Medicinski podmladak

Medical Youth

Review article

MICROBIOTA AND ALZHEIMER'S DISEASE: TOWARD NEW CLINICAL APPROACHES

MIKROBIOTA I ALCHAJMEROVA BOLEST: NA PUTU KA NOVIM KLINIČKIM PRISTUPIMA

Jelena Đokić[1], Miroslav Dinić[1]

[1] Univerzitet u Beogradu, Institut za molekularnu genetiku i genetičko inženjerstvo, Beograd, Srbija

Correspondence: jelena.djokich@gmail.com

Abstract

Alzheimer's disease (AD) is now viewed as a systemic disorder in which genetics, the gut microbiome and immune dysregulation converge to accelerate amyloid-β (Aβ) and tau pathology. Genetic studies have identified 70 risk loci. The strongest AD risk loci – APOE ε4, is conferring dose-dependent risk through impaired cholesterol trafficking, reduced Aβ clearance and heightened neuroinflammation. Rare mutations in TREM2 and CD33 further illustrate the centrality of microglia-mediated innate immunity. Importantly, peripheral Th17 skewing, neutrophil hyper-activation and loss of regulatory myeloid cells amplify neuroinflammation. Growing evidence links gut dysbiosis to these genetic factors. These carriers show depletion of short–chain fatty acids (SCFA)-producing taxa (*Gemmiger, Roseburia, Faecalibacterium*) and enrichment of pro-inflammatory Proteobacteria (*Escherichia/Shigella, Morganella*). In mice, APOE4-dependent loss of butyrate-producing bacteria lowers colonic and serum SCFA levels and worsens tauopathy, whereas Germ-free housing, early antibiotics and SCFA supplementation rescue pathology. Large meta-analyses correlate *Collinsella* and *Veillonella* with higher AD risk, and *Eubacterium nodatum* and *Prevotella 9* with protection. Metabolomic shifts mirror these compositional changes: acetate, propionate and butyrate, as well as trimethylamine N-oxide (TMAO) promote epigenetic regulation, blood-brain barrier (BBB) integrity and microglial homeostasis, whereas secondary bile acids compromise cognition. The microbial amyloids and lipopolysaccharide can cross-seed Aβ, activate the NLRP3 inflammasome and breach the blood-brain barrier. Intermittent fasting, ketogenic diets, specific probiotics (*Bifidobacterium breve A1, Lacticaseibacillus rhamnosus CBT-LR5*), sodium oligomannate (GV-971) or fecal microbiota transplantation can remodel gut ecology, raise beneficial SCFAs, dampen pro-inflammatory cytokines and improve cognition in patients with AD or mild cognitive impairment. Collectively, the data support a gut-immune system-brain axis in which host genotype shapes microbiota, and microbial metabolites or antigens contribute to neurodegeneration. Future work must define responder phenotypes, optimize next-generation probiotics and integrate multi-omics profiling with biomarker-driven clinical trials to translate microbiota modulation into disease-modifying therapy for AD.

Keywords:
Alzheimer's Disease,
APOE,
neuroinflammation,
microbiota,
short-chain fatty acids,
probiotics

The authors declare no conflicts of interest.

doi:10.5937/mp76-60520
Editorial board: podmladak.med.bg@gmail.com

Sažetak

Alchajmerova bolest (AB) je sistemsko oboljenje u kojem genetski faktori, crevna mikrobiota i poremećena regulacija imunskog sistema doprinose formiranju amiloid-β (Aβ) i tau patoloških promena. Genetička istraživanja identifikovala su oko 70 lokusa povezanih sa rizikom od razvoja AB, od kojih je APOE ε4 najznačajniji i dozno-zavisno povećava rizik narušavanjem transporta holesterola, smanjenjem klirensa Aβ i pojačanom neuroinflamacijom. Retke varijante u genima TREM2 i CD33 dodatno naglašavaju ključnu ulogu mikroglije u patogenezi AB. Istovremeno, na periferiji aktivacija pomoćničkih T-ćelija 17 (Th17) i neutrofila, kao i gubitak regulatornih mijeloidnih ćelija doprinose amplifikaciji neuroinflamacije. Rastući broj dokaza povezuje disbiozu u crevima sa pomenutim genetskim rizikom. Nosioci alela APOE ε4 imaju smanjenu zastupljenost bakterija koje proizvode kratkolančane masne kiseline (*Gemmiger, Roseburia, Faecalibacterium*) i porast proinflamatornih *Proteobacteriae* (*Escherichia/Shigella, Morganella*). U miševa, gubitak butirat produkujućih bakterija, specifičan za APOE4, pogoršava tauopatiju, dok gnotobiotski uslovi, rana antibiotska terapija ili suplementacija kratkolančanim masnim kiselinama ublažavaju patologiju. Metaanalize su povezale vrste *Collinsella* i *Veillonella* s većim rizikom za AB, a *Eubacterium nodatum* i *Prevotella 9* sa otpornošću na razvoj AB. Metabolomički profili reflektuju sledeće promene: acetat, propionat, butirat i trimetilamin-N-oksid (TMAO) podržavaju epigenetsku regulaciju, integritet krvno-moždane barijere i homeostazu mikrogije, dok sekundarne žučne kiseline narušavaju kognitivne funkcije. Mikrobni amiloidi i lipopolisaharidi mogu doprineti formiranju amiloidnih plakova, aktivirati inflamazom NLRP3 i narušiti funkciju krvno-moždane barijere. Povremeni post, ketogena dijeta, specifični probiotici (*Bifidobacterium breve A1, Lacticaseibacillus rhamnosus CBT-LR5*), natrijum-oligomanat (GV-971) ili fekalna transplantacija mikrobiote pokazali su da mogu remodelovati crevnu ekološku zajednicu, povećati korisne metabolite, smanjiti proinflamatorne citokine i poboljšati kogniciju kod obolelih od AB ili blagih kognitivnih poremećaja. Svi nalazi ukazuju na osovinu crevo-imunski sistem-mozak, gde genotip domaćina oblikuje mikrobiotu, a mikrobni metaboliti ili antigeni doprinose neurodegeneraciji. Buduća istraživanja treba da definišu profile pacijenata koji najbolje odgovaraju na terapiju, optimizuju „probiotike nove generacije" i integrišu multiomiks analize sa kliničkim ispitivanjima zasnovanim na biomarkerima.

Ključne reči:
Alchajmerova bolest, APOE, neuroinflamacija, mikrobiota, kratkolančane masne kiseline, probiotici

Introduction

Alzheimer's Disease (AD) is classically defined by the accumulation of extracellular amyloid-β (Aβ) plaques and intracellular neurofibrillary tangles of hyperphosphorylated tau. The diagnosis of AD has undergone a significant transformation, shifting from a symptom-based clinical assessment toward a precise, biology-driven approach. Initially, diagnosis was only possible at advanced stages, often confirmed post-mortem through analysis of brain tissue. However, with the emergence of neuroimaging techniques (such as MRI and PET) and cerebrospinal fluid (CSF) biomarkers (Aβ and tau), the field entered a new era where the disease could be detected *in vivo* and at much earlier stages (1). The introduction of the AT(N) framework in 2018 marked a turning point, enabling a classification of AD based on biomarker evidence of amyloid accumulation (A), tau pathology (T), and neurodegeneration (N). This model provided a more objective and standardized method for diagnosing and staging the disease, regardless of clinical symptoms (2). More recently, the framework has been expanded to ATN(X) with the addition of emerging biomarkers that reflect other pathological processes, including inflammation (I), vascular injury (V), and α-synuclein (S) (3). Furthermore, the incorporation of plasma biomarkers, which are less invasive and more accessible than CSF or imaging, has made it feasible to implement these diagnostic tools in everyday clinical settings.

Current studies have found that dysbiosis in gut microbiota is linked to AD pathophysiological changes, including abnormal brain Aβ aggregation, inflammatory responses, immune dysfunction, and neuronal and synaptic damage (**Figure 1**). Based on current research, the underlying biology of these associations may be crucial for further elucidating the pathophysiology of AD, potentially contributing to the development of novel diagnostic and therapeutic approaches. Therefore, this review aims to provide an overview of the currently available data and to offer future research directions concerning the associations between gut microbiota and other pathological processes characteristic of AD.

How do gut microbiota features associate with genetic markers in Alzheimer's disease?

Alzheimer's Disease is a complex genetic disorder with an estimated heritability of 60 - 80%, highlighting the significant role of genetic factors in disease risk. Despite

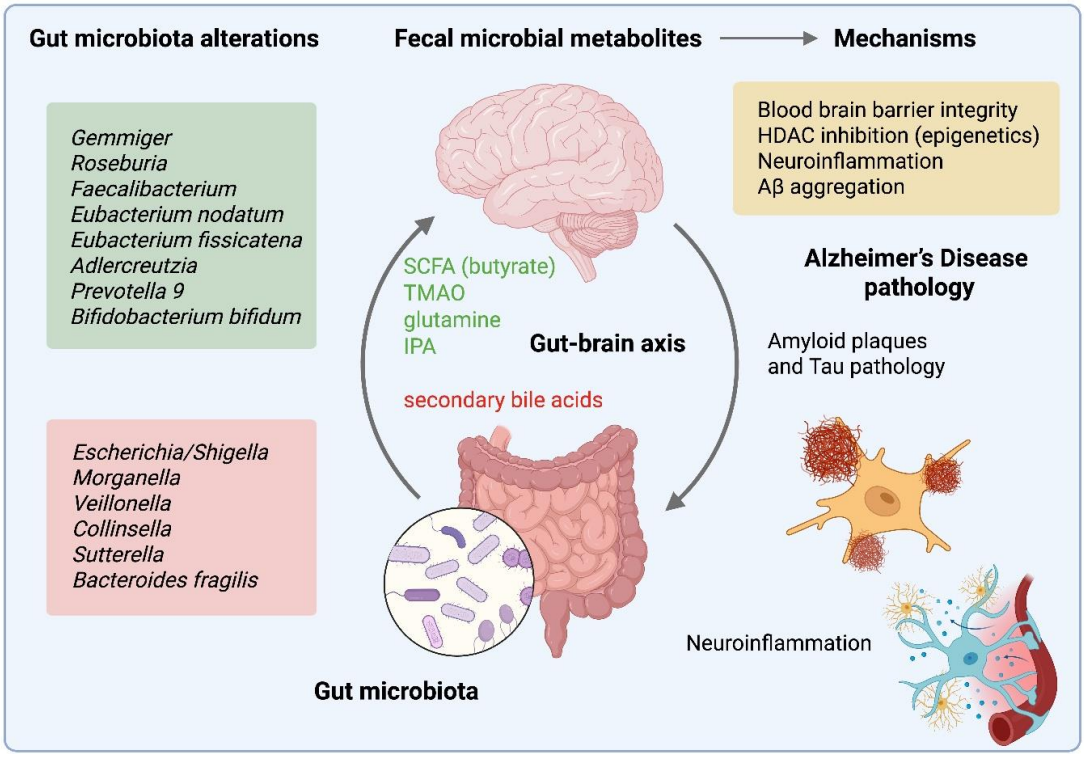

Figure 1. The schematic overview of molecular mechanisms of microbiota-brain interactions in Alzheimer's Disease
The protective bacterial taxa and metabolites are marked in green, and bacterial taxa and metabolites associated with higher risk AD are labeled red

this, identifying the genetic architecture of AD has been challenging due to its heterogeneity and the contributions of both rare and common variants. Early genetic studies focused on rare, autosomal dominant forms of early-onset AD (EOAD), leading to the identification of mutations in the amyloid-beta precursor protein-encoding gene (*APP*), presenilin 1 (*PSEN1*), and presenilin 2 (*PSEN2*) (4,5). Amyloid-beta precursor protein-encoding gene contains the amino acid stretch that forms Aβ. Generation of Aβ requires two sequential cleavages: β-secretase cuts in the ectodomain, and γ-secretase then cleaves within the transmembrane region to liberate the peptide. More than 50 highly penetrant mutations have been identified in the *APP* gene, most of which shift processing toward β-secretase or alter γ-secretase site selection, thereby increasing the production of the hydrophobic, aggregation-prone Aβ42 isoform and accelerating plaque formation (6). Pathogenic mutations in *PSEN1* (chromosome 14) and *PSEN2* (chromosome 1) bias γ-secretase toward the 42-residue cut-site, boosting production of aggregation-prone Aβ42 and accelerating plaque deposition (7). To date, more than 350 *PSEN1* variants have been catalogued, accounting for approximately 80% of EOAD. Alzheimer's disease in *PSEN* mutation carriers is usually diagnosed between 30 and 50 years of age. At the same time, the Apolipoprotein E (APOE) ε4 (APOE4) allele was identified as a significant risk factor for late-onset familial and sporadic AD (LOAD) (8). More recently, the development of next-generation sequencing (NGS) and genome-wide association studies (GWAS) based on whole-exome sequencing (WES) and whole-genome sequencing (WGS), as well as genome-wide genotyping of single-nucleotide polymorphisms (SNPs), has enabled the discovery of over 70 AD-associated loci (9). Apolipoprotein E initially identified as a lipoprotein regulator, is now recognized as the most important lipid carrier in the brain (10). Three isoforms, A*POE2, E3,* and *E4*, create six genotypes; ε3/ε3 predominates (50 - 70%), while ε4 represents 10 - 15% of alleles. Epidemiologically, ε4 confers dose-dependent AD risk: heterozygotes show 3-4-fold and homozygotes 8-15-fold elevations with earlier Aβ deposition (11). Mechanistically, ApoE4 clears cholesterol and Aβ poorly, forms neurotoxic fragments and amplifies neuroinflammation, accelerating amyloid, tau and synaptic injury (12). Single-molecule imaging shows poorly lipidated ApoE4 catalyzes Aβ42 nucleation (13). A conversely neuroprotective, slowing progression of AD is ApoE2 (12). Additionally, TREM2 promotes phagocytosis and restrains Toll-like receptor signaling. Loss-of-function variants such as R47H markedly elevate AD risk (14). Furthermore, CD33 acts as an inhibitory brake: risk alleles increase full-length CD33 and suppress Aβ uptake (15), whereas a protective splice variant lacking the sialic-acid domain enhances clearance (16). Deleting CD33 alleviates amyloidosis only when TREM2 is intact, underscoring functional cross-talk (17).

Decrease in SCFAs is associated with APOE4

The first investigation of microbiota characteristics in the context of *APOE* genotype was published by Tran et al (18). In this study, healthy participants (aged 56 - 78 years) were stratified by APOE variants: 24 APOE4 carriers (APOE3/4 and APOE4/4; 12 men and 12 women) and 32 APOE4 non-carriers (APOE2/3 and APOE3/3; 16 men and 16 women). APOE-targeted replacement mice underwent fecal 16S rRNA sequencing and metabolomic analysis. Although overall microbial diversity did not

differ, significant genotype-specific differences emerged: APOE2/E3 individuals had elevated bacteria from the genus *Gemmiger* within the family *Ruminococcaceae*, and APOE3/E3 individuals had elevated bacteria from the family *Prevotellaceae* and genus *Roseburia* (*Lachnospiraceae*). These taxa are well-known producers of short-chain fatty acids (SCFAs) and are previously associated with reduced risk of neurodegenerative diseases. In contrast, APOE4/E4 carriers showed a loss of these beneficial bacteria. Mouse models mirrored human findings, with lower levels of butyrate-producing bacteria and SCFAs in APOE4-carrier old mice. Co-inertia analysis (COIA) further confirmed that changes in microbiota composition were strongly correlated with fecal metabolomic profiles, particularly the levels of SCFAs and their precursors. Short-chain fatty acids, primarily acetate, propionate, and butyrate, are key metabolites produced by gut microbiota through the fermentation of dietary fiber and have emerged as significant mediators in the gut-brain axis, affecting AD pathogenesis. The study by Seo et al., explores the interaction between the gut microbiota, APOE genotype, and tau pathology (19). Mice expressing mutant human tau protein (P301S) alongside human APOE3 (TE3) or APOE4 (TE4) isoforms were used to assess the progression of neurodegeneration. TE4 mice raised under germ-free conditions were significantly protected from hallmark features of tauopathy, including tau accumulation, glial activation, and brain atrophy. This protection was reversed upon recolonization with microbiota, indicating a causal relationship between gut microbial composition and neurodegeneration. Further, a short antibiotic treatment during early life conferred neuroprotection in male TE3 mice, reducing tau pathology and gliosis, but had no protective effect in TE4 mice. These results suggest that the interaction between the microbiota and host is not only APOE isoform-dependent but also sex-dependent. The study also demonstrated that altered microbial composition influences the shift of immune and metabolic pathways relevant to neurodegeneration. Importantly, the administration of SCFAs to germ-free TE4 mice restored tau pathology and glial activation. For instance, treatment with butyrate has been shown to restore memory performance and enhance the expression of genes involved in associative learning in AD mouse models, through the inhibition of histone deacetylase (HDAC) (20). Moreover, valeric, butyric, and propionic acids have demonstrated the ability to interfere with the interactions between Aβ peptides, effectively hindering their aggregation into harmful neurotoxic oligomers (21). These findings suggest that SCFAs, particularly butyrate, hold therapeutic potential for AD by mitigating neuroinflammation, improving BBB function, and inhibiting Aβ pathology. However, the effects of SCFAs are complex, as butyrate consistently shows protective effects, while propionate may have beneficial or detrimental impacts depending on the pathway. Further research is needed to clarify the dual effects of other SCFAs, such as propionate, and to develop targeted interventions, including probiotics or dietary strategies. Beyond their role in regulating BBB integrity, SCFAs also exert neuroprotective effects in AD.

Taxonomic features of APOE4-carrier microbiota

However, only pathogenic bacteria belonging to the genera *Escherichia/Shigella* and *Morganella* within the family *Enterobacteriaceae*, phylum Proteobacteria, were significantly elevated in APOE4 carriers, independently of the individual's AD status (22). Furthermore, Camman et al. investigated the association between microbiota data and two SNPs, rs429358 (APOE4) and rs7412 (APOE2) (23). The analysis of the discovery sample identified six AD-risk genera: *Alistipes* and *Bacteroides* (Bacteroidetes phylum), *Lachnospira* and *Veillonella* (Firmicutes phylum), *Collinsella* (Actinobacteria phylum), and *Sutterella* (Pseudomonadota phylum), and fourteen AD-protective genera *Anaerostipes, Candidatus Soleaferrea, Catenibacterium, Eisenbergiella, Eubacterium coprostanoligenes* group, *Eubacterium fissicatena* group, *Eubacterium nodatum* group, *Intestinibacter, Lachnospiraceae* UCG-008, *Oscillibacter,* and *Roseburia* (Firmicutes phylum), *Adlercreutzia* and *Gordonibacter* (Actinobacteria phylum), and *Prevotella 9* (Bacteroidetes phylum). After testing these results on a replication sample, *Collinsella* and *Veillonella* were identified as statistically significant AD-risk-associated genera, while only *Eubacterium fissicatena* was recognized as a protective genus. Interestingly, only the genus *Collinsella* was positively correlated with AD diagnosis and APOE risk allele C at rs429358, and *Adlercreutzia, Eubacterium nodatum,* and *Prevotella 9* showed a negative correlation with APOE risk allele C at rs429358 in both discovery and replication samples. Zhao et al. demonstrated an association between the species *Bifidobacterium bifidum* and a lower risk of AD (24). On the other hand, the family *Sutterellaceae* was correlated with a higher risk of AD. The family *Sutterellaceae* belongs to the class *Actinobacteria*, which was associated in the study of Zeng et al. with a higher risk of AD (25). Further, Huang et al. study suggested the association of *Actinobacteria*, but also *Lactobacillaceae, Faecalibacterium, Ruminiclostridium,* and *Lachnoclostridium* with high genetic risk for AD, and glutamine with lower risk for AD development. Glutamate, the brain's primary excitatory neurotransmitter, is tightly regulated through the glutamate-glutamine cycle, involving metabolic collaboration between neurons and astrocytes to sustain glutamatergic neurotransmission and prevent excitotoxicity (26). In this cycle, astrocytes play a crucial role by taking up synaptically released glutamate and converting it into glutamine, which is then supplied back to neurons for the synthesis of both glutamate and GABA (γ-aminobutyric acid). Disruptions in glutamate clearance and metabolism contribute to neuronal overstimulation and neurodegeneration in AD (27). The Aβ42 reduces surface expression of the astrocytic glutamate transporter GLT-1, prolonging extracellular glutamate presence and impairing synaptic signaling specificity, an effect mitigated by the vitamin E derivative Trolox. Additionally, studies using 13C-labeled substrates in 5xFAD mouse models reveal significant astrocytic metabolic dysfunction in AD,

particularly reduced glutamine synthesis, which directly impairs neuronal GABA synthesis and disrupts synaptic excitation-inhibition balance (28). This is compounded by neuronal hypometabolism of glucose and altered metabolism of exogenous glutamine, suggesting the presence of compensatory mechanisms. These findings highlight that astrocytic glutamate transporter and metabolic dysfunction are critical in AD pathogenesis, potentially serving as therapeutic targets to restore glutamate homeostasis and mitigate neurodegeneration (26). Elucidating how host genetics shape gut microbial composition and how these microbiome shifts influence AD may identify modifiable lifestyle factors that can lower individual disease risk.

What gut microbiota markers associate with neurodegeneration in Alzheimer's Disease?

Changes in the gut microbiota affect several key mechanisms underlying neurodegeneration in AD, including Aβ deposition, neuroinflammatory responses, tau pathology, and compromised BBB integrity, which ultimately contribute to the progressive loss of specific neuronal populations and the manifestation of clinical symptoms (29).

Microbial amyloid-like proteins contribute to AD pathology

Recent findings suggest that alterations in the gut microbiota may contribute to amyloid pathology by producing microbial amyloid-like proteins that can cross-seed with human Aβ, increasing BBB permeability, and/or reducing synthesis of neuroprotective microbial metabolites such as SCFAs or neurotransmitters with multimodal activity (30). These microbial influences may not only accelerate the formation of amyloid plaques but also exacerbate downstream neurodegenerative cascades, reinforcing the concept of the gut-brain axis as a modulator of Alzheimer's pathology. Although commonly associated with neurodegenerative disorders in humans, amyloid proteins are not limited to pathological roles or the animal kingdom. Many microorganisms produce functional amyloids that serve beneficial purposes, such as structural support in biofilms (31). However, due to their structural resemblance to mammalian amyloids, these microbial proteins may also interact with host amyloidogenic proteins, influencing their misfolding and aggregation, or causing AD-like pathologies (32). Recent studies have shown that exposure to microbial amyloids, such as curli amyloid-producing *E. coli*, increases the prevalence of splenic serum amyloid A, providing the first clear evidence that microbial amyloids can initiate or exacerbate amyloid formation in mammals (33).

Microbial metabolites affect BBB function in AD

The gut microbiota is increasingly recognized as a key regulator of BBB integrity, primarily through the action of neuroprotective microbiota-derived metabolites. Experimental evidence from germ-free mice has shown that the absence of a normal gut microbiota leads to increased BBB permeability, which is associated with reduced expression of tight junction proteins (ZO-1, occludin, and claudin-5). The re-establishment of a normal microbial community was sufficient to restore tight junction expression and re-establish BBB integrity (34). Among the microbial metabolites implicated in the barrier repair process, this study identified SCFAs as active metabolites essential to upregulate tight junction protein expression, indicating that SCFA production may be a central mechanism by which the microbiota modulates BBB function. In addition to SCFAs, the gut-derived metabolite trimethylamine N-oxide (TMAO), produced from dietary methylamines, has also been found to enhance BBB integrity by regulating annexin A1, a protein involved in tight junction stability (35). Similarly, tryptophan-derived metabolites, particularly indole-3-propionic acid, have demonstrated potential in preventing the formation of amyloid-beta fibrils, highlighting their therapeutic promise in modulating neurodegenerative processes (36). In contrast to beneficial metabolites, secondary bile acids, produced by the gut microbiota, can directly influence neurodegeneration in AD. Elevated serum levels of secondary bile acids, including deoxycholic acid and its glycine- and taurine-conjugated derivatives, have been detected in AD compared to healthy controls, contributing to cognitive decline observed in patients (37). However, despite promising experimental evidence, clinical data in humans remain limited, highlighting the need for further research to confirm the translational relevance of these findings and to explore the therapeutic potential of targeting gut microbiota-derived metabolites in AD.

What is the specificity of the gut microbiota related to dysfunction of immune response in AD?

Far from being immuno-privileged, the brain hosts a finely tuned network of resident and peripheral immune cells whose protective duties can turn maladaptive, fueling neurodegeneration (38). At the core of this network are microglia, the CNS's professional phagocytes. In health, microglia patrol synapses and phagocytose debris (39). This activation is initially protective, enhancing Aβ clearance. However, prolonged signaling disrupts dendritic spine formation and accelerates tau propagation. The NLRP3 inflammasome serves as an amplifying switch, and its genetic or pharmacological ablation in APP/PS1 mice lowers caspase-1 activity, boosts Aβ uptake, and preserves cognition (40).

The role of blood myeloid cells in AD-immunopathology

In addition to microglia, mounting evidence from murine neurodegenerative disease models shows that peripheral monocytes and lymphocytes can indeed cross the BBB and infiltrate the CNS. Blood monocytes adopt an IL-1β/IL-6/TNF-α signature as dementia progresses, while an

early expansion of immunosuppressive myeloid-derived suppressor cells wanes later, lifting an anti-inflammatory brake (39). Circulating $CD11c^+$ dendritic cells fall in proportion to cognitive decline (41). In APP mice, CCR2 deficiency blocks monocyte recruitment, exacerbates plaque formation, and shortens survival (42). Interestingly, the anti-PD-1 therapy triggers IFN-γ at the choroid plexus, floods the brain with macrophages, clears plaques, and improves memory in a tauopathy mouse model (43). Neutrophils are likewise hyper-activated in AD blood, producing excess reactive oxygen species and infiltrating the brain via LFA-1, where they exacerbate pathology, and their depletion or blockade relieves cognitive deficits (44).

The role of T lymphocytes in AD-immunopathology

Both $CD4^+$ and $CD8^+$ T cells cross the BBB in AD yet exhibit blunted IFN-γ production (45). Removing adaptive immune response doubles plaque burden in 5×FAD mice (46). Patients show a Th17-skewed profile, elevated circulating Th17 cells with higher IL-17, IL-6 and IFN-γ but reduced IL-4/IL-10 (47). Also, IL-17 blockade prevents synaptic and cognitive loss in mouse models (48). Clonally expanded cytotoxic Terminally differentiated Effector Memory (TEMRA) $CD8^+$ cells accumulate in cerebrospinal fluid and correlate negatively with cognition, many recognizing Epstein-Barr viral antigens (49). At the other hand, regulatory T cell numbers are often reduced in AD blood (50). Neuroinflammation in AD is Janus-faced: tightly tuned activation removes toxic proteins and fosters repair, whereas chronic or genetically biased activation drives degeneration. Strategies that recalibrate, rather than extinguish, immune response, including TREM2 agonists, NLRP3 inhibitors, CD33 blockers, IL-17 inhibitors, and agents that disrupt pathogenic apoE-Aβ hybrids or optimize peripheral monocyte recruitment, may synergize with anti-amyloid and anti-tau therapies to durably modify disease trajectory.

Disturbance of microbiome and immune response in AD

Zhan et al. detected elevated lipopolysaccharide (LPS) and *E. coli* K99 protein in post-mortem AD brains, co-localizing with Aβ plaques and far exceeding levels in controls, implying that Gram-negative bacterial antigens sustain chronic neuroinflammation (51). By using germ-free APP/PS1 mice, Harach et al. further proved the role of microbiota in Aβ plaque formation and demonstrated 50% fewer Aβ plaques and reduced microglial activation in these animals (52). In line with these results, the recolonization of germ-free APP/PS1 mice with microbiota preparation from conventionally-raised APP/PS1 mice, but not from wild-type mice, increased cerebral Aβ pathology. Subsequent studies with different human cohorts have confirmed dysbiosis, but have also revealed regional differences. Thus, Cattaneo et al. found AD-related amyloid-PET-positive subjects carried more pro-inflammatory genera (*Escherichia/Shigella*) and fewer butyrate producers (*Eubacterium rectale*). Stool *Escherichia/Shigella* correlated positively with plasma IL-6 and IL-1β, while *E. rectale* was inversely associated, tying dysbiosis to systemic and central cytokine tone (53). Vogt et al. profiled fecal 16S rRNA in AD patients from Wisconsin AD Research Center (ADRC) in comparison to control from Wisconsin Registry for Alzheimer's Prevention (WRAP), and reported lower α-diversity, reduced SMB53 (family *Clostridiaceae*), *Dialister, Clostridium, Turicibacter,* and cc115 (family *Erysipelotrichaceae*) (Firmicutes), *Bifidobacterium* and *Adlercreutzia* (Actinobacteria), increased *Blautia*, *Phascolarctobacterium*, and *Gemella* (Firmicutes), *Bacteroides* and *Alistipes* (Bacteroidetes), *Bilophila* (Proteobacteria), and taxa linked to tau and Aβ levels in cerebrospinal fluid (54). Zhuang et al. worked with the Chinese population of AD patients and demonstrated the somewhat opposite changes in microbiota, as reduced Bacteroidetes and elevated Actinobacteria, or reduced Firmicutes and elevated Proteobacteria (55). These compositional differences in the microbiota between the AD and healthy groups from the USA and China are in accordance with the finding that microbiota composition differs among healthy individuals with different genetics and cultures, from different social groups, and different geographical origins (56). The most important factors differing between individuals from different geographical regions that impact gut microbiota composition are diet (dominance of processed food vs high-fiber diet), but also the practice of antibiotic use. This conclusion implies limited application of data on the links between microbiota composition and health obtained in individual populations to the global level, and requires studying the microbiome changes in healthy and disease populations in a specific world region. The comparison of data obtained in different laboratories is further complicated by the knowledge that microbiome data are highly affected by technical factors associated with collection, preservation, and storage of samples, the protocol for DNA extraction and the use of various sequencing platforms and application of different bioinformatics tools and databases. Despite these differences in the composition of the microbiome, all studies agreed on the depletion of butyrate-producing symbionts and the enrichment of opportunistic, pro-inflammatory bacteria. Thus, the data on metabolic and functional activity of microbiome seems more valuable than the analysis of relative abundances of specific taxa in microbiome, indicating the importance of shotgun sequencing of microbiome DNA (whole genome sequencing) and metabolomics analysis.

In parallel with the changes in commensal microbiota, the role of pathogenic bacteria in neuroinflammation associated with AD pathology was investigated. Dominy et al. investigated the oral pathogen P*orphyromonas gingivalis* and showed that bacteria and their gingipains (toxic proteases) infiltrate AD brains (57). Repeated oral infections in mice worsened neuroinflammation and amyloidosis, suggesting that chronic periodontal infection may act as a systemic trigger. Also, the authors demonstrated that small molecule inhibitors of gingipains reduced the bacterial

load of *P. gingivalis*, and reduced associated AD pathology, such as production of Aβ42, and neuroinflammation. A landmark Cell Research paper revealed that dysbiosis in 5×FAD mice elevates circulating phenylalanine and isoleucine, driving peripheral Th1 expansion, the infiltration of these cells in the brain and the association with activated M1 microglial cells, and cognition loss. Notably, the authors observed a parallel in humans: patients with mild cognitive impairment due to AD had elevated blood phenylalanine/isoleucine levels and increased Th1 cell frequencies compared to healthy controls. Furthermore, they demonstrated that algal oligosaccharide GV-971 normalized the microbiota, lowered these amino acids, suppressed Th1 cell differentiation, reduced neuroinflammation, and rescued cognition in mice (58). Chen et al. uncovered that gut microbiota dysbiosis activates CCAAT/enhancer binding protein β/asparagine endopeptidase (C/EBPβ/AEP) in gut and brain, accelerating Aβ and tau pathology. Also, antibiotics or a prebiotic that enriched *Lactobacillus salivarius* suppressed gut leakage, oxidative stress, reduced amyloid aggregates in the gut, and improved cognition (59). Deep dives into the mechanisms of the gut-immune system-brain axis associated with AD pathology have led to the latest advances and direction of future research. Thus, following studies that reported an increase in Bacteroidetes, Xia et al. identified virulent *Bacteroides fragilis* strains whose metabolites, 12-hydroxy-heptadecatrienoic acid (12-HHTrE) and Prostaglandin E2 (PGE2), directly activate microglia and worsen Aβ and tau pathology in neuronal C/EBPβ transgenic mice (60). In the next year, Wasén et al. selectively depleted Bacteroidetes in APP/PS1-21 mice, resulting in a decrease in Aβ plaques and an enhancement of microglial phagocytic genes, pro-inflammatory cytokine signaling, and lysosomal activity in females. Conversely, supplementation of 5xFAD mice with *B. fragilis* increased plaques and impaired microglial clearance by down-regulating phagocytosis genes, including CD33, P2ry12, and Jun, in aged male and female mice, implicating specific taxa in suppressing Aβ removal (61). Following numerous studies, some of which are cited here, showing the reduction of SCFA-producing bacteria, Colombo et al. performed metabolite-focused studies that probed SCFAs in AD germ-free and SPF mice (62). Surprisingly, the study showed SCFAs promote plaque deposition and, among other changes in transcriptomic profile, induce upregulation of ApoE in microglial cells, but with a lower level of intracellular Aβ in germ-free AD mice, indicating not a uniform and simple function of SCFAs as just immune modulators but a broader metabolic agent with stage-dependent effects.

Most compellingly, a recent fecal microbiota transplant experiment demonstrated a causal microbiome-immune-AD effect across species. Researchers transplanted gut microbiota from AD patients into young, healthy mice and observed that the recipient mice developed memory impairments and AD-like brain changes (63). The mice receiving Alzheimer-derived microbiota produced fewer new neurons in the hippocampus and showed signs of neuroinflammation, mirroring deficits seen in the patients. Notably, the AD patients' microbiota contained an excess of inflammation-promoting bacteria, and the cognitive decline in recipient mice was associated with elevated peripheral inflammatory signals, suggesting the human gut microbes induced a deleterious immune response in the mice. This transmissible effect confirms that gut dysbiosis can drive AD-associated cognitive and immune changes.

Based on this part of the review, AD is a systemic disorder in which the gut microbiota and immune system are integral drivers. Dysbiosis skews immune responses toward chronic inflammation, fueling Aβ and tau pathology. Restoring microbial balance, by targeting harmful taxa/metabolites or promoting beneficial butyrate producers, emerges as a promising strategy to temper neuroinflammatory cascades and slow AD progression. Future clinical trials will determine whether remodeling the microbiota can complement traditional anti-amyloid therapies, underscoring that in AD, the gut may be as crucial as the brain.

Microbiome-based interventions for Alzheimer's Disease across diverse model systems

The growing recognition of the gut-brain axis in AD has prompted increasing interest in microbiota-based interventions, particularly classical probiotics (lactic acid bacteria and *Bifidobacteria*) and Next-Generation Probiotics (gut anaerobic bacteria), as potential modulators of neurodegenerative processes (**Table 1**). To investigate these interactions, a range of experimental models has been developed, each offering unique advantages for dissecting host-microbe dynamics, neuroinflammation, and amyloid or tau pathology.

Some lifestyle interventions proved effective: intermittent fasting shifted the microbiome toward *Lactobacillus* and *Prevotellaceae*, restored hippocampal NAD^+ metabolism, lowered systemic IL-1β/IL-6 and normalized microglial homeostatic genes, conferring cognitive protection in 5×FAD mice (64). More recently, oral re-introduction of the butyrate producer *Agathobacter rectalis* elevated colonic butyrate, blocked microglial Akt/NF-κB signaling and improved memory in APP/PS1 mice (65). A study by Prajapati et al. in 2025 extended these insights with a ten-strain *Lactobacillus/Enterococcus* cocktail that repaired gut permeability, reduced serum IL-6/IL-1β, curtailed microglial M1 markers and limited amyloid aggregation in humanized APP/PS1 mice (66).

Further, invertebrate models such as *Caenorhabditis elegans* and *Drosophila melanogaster* provide rapid, cost-effective platforms to assess neuroprotective effects of probiotic bacteria and their metabolites, often using behavioral assays or Aβ-induced paralysis phenotypes. For example, transgenic *C. elegans* expressing human Aβ and tau proteins exhibit hallmark neurodegenerative traits, including impaired chemotaxis, defective associative

Table 1. Probiotic interventions in different Alzheimer's Disease model systems

Model	Probiotic (Bacterial Strain)	Key Findings
APP/PS1 transgenic mice	*Bifidobacterium breve* HNXY26M4 (70)	Prevented memory loss, reduced Aβ production, microglial activation, improved cognition and SCFA production
	Clostridium butyricum (71)	Shown to attenuate Aβ accumulation and cognitive decline in various studies
Caenorhabditis elegans expressing human Aβ42	*Bacillus subtilis* R0179 and *Lacticaseibacillus rhamnosus* (72)	Delayed Aβ-induced paralysis; longevity effects via mitophagy, restored behavior in neuron-expressing Aβ lines Reduced paralysis via modulation of fatty acid metabolism
Drosophila melanogaster expressing Aβ42	*Lactobacillus plantarum* DR7 (73)	Rescued eye neurodegeneration and restored microbiome balance
Aluminum-induced cognitive impairment and AD-like pathology in zebrafish	Pasteurized *Akkermansia muciniphila* (74)	Improved memory and emotional behavior in T-maze and novel tank tests, increased social preference, reduced aggression/anxiety, and improved microbiome composition linked to Aβ clearance
Aβ proteotoxicity in 2D neuronal cell cultures	Heat-killed *Ruminococcus albus* (75)	Protect SH-SY5Y neurons against Aβ-induced apoptosis, reducing oxidative stress and DNA damage

learning, and reduced mobility (67). Zebrafish models have also been used to evaluate probiotic effects on social behavior and anxiety, further linking gut microbial changes with neurological outcomes.

At the cellular level, 2D neuronal cultures (e.g., SH-SY5Y cells) have allowed high-throughput screening of probiotic-derived metabolites and supernatants, showing protective effects against Aβ-induced proteotoxicity (68). Meanwhile, 3D cerebral organoids, including brain-on-chip and BBB-on-chip technologies, represent emerging systems that more closely mimic human brain microenvironments. However, direct testing of probiotics in these platforms remains limited. Moreover, recent advances in iPSC-based modeling have opened new avenues to personalize the investigation of host-microbe interactions in neurodegeneration. Patient-derived iPSCs, combined with organ-on-chip platforms mimicking the BBB and gut barriers, offer a powerful, physiologically relevant approach to study individual-specific responses to microbial metabolites or probiotics (69).

Clinical studies investigating the gut microbiome in Alzheimer's Disease and mild cognitive impairment

Randomized, double-blind trials provide the most substantial evidence so far. Daily intake of a four-strain probiotic milk for 12 weeks significantly raised Mini-Mental State Examination (MMSE) scores (28% increase versus decline in placebo) and reduced serum C-reactive protein, malondialdehyde and indices of insulin resistance, indicating concurrent anti-inflammatory and metabolic benefits (76). In a larger three-arm trial, co-supplementation with selenium and the multi-strain probiotic for 12 weeks produced the most significant cognitive gain (MMSE + 1.5), alongside larger decreases in high-sensitivity CRP and improvements in total antioxidant capacity and glutathione compared with selenium alone or placebo (77). A 90-day open-label study using kefir (a synbiotic containing live bacteria and yeast plus fermentation metabolites) reported broad improvements across memory, visuospatial, and language domains, accompanied by 30% reductions in circulating pro-inflammatory cytokines and reactive oxygen species and a doubling of nitric-oxide bioavailability (78). A four-week open-label pilot in 18 outpatients linked multispecies probiotic intake to reduced fecal ZO-1 and increased *Faecalibacterium prausnitzii*, alongside activation of the kynurenine pathway without short-term cognitive change (79). A separate 12-week double-blind pilot observed that probiotic supplementation (various *Lactobacillus*/*Bifidobacterium* strains) modestly improved cognition and metabolic indices compared with placebo, although sample size was only twenty (80). A 100-participant RCT in MCI showed that 12 weeks of *Lactobacillus plantarum* C29-fermented soybean significantly improved composite cognition, particularly attention, and increased serum BDNF (81). *Bifidobacterium breve* A1 has been repeatedly evaluated. An open-label 24-week trial raised MMSE by 1.7 points (82). In a 121-subject placebo-controlled RCT, global effects were neutral, but the probiotic conferred superior immediate memory and MMSE in participants with lower baseline scores (83). The longest study (24 weeks) with *B. breve* MCC1274 reported attenuated hippocampal and whole-brain atrophy on MRI, despite only subtle cognitive benefits, suggesting disease-modifying potential (84). A 12-week pilot comparing two doses of *Lacticaseibacillus rhamnosus* CBT-LR5 found meaningful MoCA and ADAS-Cog13 improvements together with a reduction in plasma Aβ40/Aβ42 ratio and enrichment of *Lacticaseibacillus* in feces (85).

Sodium oligomannate (GV-971), an oral algal oligosaccharide with documented microbiota-modulating properties, met its primary efficacy endpoint in an 818-patient, 36-week phase-3 RCT: ADAS-Cog12 scores improved by an additional 2.15 points over placebo, with a safety profile indistinguishable from placebo (86). Mechanistic work suggests that GV-971 restores eubiosis and reduces peripheral immune cell trafficking to the CNS, thereby mitigating neuroinflammation. A six-week crossover study

Table 2. Clinical and Interventional Studies Modulating the Gut Microbiota in AD/MCI

Study	Microbiota Modulation	Participants	Key Findings
Akbari et al. (76)	Four-strain 2 × 10^9 CFU/g for each (*Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum*, and *Lactobacillus fermentum*) probiotic milk daily for 12 weeks	AD patients (30 test and 30 control); randomized, double-blind, placebo-controlled	↑ MMSE (28% vs decline in placebo) ↓ CRP, malondialdehyde, insulin resistance
Tamtaji et al. (77)	Selenium (200 μg/day) and/or four-strain 2 × 10^9 CFU/g for each (*Lactobacillus acidophilus, Bifidobacterium bifidum*, and *Bifidobacterium longum*) probiotic milk daily for 12 weeks	AD patients (27 only selenium, 26 in selenium plus probiotic, 26 only probiotic); three-arm randomized, double-blind trial	↑ MMSE (+ 1.5), total antioxidant capacity, and glutathione vs selenium alone/ placebo ↓hs-CRP
Ton et al. (78)	Kefir synbiotic drink fermented for 24 h (4% kefir grains containing the species *Acetobacter aceti*, *Acetobacter* sp., *Lactobacillus delbrueckii*, *Lactobacillus fermentum*, *Lactobacillus fructivorans*, *Enterococcus faecium*, *Leuconostoc* spp., *Lactobacillus kefiranofaciens*, *Candida famata*, and *Candida krusei*) for 90 days	13 AD patients; open-label	↑ memory/visuospatial/language ↓ pro-inflammatory cytokines and ROS
Leblhuber et al. (79)	Multispecies probiotic (*Lactobacillus casei W56, Lactococcus lactis W19, Lactobacillus acidophilus W22, Bifidobacterium lactis W52, Lactobacillus paracasei W20, Lactobacillus plantarum W62, Bifidobacterium lactis W51, Bifidobacterium bifidum W23 and Lactobacillus salivarius W24)* for 4 weeks	18 AD patients; open-label pilot	no short-term cognitive change ↑ *Faecalibacterium prausnitzii*, kynurenine pathway ↓ fecal ZO-1
Agahi et al. (80)	*Lactobacillus fermentum, Lactobacillus plantarum*, *Lactobacillus acidophilus, Bifidobacterium lactis, Bifidobacterium bifidum*, and *Bifidobacterium longum* strains for 12 weeks	AD patients (23 patients in the placebo and 25 in the probiotic group); randomized, double-blind pilot	Modest cognitive and metabolic improvement
Hwang et al. (81)	*Lactobacillus plantarum* C29-fermented soybean (DW2009) for 12 weeks	MCI subjects (50 patients in the placebo and 50 in the probiotic group); multicenter RCT	↑ composite cognition (especially attention), serum BDNF
Kobayashi et al. (82)	*Bifidobacterium breve* A1 for 24 weeks	19 older adults with MCI; open-label single-arm	↑ MMSE (+ 1.7), cognition
Kobayashi et al. (83)	*Bifidobacterium breve* A1 for 12 weeks	117 older adults with memory complaints (probiotic and placebo groups); RCT	↑ immediate memory, MMSE in low-baseline subgroup
Asaoka et al. (84)	*Bifidobacterium breve* MCC1274 for 24 weeks	Older adults with suspected MCI (55 patients in the probiotic, and 60 in the placebo group); double-blind RCT	↓ hippocampal, whole-brain atrophy on MRI subtle cognitive benefits
Jung et al. (85)	*Lacticaseibacillus rhamnosus* CBT-LR5 (two doses) for 12 weeks	20 elderly with MCI; randomized, double-blind study	↑ MoCA, ADAS-Cog13, fecal *Lacticaseibacillus* ↓ plasma Aβ40/ Aβ42 ratio
Xiao et al. (86)	Sodium oligomannate isolated from the marine algae *Ecklonia kurome* (GV-971) for 36 weeks	AD patients (408 patients in the GV-971, and 410 in the placebo group); phase 3 multicenter RCT	↑ ADAS-Cog12 (+ 2.15)
Nagpal et al. (87)	Modified Mediterranean ketogenic diet (MMKD) vs American Heart Association Diet (AHAD) for 6 weeks	11 MCI and 6 control participants; crossover design vs low-fat control	↑ *Akkermansia*, butyrate favorable CSF Aβ42 and tau biomarker shifts in MMKD vs AHAD
Hazan et al. (88)	Single fecal microbiota transplant (FMT, from patient's wife) for *C. difficile*	AD patient, 82-year-old man; case report	↑ MMSE after 6 months, mood, memory
Park et al. (89)	Single fecal microbiota transplant (FMT) from healthy 27-year-old man	AD patient, 90-year-old female; case report	↑ MMSE, MoCA, CDR after 1 month, SCFA-producing taxa
Park et al. (90)	FMT vs standard antibiotics (controlled case series)	Dementia patients with recurrent *C. difficile* (10 patients in the FMT, and 10 in the antibiotic group)	Only FMT: ↑ cognitive gains and gut α-diversity

showed that a modified Mediterranean ketogenic diet, but not a low-fat control diet, enriched *Akkermansia* and butyrate, and favorably shifted CSF Aβ42 and tau markers in MCI participants (87).

In the first report of administration of a single FMT to an AD patient with *Clostridioides difficile* infection raised MMSE from 20 to 29 within six months and alleviated mood and memory complaints (88). A subsequent case involving a 90-year-old female demonstrated parallel improvements in MMSE, Montreal Cognitive Assessment and Clinical Dementia Rating one month post-FMT, with 16S profiling revealing increased SCFA-producing taxa (89). Extending these observations, a controlled case series of ten dementia patients with recurrent *C. difficile* showed that FMT, but not standard antibiotic therapy, enhanced cognitive scores and shifted the gut microbiome towards higher α-diversity; microbial changes correlated with the cognitive gains (90). Together, these data imply that profound microbiota restructuring can acutely benefit cognition in susceptible AD brains.

Conclusion

Collectively, these trials (**Table 2**) indicate that manipulating the intestinal ecosystem via live microbial consortia, targeted prebiotics, whole-food synbiotics or complete microbiome replacement can translate into cognitive benefit, biomarker improvement and, in some instances, structural neuroprotection. While heterogeneity in designs, strains, doses and endpoints delays firm clinical recommendations, findings from different independent approaches support the gut-brain axis as a modifiable contributor to AD pathophysiology. Larger, longer and mechanism-focused trials, including rigorous FMT studies and multi-omics microbiome profiling, are now warranted to optimize microbial therapeutics for preventing or slowing neurodegeneration.

Literature

1. Murray ME, Graff-Radford NR, Ross OA, Petersen RC, Duara R, Dickson DW. Neuropathologically defined subtypes of Alzheimer's disease with distinct clinical characteristics: a retrospective study. Lancet Neurol. 2011; 10(9):785-96.
2. Jack CR, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, et al. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. Alzheimers Dement J Alzheimers Assoc. 2018; 14(4):535-62.
3. Jack CR, Andrews JS, Beach TG, Buracchio T, Dunn B, Graf A, et al. Revised criteria for diagnosis and staging of Alzheimer's disease: Alzheimer's Association Workgroup. Alzheimers Dement J Alzheimers Assoc. 2024; 20(8):5143-69.
4. Sherrington R, Froelich S, Sorbi S, Campion D, Chi H, Rogaeva EA, et al. Alzheimer's Disease Associated with Mutations in Presenilin 2 is Rare and Variably Penetrant. Hum Mol Genet. 1996; 5(7):985-8.
5. Goate A, Chartier-Harlin MC, Mullan M, Brown J, Crawford F, Fidani L, et al. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature. 1991; 349(6311):704-6.
6. Tcw J, Goate AM. Genetics of β-Amyloid Precursor Protein in Alzheimer's Disease. Cold Spring Harb Perspect Med. 2017; 7(6):a024539.
7. Thinakaran G, Koo EH. Amyloid precursor protein trafficking, processing, and function. J Biol Chem. 2008; 283(44):29615-9.
8. Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, et al. Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci USA. 1993; 90(5):1977-81.
9. Bellenguez C, Küçükali F, Jansen IE, Kleineidam L, Moreno-Grau S, Amin N, et al. New insights into the genetic etiology of Alzheimer's disease and related dementias. Nat Genet. 2022; 54(4):412-36.
10. Shore VG, Shore B. Heterogeneity of human plasma very low density lipoproteins. Separation of species differing in protein components. Biochemistry. 1973; 12(3):502-7.
11. Huang Y, Weisgraber KH, Mucke L, Mahley RW. Apolipoprotein E: diversity of cellular origins, structural and biophysical properties, and effects in Alzheimer's disease. J Mol Neurosci. 2004; 23(3):189-204.
12. Han X. The role of apolipoprotein E in lipid metabolism in the central nervous system. Cell Mol Life Sci. 2004; 61(15):1896-906.
13. Xia Z, Prescott EE, Urbanek A, Wareing HE, King MC, Olerinyova A, et al. Co-aggregation with Apolipoprotein E modulates the function of Amyloid-β in Alzheimer's disease. Nat Commun. 2024; 15(1):4695.
14. Jay TR, Miller CM, Cheng PJ, Graham LC, Bemiller S, Broihier ML, et al. TREM2 deficiency eliminates TREM2+ inflammatory macrophages and ameliorates pathology in Alzheimer's disease mouse models. J Exp Med. 2015; 212(3):287-95.
15. Bradshaw EM, Chibnik LB, Keenan BT, Ottoboni L, Raj T, Tang A, et al. CD33 Alzheimer's disease locus: altered monocyte function and amyloid biology. Nat Neurosci. 2013; 16(7):848-50.
16. Bhattacherjee A, Jung J, Zia S, Ho M, Eskandari-Sedighi G, St Laurent CD, et al. The CD33 short isoform is a gain-of-function variant that enhances Aβ1-42 phagocytosis in microglia. Mol Neurodegener. 2021; 16(1):19.
17. Griciuc A, Patel S, Federico AN, Choi SH, Innes BJ, Oram MK, et al. TREM2 Acts Downstream of CD33 in Modulating Microglial Pathology in Alzheimer's Disease. Neuron. 2019; 103(5):820-35. e7.
18. Tran TTT, Corsini S, Kellingray L, Hegarty C, Le Gall G, Narbad A, et al. APOE genotype influences the gut microbiome structure and function in humans and mice: relevance for Alzheimer's disease pathophysiology. FASEB J. 2019; 33(7):8221-31.
19. Seo DO, O'Donnell D, Jain N, Ulrich JD, Herz J, Li Y, et al. ApoE isoform and microbiota-dependent progression of neurodegeneration in a mouse model of tauopathy. Science. 2023; 379(6628):eadd1236.
20. Govindarajan N, Agis-Balboa RC, Walter J, Sananbenesi F, Fischer A. Sodium butyrate improves memory function in an Alzheimer's disease mouse model when administered at an advanced stage of disease progression. J Alzheimers Dis. 2011;26(1):187-97.
21. Ho L, Ono K, Tsuji M, Mazzola P, Singh R, Pasinetti GM. Protective roles of intestinal microbiota derived short chain fatty acids in Alzheimer's disease-type beta-amyloid neuropathological mechanisms. Expert Rev Neurother. 2018; 18(1):83-90.
22. Hou M, Xu G, Ran M, Luo W, Wang H. APOE-ε4 Carrier Status and Gut Microbiota Dysbiosis in Patients With Alzheimer Disease. Front Neurosci. 2021; 15:619051.
23. Cammann D, Lu Y, Cummings MJ, Zhang ML, Cue JM, Do J, et al. Genetic correlations between Alzheimer's disease and gut microbiome genera. Sci Rep. 2023; 13:5258.
24. Zhao Q, Baranova A, Cao H, Zhang F. Evaluating Causal Effects of Gut Microbiome on Alzheimer's Disease. J Prev Alzheimers Dis. 2024; 11(6):1843-8.
25. Zeng H, Zhou K, Zhuang Y, Li A, Luo B, Zhang Y. Unraveling the connection between gut microbiota and Alzheimer's disease: a two-sample Mendelian randomization analysis. Front Aging Neurosci. 2023;15:1273104.
26. Andersen JV, Markussen KH, Jakobsen E, Schousboe A, Waagepetersen HS, Rosenberg PA, et al. Glutamate metabolism

and recycling at the excitatory synapse in health and neurodegeneration. Neuropharmacology. 2021; 196:108719.
27. Scimemi A, Meabon JS, Woltjer RL, Sullivan JM, Diamond JS, Cook DG. Amyloid-β1-42 slows clearance of synaptically released glutamate by mislocalizing astrocytic GLT-1. J Neurosci. 2013; 33(12):5312-8.
28. Andersen JV, Christensen SK, Westi EW, Diaz-delCastillo M, Tanila H, Schousboe A, et al. Deficient astrocyte metabolism impairs glutamine synthesis and neurotransmitter homeostasis in a mouse model of Alzheimer's disease. Neurobiol Dis. 2021; 148:105198.
29. Chandra S, Sisodia SS, Vassar RJ. The gut microbiome in Alzheimer's disease: what we know and what remains to be explored. Mol Neurodegener. 2023; 18(1):9.
30. Ahmed H, Leyrolle Q, Koistinen V, Kärkkäinen O, Layé S, Delzenne N, et al. Microbiota-derived metabolites as drivers of gut-brain communication. Gut Microbes. 2022;14(1):2102878.
31. Sampson T. Microbial amyloids in neurodegenerative amyloid diseases. FEBS J. 2025; 292(6):1265-81.
32. Blanco-Míguez A, Tamés H, Ruas-Madiedo P, Sánchez B. Microbiota-Derived β-Amyloid-like Peptides Trigger Alzheimer's Disease-Related Pathways in the SH-SY5Y Neural Cell Line. Nutrients. 2021; 13(11):3868.
33. Lundmark K, Westermark GT, Olsén A, Westermark P. Protein fibrils in nature can enhance amyloid protein A amyloidosis in mice: Cross-seeding as a disease mechanism. Proc Natl Acad Sci USA. 2005; 102(17):6098-102.
34. Braniste V, Al-Asmakh M, Kowal C, Anuar F, Abbaspour A, Tóth M, et al. The gut microbiota influences blood-brain barrier permeability in mice. Sci Transl Med. 2014; 6(263):263ra158.
35. Hoyles L, Pontifex MG, Rodriguez-Ramiro I, Anis-Alavi MA, Jelane KS, Snelling T, et al. Regulation of blood-brain barrier integrity by microbiome-associated methylamines and cognition by trimethylamine N-oxide. Microbiome. 2021; 9(1):235.
36. Pappolla MA, Perry G, Fang X, Zagorski M, Sambamurti K, Poeggeler B. Indoles as essential mediators in the gut-brain axis. Their role in Alzheimer's disease. Neurobiol Dis. 2021; 156:105403.
37. MahmoudianDehkordi S, Arnold M, Nho K, Ahmad S, Jia W, Xie G, et al. Altered Bile Acid Profile Associates with Cognitive Impairment in Alzheimer's Disease - An Emerging Role for Gut Microbiome. Alzheimers Dement J Alzheimers Assoc. 2019; 15(1):76-92.
38. De Strooper B, Karran E. The Cellular Phase of Alzheimer's Disease. Cell. 2016; 164(4):603-15.
39. Thome AD, Faridar A, Beers DR, Thonhoff JR, Zhao W, Wen S, et al. Functional alterations of myeloid cells during the course of Alzheimer's disease. Mol Neurodegener. 2018; 13(1):61.
40. Heneka MT, Kummer MP, Stutz A, Delekate A, Schwartz S, Vieira-Saecker A, et al. NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. Nature. 2013; 493(7434):674-8.
41. Ciaramella A, Salani F, Bizzoni F, Orfei MD, Caltagirone C, Spalletta G, et al. Myeloid dendritic cells are decreased in peripheral blood of Alzheimer's disease patients in association with disease progression and severity of depressive symptoms. J Neuroinflammation. 2016; 13:18.
42. Naert G, Rivest S. CC chemokine receptor 2 deficiency aggravates cognitive impairments and amyloid pathology in a transgenic mouse model of Alzheimer's disease. J Neurosci. 2011; 31(16):6208-20.
43. Rosenzweig N, Dvir-Szternfeld R, Tsitsou-Kampeli A, Keren-Shaul H, Ben-Yehuda H, Weill-Raynal P, et al. PD-1/PD-L1 checkpoint blockade harnesses monocyte-derived macrophages to combat cognitive impairment in a tauopathy mouse model. Nat Commun. 2019; 10(1):465.
44. Dong Y, Lagarde J, Xicota L, Corne H, Chantran Y, Chaigneau T, et al. Neutrophil hyperactivation correlates with Alzheimer's disease progression. Ann Neurol. 2018; 83(2):387-405.
45. Ferretti MT, Merlini M, Späni C, Gericke C, Schweizer N, Enzmann G, et al. T-cell brain infiltration and immature antigen-presenting cells in transgenic models of Alzheimer's disease-like cerebral amyloidosis. Brain Behav Immun. 2016; 54:211-25.
46. Marsh SE, Abud EM, Lakatos A, Karimzadeh A, Yeung ST, Davtyan H, et al. The adaptive immune system restrains Alzheimer's disease pathogenesis by modulating microglial function. Proc Natl Acad Sci USA. 2016; 113(9):E1316-25.
47. Oberstein TJ, Taha L, Spitzer P, Hellstern J, Herrmann M, Kornhuber J, et al. Imbalance of Circulating Th17 and Regulatory T Cells in Alzheimer's Disease: A Case Control Study. Front Immunol. 2018; 9:1213.
48. Brigas HC, Ribeiro M, Coelho JE, Gomes R, Gomez-Murcia V, Carvalho K, et al. IL-17 triggers the onset of cognitive and synaptic deficits in early stages of Alzheimer's disease. Cell Rep. 2021; 36(9):109574.
49. Gate D, Saligrama N, Leventhal O, Yang AC, Unger MS, Middeldorp J, et al. Clonally expanded CD8 T cells patrol the cerebrospinal fluid in Alzheimer's disease. Nature. 2020; 577(7790):399-404.
50. Ciccocioppo F, Lanuti P, Pierdomenico L, Simeone P, Bologna G, Ercolino E, et al. The Characterization of Regulatory T-Cell Profiles in Alzheimer's Disease and Multiple Sclerosis. Sci Rep. 2019; 9(1):8788.
51. Zhan X, Stamova B, Jin LW, DeCarli C, Phinney B, Sharp FR. Gram-negative bacterial molecules associate with Alzheimer disease pathology. Neurology. 2016; 87(22):2324-32.
52. Harach T, Marungruang N, Duthilleul N, Cheatham V, Mc Coy KD, Frisoni G, et al. Reduction of Abeta amyloid pathology in APPPS1 transgenic mice in the absence of gut microbiota. Sci Rep. 2017; 7:41802.
53. Cattaneo A, Cattane N, Galluzzi S, Provasi S, Lopizzo N, Festari C, et al. Association of brain amyloidosis with pro-inflammatory gut bacterial taxa and peripheral inflammation markers in cognitively impaired elderly. Neurobiol Aging. 2017; 49:60-8.
54. Vogt NM, Kerby RL, Dill-McFarland KA, Harding SJ, Merluzzi AP, Johnson SC, et al. Gut microbiome alterations in Alzheimer's disease. Sci Rep. 2017; 7(1):13537.
55. Zhuang ZQ, Shen LL, Li WW, Fu X, Zeng F, Gui L, et al. Gut Microbiota is Altered in Patients with Alzheimer's Disease. J Alzheimers Dis. 2018; 63(4):1337-46.
56. Abdill RJ, Graham SP, Rubinetti V, Ahmadian M, Hicks P, Chetty A, et al. Integration of 168,000 samples reveals global patterns of the human gut microbiome. Cell. 2025; 188(4):1100-18.e17.
57. Dominy SS, Lynch C, Ermini F, Benedyk M, Marczyk A, Konradi A, et al. Porphyromonas gingivalis in Alzheimer's disease brains: Evidence for disease causation and treatment with small-molecule inhibitors. Sci Adv. 2019; 5(1):eaau3333.
58. Wang X, Sun G, Feng T, Zhang J, Huang X, Wang T, et al. Sodium oligomannate therapeutically remodels gut microbiota and suppresses gut bacterial amino acids-shaped neuroinflammation to inhibit Alzheimer's disease progression. Cell Res. 2019; 29(10):787-803.
59. Chen C, Ahn EH, Kang SS, Liu X, Alam A, Ye K. Gut dysbiosis contributes to amyloid pathology, associated with C/EBPβ/AEP signaling activation in Alzheimer's disease mouse model. Sci Adv. 2020; 6(31):eaba0466.
60. Xia Y, Xiao Y, Wang ZH, Liu X, Alam AM, Haran JP, et al. Bacteroides Fragilis in the gut microbiomes of Alzheimer's disease activates microglia and triggers pathogenesis in neuronal C/EBPβ transgenic mice. Nat Commun. 2023; 14(1):5471.
61. Wasén C, Beauchamp LC, Vincentini J, Li S, LeServe DS, Gauthier C, et al. Bacteroidota inhibit microglia clearance of amyloid-beta and promote plaque deposition in Alzheimer's disease mouse models. Nat Commun. 2024; 15(1):3872.
62. Colombo AV, Sadler RK, Llovera G, Singh V, Roth S, Heindl S, et al. Microbiota-derived short chain fatty acids modulate microglia and promote Aβ plaque deposition. eLife. 2021; 10:e59826.
63. Grabrucker S, Marizzoni M, Silajdžić E, Lopizzo N, Mombelli E, Nicolas S, et al. Microbiota from Alzheimer's patients induce deficits in cognition and hippocampal neurogenesis. Brain J Neurol. 2023; 146(12):4916-34.

64. Pan RY, Zhang J, Wang J, Wang Y, Li Z, Liao Y, et al. Intermittent fasting protects against Alzheimer's disease in mice by altering metabolism through remodeling of the gut microbiota. Nat Aging. 2022; 2(11):1024-39.
65. Lv X, Zhan L, Ye T, Xie H, Chen Z, Lin Y, et al. Gut commensal Agathobacter rectalis alleviates microglia-mediated neuroinflammation against pathogenesis of Alzheimer disease. iScience. 2024; 27(11):111116.
66. Prajapati SK, Wang S, Mishra SP, Jain S, Yadav H. Protection of Alzheimer's disease progression by a human-origin probiotics cocktail. Sci Rep. 2025; 15(1):1589.
67. Cao SQ, Wang HL, Palikaras K, Tavernarakis N, Fang EF. Chemotaxis assay for evaluation of memory-like behavior in wild-type and Alzheimer's-disease-like C. elegans models. STAR Protoc. 2023; 4(2):102250.
68. Sirin S, Aslim B. Characterization of lactic acid bacteria derived exopolysaccharides for use as a defined neuroprotective agent against amyloid beta1-42-induced apoptosis in SH-SY5Y cells. Sci Rep. 2020; 10(1):8124.
69. Mihailovich M, Soković Bajić S, Dinić M, Đokić J, Živković M, Radojević D, et al. Cutting-Edge iPSC-Based Approaches in Studying Host-Microbe Interactions in Neuropsychiatric Disorders. Int J Mol Sci. 2024; 25(18):10156.
70. Zhu G, Zhao J, Wang G, Chen W. Bifidobacterium breve HNXY26M4 Attenuates Cognitive Deficits and Neuroinflammation by Regulating the Gut-Brain Axis in APP/PS1 Mice. J Agric Food Chem. 2023; 71(11):4646-55.
71. Sun J, Xu J, Yang B, Chen K, Kong Y, Fang N, et al. Effect of Clostridium butyricum against Microglia-Mediated Neuroinflammation in Alzheimer's Disease via Regulating Gut Microbiota and Metabolites Butyrate. Mol Nutr Food Res. 2020; 64(2):e1900636.
72. Foster SG, Mathew S, Labarre A, Parker JA, Tompkins TA, Binda S. Lacticaseibacillus rhamnosus HA-114 and Bacillus subtilis R0179 Prolong Lifespan and Mitigate Amyloid-β Toxicity in C. elegans via Distinct Mechanisms. J Alzheimers Dis. 2024;101(1):49-60.
73. 73. Tan FHP, Liu G, Lau SYA, Jaafar MH, Park YH, Azzam G, et al. Lactobacillus probiotics improved the gut microbiota profile of a Drosophila melanogaster Alzheimer's disease model and alleviated neurodegeneration in the eye. Benef Microbes. 2020; 11(1):79-89.
74. Qu L, Liu F, Fang Y, Wang L, Chen H, Yang Q, et al. Improvement in Zebrafish with Diabetes and Alzheimer's Disease Treated with Pasteurized Akkermansia muciniphila. Microbiol Spectr. 2023; 11(3):e0084923.
75. Choo S, An M, Lim YH. Protective Effects of Heat-Killed Ruminococcus albus against β-Amyloid-Induced Apoptosis on SH-SY5Y Cells. J Microbiol Biotechnol. 2024; 34(1):85-93.
76. Akbari E, Asemi Z, Daneshvar Kakhaki R, Bahmani F, Kouchaki E, Tamtaji OR, et al. Effect of Probiotic Supplementation on Cognitive Function and Metabolic Status in Alzheimer's Disease: A Randomized, Double-Blind and Controlled Trial. Front Aging Neurosci. 2016; 8:256.
77. Tamtaji OR, Heidari-Soureshjani R, Mirhosseini N, Kouchaki E, Bahmani F, Aghadavod E, et al. Probiotic and selenium co-supplementation, and the effects on clinical, metabolic and genetic status in Alzheimer's disease: A randomized, double-blind, controlled trial. Clin Nutr Edinb Scotl. 2019; 38(6):2569-75.
78. Ton AMM, Campagnaro BP, Alves GA, Aires R, Côco LZ, Arpini CM, et al. Oxidative Stress and Dementia in Alzheimer's Patients: Effects of Synbiotic Supplementation. Oxid Med Cell Longev. 2020; 2020:2638703.
79. Leblhuber F, Steiner K, Schuetz B, Fuchs D, Gostner JM. Probiotic Supplementation in Patients with Alzheimer's Dementia - An Explorative Intervention Study. Curr Alzheimer Res. 2018; 15(12):1106-13.
80. Agahi A, Hamidi GA, Daneshvar R, Hamdieh M, Soheili M, Alinaghipour A, et al. Does Severity of Alzheimer's Disease Contribute to Its Responsiveness to Modifying Gut Microbiota? A Double Blind Clinical Trial. Front Neurol. 2018; 9:662.
81. Hwang YH, Park S, Paik JW, Chae SW, Kim DH, Jeong DG, et al. Efficacy and Safety of Lactobacillus Plantarum C29-Fermented Soybean (DW2009) in Individuals with Mild Cognitive Impairment: A 12-Week, Multi-Center, Randomized, Double-Blind, Placebo-Controlled Clinical Trial. Nutrients. 2019; 11(2):305.
82. Kobayashi Y, Kinoshita T, Matsumoto A, Yoshino K, Saito I, Xiao JZ. Bifidobacterium Breve A1 Supplementation Improved Cognitive Decline in Older Adults with Mild Cognitive Impairment: An Open-Label, Single-Arm Study. J Prev Alzheimers Dis. 2018; 6(1):70-5.
83. Kobayashi Y, Kuhara T, Oki M, Xiao JZ. Effects of Bifidobacterium breve A1 on the cognitive function of older adults with memory complaints: a randomised, double-blind, placebo-controlled trial. Benef Microbes. 2019; 10(5):511-20.
84. Asaoka D, Xiao J, Takeda T, Yanagisawa N, Yamazaki T, Matsubara Y, et al. Effect of Probiotic Bifidobacterium breve in Improving Cognitive Function and Preventing Brain Atrophy in Older Patients with Suspected Mild Cognitive Impairment: Results of a 24-Week Randomized, Double-Blind, Placebo-Controlled Trial. J Alzheimers Dis. 2022;88(1):75-95.
85. Jung SJ, Cho K, Jung ES, Son D, Byun JS, Kim SI, et al. Augmenting Cognitive Function in the Elderly with Mild Cognitive Impairment Using Probiotic Lacticaseibacillus rhamnosus CBT-LR5: A 12-Week Randomized, Double-Blind, Parallel-Group Non-Comparative Study. Nutrients. 2025; 17(4):691.
86. Xiao S, Chan P, Wang T, Hong Z, Wang S, Kuang W, et al. A 36-week multicenter, randomized, double-blind, placebo-controlled, parallel-group, phase 3 clinical trial of sodium oligomannate for mild-to-moderate Alzheimer's dementia. Alzheimers Res Ther. 2021; 13(1):62.
87. Nagpal R, Neth BJ, Wang S, Craft S, Yadav H. Modified Mediterranean-ketogenic diet modulates gut microbiome and short-chain fatty acids in association with Alzheimer's disease markers in subjects with mild cognitive impairment. EBioMedicine. 2019; 47:529-42.
88. Hazan S. Rapid improvement in Alzheimer's disease symptoms following fecal microbiota transplantation: a case report. J Int Med Res. 2020; 48(6):300060520925930.
89. Park SH, Lee JH, Shin J, Kim JS, Cha B, Lee S, et al. Cognitive function improvement after fecal microbiota transplantation in Alzheimer's dementia patient: a case report. Curr Med Res Opin. 2021; 37(10):1739-44.
90. Park SH, Lee JH, Kim JS, Kim TJ, Shin J, Im JH, et al. Fecal microbiota transplantation can improve cognition in patients with cognitive decline and Clostridioides difficile infection. Aging. 2022; 14(16):6449-66.