A. M., Cammann, D., Powell, J., & Chen, J. (2024). Anti-amyloid monoclonal antibodies for the treatment of Alzheimer's disease. *BioDrugs, 38*(1), 5–22. https://doi.org/10.1007/s40259-023-00633-2

Deng, J., Huang, Q., Wang, F., Liu, Y., Wang, Z., Wang, Z., Zhang, Q., Lei, B., & Cheng, Y. (2012). The role of caveolin-1 in blood-brain barrier disruption induced by focused ultrasound combined with microbubbles. *Journal of Molecular Neuroscience, 46*(3), 677–687. https://doi.org/10.1007/s12031-011-9629-9

Dib, M., Ampil, E., Kee, H. F., Krespi, Y., & Al Mahdawi, A. (2022). A review of NeuroAiD™ II (MLC901) development in Alzheimer's disease treatment: Promises of a multimodal pathway. *Journal of Neurology Research Reviews & Reports, 4*, 160. https://doi.org/10.47363/JNRRR/2022(4)160

Elmaleh, D. R., Farlow, M. R., Conti, P. S., Tompkins, R. G., Kundakovic, L., & Tanzi, R. E. (2019). Developing effective Alzheimer's disease therapies: Clinical experience and future directions. *Journal of Alzheimer's Disease, 71*(3), 715–732. https://doi.org/10.3233/JAD-190507

Gao, L., Wang, J., & Bi, Y. (2025). Nanotechnology for neurodegenerative diseases: Recent progress in brain-targeted delivery, stimuli-responsive platforms, and organelle-specific therapeutics. *International Journal of Nanomedicine, 20*, 11015–11044. https://doi.org/10.2147/IJN.S549893

Gao, Y., Liu, Y., & Li, Y. (2024). Safety and efficacy of acetylcholinesterase inhibitors for Alzheimer's disease: A systematic review and meta-analysis. *Advances in Clinical and Experimental Medicine, 33*(11), 1179–1187. https://doi.org/10.17219/acem/176051

Geldmacher, D. (2024). Treatment of Alzheimer disease. *Continuum, 30*(6), 1823–1844. https://doi.org/10.1212/CON.0000000000001503

Gudla, S. S., Vadaga, A. K., Bhumireddy, S. K. A., Kumari R, K., & Sharma, K. (2025). Nanotechnology in neuroscience transforming Alzheimer's therapy. In R. Pal, S. Shukla, A. Rai, & A. Sharma (Eds.), *Nano-brain revolution in Alzheimer's disease: A new era of brain drug delivery* (pp. 31–54). Deep Science Publishing. https://doi.org/10.70593/978-93-7185-149-7_2

Guo, Q., Li, Y., Xu, S., Wang, P., Qian, K., Yang, P., Sheng, D., Wang, L., Chen, Y., Meng, R., Cao, J., Luo, H., Wei, Y., & Zhang, Q. (2023). Brain-neuron targeted nanoparticles for peptide synergy therapy at dual-target of Alzheimer's disease. *Journal of Controlled Release, 355*, 483–497. https://doi.org/10.1016/j.jconrel.2023.01.074

Gupta, D. K., & Chaudhuri, A. (2026). CRISPR gene therapy for Alzheimer's disease: A systematic review of advances and challenges. *Medical Journal of Dr. D. Y. Patil Vidyapeeth, 19*(4), 319. https://doi.org/10.4103/mjdrdypu.mjdrdypu_443_25

Hafez, M. M., Abbas, H. A., Shoman, N. A., Soubh, A. A., Aly, O., Sallam, M. F., Seliem, M., & Malaak, F. A. (2026). A new era in neuropharmacology: Assessing the efficacy and safety of novel anti-amyloid and non-amyloid drug targets for Alzheimer's disease. *Journal of Neurology, 273*(5), 303. https://doi.org/10.1007/s00415-026-13829-7

Hanson, L. R., & Frey, W. H., II. (2008). Intranasal delivery bypasses the blood-brain barrier to target therapeutic agents to the central nervous system and treat neurodegenerative disease. *BMC Neuroscience, 9*(Suppl. 3), S5. https://doi.org/10.1186/1471-2202-9-S3-S5

Hossain, M. S., & Hussain, M. H. (2025). Multi-target drug design in Alzheimer's disease treatment: Emerging technologies, advantages, challenges, and limitations. *Pharmacology Research & Perspectives, 13*(1), e70131. https://doi.org/10.1002/prp2.70131

Jang, Y. J., Kang, S. J., Park, H. S., Lee, D. H., Kim, J. H., Kim, J. E., Kim, D. I., Chung, C. H., Yoon, J. K., & Bhang, S. H. (2025). Drug delivery strategies with lipid-based nanoparticles for Alzheimer's disease treatment. *Journal of Nanobiotechnology, 23*(1), 99. https://doi.org/10.1186/s12951-025-03109-3

Jiang, D. F. (2023). A review of the pharmacological treatments of Alzheimer's disease (AD). *Journal of Student Research, 11*(3). https://doi.org/10.47611/jsrhs.v11i3.347

Johnston, J. L., Reda, S. M., Setti, S. E., Taylor, R. W., Berthiaume, A. A., Walker, W. E., . . . Church, K. J. (2023). Fosgonimeton, a novel positive modulator of the HGF/MET system, promotes neurotrophic and procognitive effects in models of dementia. *Neurotherapeutics, 20*(2), 431–451. https://doi.org/10.1007/s13311-022-01325-5

Kamila, P., Kar, K., Chowdhury, S., Chakraborty, P., Dutta, R., Singh, A., & Prajapati, B. G. (2025). Effect of neuroinflammation on the progression of Alzheimer's disease and its significant ramifications for novel anti-inflammatory treatments. *IBRO Neuroscience Reports, 18*, 771–782. https://doi.org/10.1016/j.ibneur.2025.05.005

Khaled, M. A. Q. R., & Hazar-Yavuz, A. N. (2025). Comparative analysis of FDA-approved Alzheimer's therapies: Symptomatic and disease-modifying approaches. *Journal of Research in Pharmacy, 29*(5), 2165–2179. https://doi.org/10.12991/jrespharm.1707084

Khalefa, E. M. M., Altumi, S. A., & Eldawi, N. S. (2024). Targeting Alzheimer's disease: Current pharmacological strategies and emerging therapies. *Libyan Journal of Medical and Applied Sciences, 2*(1). https://doi.org/10.64943/ljmas.v2i1.25

Kim, A., Jerdi, S. A., MacDonald, R., Triggle, C., Blokland, A., & Femminella, G. (2024). Alzheimer's disease and its treatment–yesterday, today, and tomorrow. *Frontiers in Pharmacology, 15*, 1399121. https://doi.org/10.3389/fphar.2024.1399121

Kim, C. K., Lee, Y. R., Ong, L., Gold, M., Kalali, A., & Sarkar, J. (2022). Alzheimer's disease: Key insights from two decades of clinical trial failures. *Journal of Alzheimer's Disease, 87*(1), 83–100. https://doi.org/10.3233/JAD-215699

Kochman, U., Sitka, H., Kuźniar, J., Czaja, M., Kozubek, P., Beszłej, J. A., & Leszek, J. (2026). Targeted nanoparticles for drug delivery across the blood-brain barrier in early and late stages of Alzheimer's disease: A review. *Molecular Neurobiology, 63*(1), 75. https://doi.org/10.1007/s12035-025-05417-z

Le, H., Vu, G. T., Abioye, A. O., & Adejare, A. (2026). Recent advances in nanoparticle-based drug delivery strategies to cross the blood-brain barrier in targeted treatment of Alzheimer's disease. *Pharmaceutics, 18*(2), 192. https://doi.org/10.3390/pharmaceutics18020192

Li, B.-F., Chen, X., Xie, R., Mo, Y.-F., Wu, Y.-Z., Meng, Y., Han, X., Chen, M.-H., & Peng, Y. (2026). Targeting microglia-mediated neuroinflammation in Alzheimer's disease: Mechanisms and therapeutic approaches. *Frontiers in Immunology, 17*, 1818802. https://doi.org/10.3389/fimmu.2026.1818802

Li, J., Guo, L.-T., Cai, W., Mei, J., Liu, J., & Liu, Y. (2026). Overcoming the blood-brain barrier: Nanomedicine strategies for targeted delivery and multimodal therapy in Alzheimer's disease.

Drug Delivery, 33(1), 2645830. https://doi.org/10.1080/10717544.2026.2645830

Li, Z., & Gong, C. (2025). NLRP3 inflammasome in Alzheimer's disease: Molecular mechanisms and emerging therapies. *Frontiers in Immunology, 16*, 1583886. https://doi.org/10.3389/fimmu.2025.1583886

Lipsman, N., Meng, Y., Bethune, A. J., Huang, Y., Lam, B., Masellis, M., Herrmann, N., Heyn, C., Aubert, I., Boutet, A., Smith, G. S., Hynynen, K., & Black, S. E. (2018). Blood-brain barrier opening in Alzheimer's disease using MR-guided focused ultrasound. *Nature Communications, 9*(1), 2336. https://doi.org/10.1038/s41467-018-04529-6

Lista, S., Vergallo, A., Teipel, S., Lemercier, P., Giorgi, F., Gabelle, A., Garaci, F., Mercuri, N., Babiloni, C., Gaire, B., Koronyo, Y., Koronyo-Hamaoui, M., Hampel, H., & Nisticò, R. (2023). Determinants of approved acetylcholinesterase inhibitor response outcomes in Alzheimer's disease: Relevance for precision medicine in neurodegenerative diseases. *Ageing Research Reviews, 84*, 101819. https://doi.org/10.1016/j.arr.2022.101819

Liu, X., Jiao, B., & Shen, L. (2018). The epigenetics of Alzheimer's disease: Factors and therapeutic implications. *Frontiers in Genetics, 9*, 579. https://doi.org/10.3389/fgene.2018.00579

Long, H., Simmons, A., Mayorga, A., Burgess, B., Nguyen, T., Budda, B., . . . Rosenthal, A. (2024). Preclinical and first-in-human evaluation of AL002, a novel TREM2 agonistic antibody for Alzheimer's disease. *Alzheimer's Research & Therapy, 16*(1), 235. https://doi.org/10.1186/s13195-024-01599-1

Lu, L., Yu, X., Cai, Y., Sun, M., & Yang, H. (2021). Application of CRISPR/Cas9 in Alzheimer's disease. *Frontiers in*

Neuroscience, 15, 803894. https://doi.org/10.3389/fnins.2021.803894

Ma, X., Zhao, Y., Gong, N., Wu Chen, X.-L., Liu, G., & Xiao, J. (2022). Integration of network pharmacology and molecular docking to explore the molecular mechanism of cordycepin in the treatment of Alzheimer's disease. *Frontiers in Aging Neuroscience, 14*, 1058780. https://doi.org/10.3389/fnagi.2022.1058780

Ma, Y., Wang, W., Liu, S., Qiao, X., Xing, Y., Zhou, Q., & Zhang, Z. (2023). Epigenetic regulation of neuroinflammation in Alzheimer's disease. *Cells, 13*(1), 79. https://doi.org/10.3390/cells13010079

McManus, R. M., Komes, M. P., Griep, A., Santarelli, F., Schwartz, S., Perea, J. R., Schlachetzki, J. C. M., Bouvier, D. S., Khalil, M.-A., Lauterbach, M. A., Heinemann, L., Schlüter, T., Shaban Pour, M., Lovotti, M., Stahl, R., Duthie, F., Rodríguez-Alcázar, J. F., Schmidt, S. V., Spitzer, J., . . . Heneka, M. T. (2025). NLRP3-mediated glutaminolysis controls microglial phagocytosis to promote Alzheimer's disease progression. *Immunity, 58*(2), 326–343.e11. https://doi.org/10.1016/j.immuni.2025.01.007

Morató, X., Pytel, V., Jofresa, S., Ruiz, A., & Boada, M. (2022). Symptomatic and disease-modifying therapy pipeline for Alzheimer's disease: Towards a personalized polypharmacology patient-centered approach. *International Journal of Molecular Sciences, 23*(16), 9305. https://doi.org/10.3390/ijms23169305

Moreta, M. P.-G., Burgos-Alonso, N., Torrecilla, M., Marco-Contelles, J., & Bruzos-Cidón, C. (2021). Efficacy of acetylcholinesterase inhibitors on cognitive function in Alzheimer's disease: Review of reviews. *Biomedicines, 9*(11), 1689. https://doi.org/10.3390/biomedicines9111689

Mostafa, A., Tiu, S., Khan, F., & Baig, N. A. (2025). The efficacy of anti-amyloid monoclonal antibodies in early Alzheimer's dementia: A systematic review. *Annals of Indian Academy of Neurology, 28*, 333–343. https://doi.org/10.4103/aian.aian_547_24

Mumtaz, M., Unnithan, D., Bano, A., Pratap, A., Chauhan, S., Ali, J., & Khan, M. A. (2025). Targeting Alzheimer's disease pathology: Influence of nano-based drug delivery systems loaded with a combination of herbal and synthetic drugs. *Expert Opinion on Drug Delivery, 22*(8), 1155–1170. https://doi.org/10.1080/17425247.2025.2513440

Nakano-Kobayashi, A., Canela, A., Yoshihara, T., & Hagiwara, M. (2023). Astrocyte-targeting therapy rescues cognitive impairment caused by neuroinflammation via the Nrf2 pathway. *Proceedings of the National Academy of Sciences of the United States of America, 120*(33), e2303809120. https://doi.org/10.1073/pnas.2303809120

Nguyen, V. T. T., Sallbach, J., dos Santos Guilherme, M., & Endres, K. (2021). Influence of acetylcholine esterase inhibitors and memantine, clinically approved for Alzheimer's dementia treatment, on intestinal properties of the mouse. *International Journal of Molecular Sciences, 22*(3), 1015. https://doi.org/10.3390/ijms22031015

Ouyang, Q., Meng, Y., Zhou, W., Tong, J., Cheng, Z., & Zhu, Q. (2022). New advances in brain-targeting nano-drug delivery systems for Alzheimer's disease. *Journal of Drug Targeting, 30*(1), 61–81. https://doi.org/10.1080/1061186X.2021.1927055

Pan, T. T., Sun, Y. Y., Shi, Y. F., Zhao, M., Khan, N. U., Chen, H. Y., . . . Ma, Q. H. (2025). Endothelial delivery of simvastatin by LRP1-targeted nanoparticles ameliorates pathogenesis of Alzheimer's disease in a mouse model. *Alzheimer's Research & Therapy, 17*(1), 193. https://doi.org/10.1186/s13195-025-01840-5

Pardridge, W. M. (2020). Treatment of Alzheimer's disease and blood-brain barrier drug delivery. *Pharmaceuticals, 13*(11), 394. https://doi.org/10.3390/ph13110394

Passeri, E., Elkhoury, K., Morsink, M., Broersen, K., Linder, M., Tamayol, A., Malaplate, C., Yen, F., & Arab-Tehrany, E. (2022). Alzheimer's disease: Treatment strategies and their limitations. *International Journal of Molecular Sciences, 23*(22), 13954. https://doi.org/10.3390/ijms232213954

Patel, P., Noyda, I., & Kagathara, M. (2023). Alzheimer's disease: The review from pathophysiology to future direction. *Technoarete Transactions on Recent Research in Applied Microbiology and Biotechnology, 2*(1), 6–12. https://doi.org/10.36647/TTRRAMB/02.01.A002

Peng, Y., Jin, H., Xue, Y., Chen, Q., Yao, S., Du, M.-Q., & Liu, S. (2023). Current and future therapeutic strategies for Alzheimer's disease: An overview of drug development bottlenecks. *Frontiers in Aging Neuroscience, 15*, 1206572. https://doi.org/10.3389/fnagi.2023.1206572

Polydoro, M., Geric, I., Zheng, J., Bisgaard, T. S., Comer, A., Sans, L., . . . Plath, N. (2025). A novel TREM2 small molecule agonist MTX46943 re-programs human microglia and reduces amyloid pathology in vivo. *Alzheimer's & Dementia, 21*(Suppl. 5), e100777. https://doi.org/10.1002/alz70859_100777

Qi, X., Nizamutdinov, D., Yi, S. S., Wu, E., & Huang, J. H. (2024). Disease modifying monoclonal antibodies and symptomatic pharmacological treatment for Alzheimer's disease. *Biomedicines, 12*(11), 2636. https://doi.org/10.3390/biomedicines12112636

Roamcharern, N., & Yubolphan, R. (2026). Overcoming the blood-brain barrier in Alzheimer's disease: Translational perspectives on advanced drug delivery platforms. *Frontiers in*

Neuroscience, 20, 1810486. https://doi.org/10.3389/fnins.2026.1810486

Saad, H., Atef, E., Aboushouk, A., Waheeb, T. S., Elsayed, A. E., & Mohammed, S. A. A. (2026). Cross-talk between pyroptosis and miRNAs in Alzheimer disease neuropathology, therapeutic targeting of NLRP inflammasomes, and recent advances in nanoparticle-targeted therapy. *Journal of Neuropathology & Experimental Neurology*, nlag014. https://doi.org/10.1093/jnen/nlag014

Safieh, M., Korczyn, A. D., & Michaelson, D. M. (2019). ApoE4: An emerging therapeutic target for Alzheimer's disease. *BMC Medicine, 17*(1), 64. https://doi.org/10.1186/s12916-019-1299-4

Satyanarayana, B., Wajid, S., Kumar, M. R., & Paladugu, N. D. (2026). Blood-brain barrier-targeted nanocarriers for Alzheimer's disease: Molecular mechanisms, therapeutic advances, clinical translation and future perspectives. *International Journal of Drug Delivery Technology*. https://doi.org/10.25258/ijddt.16.61s.114

Saunders, A. M., Burns, D. K., & Gottschalk, W. K. (2021). Reassessment of pioglitazone for Alzheimer's disease. *Frontiers in Neuroscience, 15*, 666958. https://doi.org/10.3389/fnins.2021.666958

Saunders, S., Muniz-Terrera, G., Sheehan, S., Ritchie, C. W., & Luz, S. (2021). A UK-wide study employing natural language processing to determine what matters to people about brain health to improve drug development: The electronic Person-Specific Outcome Measure (ePSOM) Programme. *The Journal of Prevention of Alzheimer's Disease, 8*(4), 448–456. https://doi.org/10.14283/jpad.2021.30

Savonenko, A. V., Melnikova, T., Hiatt, A., Li, T., Worley, P. F., Troncoso, J. C., . . . Price, D. L. (2012). Alzheimer's therapeutics: Translation of preclinical science to clinical drug development. *Neuropsychopharmacology, 37*(1), 261–277. https://doi.org/10.1038/npp.2011.211

Shen, X., Li, H., Zhang, B., Li, Y., & Zhu, Z. (2025). Targeting transferrin receptor 1 for enhancing drug delivery through the blood-brain barrier for Alzheimer's disease. *International Journal of Molecular Sciences, 26*(19), 9793. https://doi.org/10.3390/ijms26199793

Shim, G. H., Lau, E. C. Y., Huynh, A. L. H., Lu, C. Y., & Tan, E. C. K. (2026). Influence of patient characteristics on efficacy and safety of anti-amyloid monoclonal antibodies in Alzheimer's disease: A systematic review and meta-analysis. *Ageing Research Reviews, 114*, 102981. https://doi.org/10.1016/j.arr.2025.102981

Sims, J. R., Zimmer, J. A., Evans, C. D., Lu, M., Ardayfio, P., Sparks, J., Wessels, A. M., Shcherbinin, S., Wang, H., Monkul Nery, E. S., Collins, E. C., Solomon, P., Salloway, S., Apostolova, L. G., Hansson, O., Ritchie, C., Brooks, D. A., Mintun, M. A., & Skovronsky, D. M. (2023). Donanemab in early symptomatic Alzheimer's disease: The TRAILBLAZER-ALZ 2 randomized clinical trial. *JAMA, 330*(6), 512–527. https://doi.org/10.1001/jama.2023.13239

Sood, S., Jain, K., & Gowthamarajan, K. (2014). Intranasal therapeutic strategies for management of Alzheimer's disease. *Journal of Drug Targeting, 22*(4), 279–294. https://doi.org/10.3109/1061186X.2013.876644

Sumbria, R. K., & Boado, R. J. (2026). Brain delivery of antibody-derived biologicals for Alzheimer's disease: An updated narrative review. *Antibodies, 15*(2), 37. https://doi.org/10.3390/antib15020037

Thangwaritorn, S., Lee, C., Metchikoff, E., Razdan, V., Ghafary, S., Rivera, D., Pinto, A., & Pemminati, S. (2024). A review of recent advances in the management of Alzheimer's disease. *Cureus, 16*(4), e58416. https://doi.org/10.7759/cureus.58416

Thawabteh, A. M., Ghanem, A. W., AbuMadi, S., Thaher, D., Jaghama, W., Karaman, D., & Karaman, R. (2024). Recent advances in therapeutics for the treatment of Alzheimer's disease. *Molecules, 29*(21), 5131. https://doi.org/10.3390/molecules29215131

Toader, C., Șerban, M., Munteanu, O., Covache-Busuioc, R.-A., Enyedi, M., Ciurea, A., & Tătaru, C. (2025). From synaptic plasticity to neurodegeneration: BDNF as a transformative target in medicine. *International Journal of Molecular Sciences, 26*(9), 4271. https://doi.org/10.3390/ijms26094271

Tonegawa-Kuji, R., Hou, Y., Hu, B., Lorincz-Comi, N., Pieper, A. A., Tousi, B., Leverenz, J. B., & Cheng, F. (2025). Efficacy and safety of passive immunotherapies targeting amyloid beta in Alzheimer's disease: A systematic review and meta-analysis. *PLOS Medicine, 22*(3), e1004568. https://doi.org/10.1371/journal.pmed.1004568

Tung, Y. S., Vlachos, F., Feshitan, J. A., Borden, M. A., & Konofagou, E. E. (2011). The mechanism of interaction between focused ultrasound and microbubbles in blood-brain barrier opening in mice. *The Journal of the Acoustical Society of America, 130*(5), 3059–3067. https://doi.org/10.1121/1.3646905

Tutubala, T. E., Egunlusi, A. O., Fisher, D., Dube, A., & Joubert, J. (2025). Nanomedicine solutions for Alzheimer's disease: A critical review of therapeutic nanoparticle strategies. *ACS Omega, 10*(48), 53633–53657. https://doi.org/10.1021/acsomega.4c04887

U.S. Food and Drug Administration. (2025). *Highlights of prescribing information: LEQEMBI (lecanemab-irmb) injection, for*

intravenous or subcutaneous use (Reference ID 5651461). https://www.accessdata.fda.gov/drugsatfda_docs/label/2025/761375s000lbl.pdf

U.S. National Library of Medicine. (2018). *Gene therapy for APOE4 homozygote of Alzheimer's disease* (ClinicalTrials.gov Identifier NCT03634007). ClinicalTrials.gov. https://clinicaltrials.gov/study/NCT03634007

U.S. National Library of Medicine. (2022). *Long-term follow-up of gene therapy for APOE4 homozygote Alzheimer's disease* (ClinicalTrials.gov Identifier NCT05400330). ClinicalTrials.gov. https://clinicaltrials.gov/study/NCT05400330

van Dyck, C. H., Swanson, C. J., Aisen, P., Bateman, R. J., Chen, C., Gee, M., Kanekiyo, M., Li, D., Reyderman, L., Cohen, S., Froelich, L., Katayama, S., Sabbagh, M., Vellas, B., Watson, D., Dhadda, S., Irizarry, M., Kramer, L. D., & Iwatsubo, T. (2023). Lecanemab in early Alzheimer's disease. *The New England Journal of Medicine, 388*(1), 9–21. https://doi.org/10.1056/NEJMoa2212948

Varadharajan, A., Davis, A. D., Ghosh, A., Jagtap, T., Xavier, A., Menon, A. J., Roy, D., Gandhi, S., & Issac, T. G. (2023). Guidelines for pharmacotherapy in Alzheimer's disease–A primer on FDA-approved drugs. *Journal of Neurosciences in Rural Practice*. https://doi.org/10.25259/JNRP_356_2023

Veroniki, A. A., Ashoor, H. M., Rios, P., Seitidis, G., Stewart, L., Clarke, M., Tudur-Smith, C., Mavridis, D., Hemmelgarn, B. R., Holroyd-Leduc, J., Straus, S. E., & Tricco, A. C. (2022). Comparative safety and efficacy of cognitive enhancers for Alzheimer's dementia: A systematic review with individual patient data network meta-analysis. *BMJ Open, 12*(4), e053012. https://doi.org/10.1136/bmjopen-2021-053012

Vishwas, S., et al. (2023). The overview of drugs used in Alzheimer's disease and their molecular targets. In *Advances in neuropharmacology* (pp. 351–376). Springer. https://doi.org/10.1007/978-981-99-2657-2_15

Wang, K., Yang, R., Li, J., Wang, H., Wan, L., & He, J. (2025). Nanocarrier-based targeted drug delivery for Alzheimer's disease: Addressing neuroinflammation and enhancing clinical translation. *Frontiers in Pharmacology, 16*, 1591438. https://doi.org/10.3389/fphar.2025.1591438

Wang, T., Liu, X., Wang, X., Hua, F., & Yan, L. (2026). TREM2 and microglial immunity in Alzheimer's disease: Mechanisms, genetics, and therapeutic opportunities. *Frontiers in Immunology, 17*, 1739875. https://doi.org/10.3389/fimmu.2026.1739875

Wei, Y., & Li, H. (2025). The efficacy and safety of amyloid beta-directed monoclonal antibodies for Alzheimer's disease: A systematic review and meta-analysis of phase III randomized controlled trials. *Human Psychopharmacology: Clinical and Experimental, 40*(5), e70017. https://doi.org/10.1002/hup.70017

Wong, C. Y. J., Baldelli, A., Hoyos, C. M., Tietz, O., Ong, H. X., & Traini, D. (2024). Insulin delivery to the brain via the nasal route: Unraveling the potential for Alzheimer's disease therapy. *Drug Delivery and Translational Research, 14*(7), 1776–1793. https://doi.org/10.1007/s13346-024-01558-1

Wong, E., Malviya, M., Jain, T., Liao, G. P., Kehs, Z., Chang, J. C., . . . Li, Y. M. (2024). HuM195 and its single-chain variable fragment increase Aβ phagocytosis in microglia via elimination of CD33 inhibitory signaling. *Molecular Psychiatry, 29*(7), 2084–2094. https://doi.org/10.1038/s41380-024-02474-z

Wu, L., Rosa-Neto, P., & Gauthier, S. (2011). Use of biomarkers in clinical trials of Alzheimer disease: From concept to application. *Molecular Diagnosis & Therapy, 15*(6), 313–325. https://doi.org/10.1007/BF03256467

Wu, W., Ji, Y., Wang, Z., Wu, X., Li, J., Gu, F., Chen, Z., & Wang, Z. (2023). The FDA-approved anti-amyloid-β monoclonal antibodies for the treatment of Alzheimer's disease: A systematic review and meta-analysis of randomized controlled trials. *European Journal of Medical Research, 28*(1), 544. https://doi.org/10.1186/s40001-023-01512-w

Xiao, C., Davis, F. J., Chauhan, B. C., Viola, K. L., Lacor, P. N., Velasco, P. T., . . . Chauhan, N. B. (2013). Brain transit and ameliorative effects of intranasally delivered anti-amyloid-β oligomer antibody in 5XFAD mice. *Journal of Alzheimer's Disease, 35*(4), 777–788. https://doi.org/10.3233/JAD-122419

Xu, Y., Ye, X., Du, Y., Yang, W., Tong, F., Li, W., . . . Zhang, W. (2025). Nose-to-brain delivery of targeted lipid nanoparticles as two-pronged β-amyloid nanoscavenger for Alzheimer's disease therapy. *Acta Pharmaceutica Sinica B, 15*(6), 2884–2899. https://doi.org/10.1016/j.apsb.2025.02.035

Yaari, R., & Hake, A. (2015). Alzheimer's disease clinical trials: Past failures and future opportunities. *Clinical Investigation, 5*(3), 297–309. https://doi.org/10.4155/cli.14.127

Yang, S. S., Zhang, R., Wang, G., & Zhang, Y. F. (2017). The development prospection of HDAC inhibitors as a potential therapeutic direction in Alzheimer's disease. *Translational Neurodegeneration, 6*(1), 19. https://doi.org/10.1186/s40035-017-0089-1

Yang, X., Yang, W., Xia, X., Lei, T., Yang, Z., Jia, W., Zhou, Y., Cheng, G., & Gao, H. (2022). Intranasal delivery of BACE1

siRNA and rapamycin by dual targets modified nanoparticles for Alzheimer's disease therapy. *Small, 18*(38), e2203182. https://doi.org/10.1002/smll.202203182

Yiannopoulou, K. G., Anastasiou, A. I., Zachariou, V., & Pelidou, S. H. (2019). Reasons for failed trials of disease-modifying treatments for Alzheimer disease and their contribution in recent research. *Biomedicines, 7*(4), 97. https://doi.org/10.3390/biomedicines7040097

Yin, J., Zhao, F., Chojnacki, J. E., Fulp, J., Klein, W. L., Zhang, S., & Zhu, X. (2018). NLRP3 inflammasome inhibitor ameliorates amyloid pathology in a mouse model of Alzheimer's disease. *Molecular Neurobiology, 55*(3), 1977–1987. https://doi.org/10.1007/s12035-017-0467-9

Zhang, J., Zhang, Y., Wang, J., Xia, Y., Zhang, J., & Chen, L. (2024). Recent advances in Alzheimer's disease: Mechanisms, clinical trials and new drug development strategies. *Signal Transduction and Targeted Therapy, 9*(1), 212. https://doi.org/10.1038/s41392-024-01911-3

Zhang, Y., Li, Z., Guan, H., Qiu, Z., & Zou, C. (2025). Engineering exosomes for Alzheimer's disease: Multi-target therapeutic strategies from pathogenesis to clinical translation. *Clinical and Translational Medicine, 15*(2), e70548. https://doi.org/10.1002/ctm2.70548


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