Review

The mechanism of the gut microbiota affecting the development of Alzheimer's disease and expectations on therapeutic methods

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Abstract:

Alzheimer's disease (AD) is the most common neurological disease that causes dementia, affecting millions of people every year. Recent studies have shown that there is a potential relationship between AD and gut microbiota. The changes of gut microbiota and their metabolites are related to neuroinflammation and can affect the disease progression of all stages of AD. This paper summarizes the changes of gut microbiota in each stage of AD, the mechanism of gut microbiota affecting the progression of AD and the means of treating AD by changing gut microbiota. This work may play an important role in the diagnosis and treatment of AD staging.

Keywords: Alzheimer's disease (AD), Gut microbiota, Neuroinflammation, Stages of AD, Antibiotic

Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disease leading to dementia and affects millions of people and their guardians and families every year. Its main pathological features exist in the deposition of Aβ amyloid oligomer and protein outside neurons, production of neurofibrillary tangles composed of hyperphosphorylated tau protein inside neurons, regional cerebral glucose metabolism decline, synaptic dysfunction and mitochondrial dysfunction [1]. AD is generally divided into three stages, subjective cognitive decline (SCD), mild cognitive impairment (MCI) and dementia, while it takes many years to develop from SCD to dementia. The long pre-clinical stage provides opportunities for the treatment of this disorder. However, the specific pathogenesis of AD is unclear, and it is still difficult to diagnose AD before the

onset of dementia symptoms [2]. Reportedly, symbiotic microbiota, such as the gut microbiota, can regulate host immunity, affect brain function, and cause effects on neurodegenerative diseases such as AD. Once the antibiotics are administered to AD model mice to interfere with their gut microbiota, the concentration of neuroinflammatory response and blood Aβ will decrease. There is a symbiotic microbiota in the human intestinal tract. Over the past years, researchers have found that there is also a complex relationship among the gut microbiota and brain function and behavior. The two-way relationship between the gut microbiota and the brain is known as the brain-gut axis [3]. The gut microbiota is also different at different stages of AD, so understanding the changes of the gut microbiota may help to diagnose the staging of AD, the process of AD and its treatment. This paper aims to explore the changes of the gut microbiota in stages of AD, expound the mechanism of the gut microbiota affecting the development of AD, and

delve into the methods to treat AD by changing the gut microbiota.

Staging of AD

According to the AD Research Framework of National Institute on Aging and Alzheimer's Association (NIA-AA), AD is divided into totally four stages, respectively are asymptomatic stage, transitional stage, mild cognitive impairment (MCI) and dementia. SCD corresponds to the transition phase for this framework [4]. SCD refers to a significant decline in memory or other cognitive abilities of individuals relative to their previous performance in the absence of objective neuropsychological defects. It occurs before cognitive impairment, and patients have only slight neuronal damage and can make functional compensation. Two research criteria are included in this study: first, the cognitive ability continues to decline compared with the previous normal state, and this decline is not caused by unexpected events; second, in the standardized cognitive test used to classify MCI or precursor AD, there is a normal performance after adjustment in terms of age, gender and education [5]. MCI is a symptomatic predementia stage in the continuous process of cognitive decline. Clinical features include self- or guardian-reported cognitive symptoms, the presence of objective cognitive impairment, functional independence, and the absence of dementia. MCI patients can be divided into two categories: amnestic MCI (a-MCI), which underperforms on neuropsychological tests of episodic memory, and non-amnestic MCI (na-MCI), which underperforms on neuropsychological tests covering cognitive domains other than memory (e.g., executive function, language, or visuospatial ability) [6]. Dementia stages are characterized by severe progressive cognitive impairment that affects multiple domains and neurobehavioral symptoms. These conditions may be reported by the individual or guardian or observed through changes in a longitudinal cognitive testing. Among them, cognitive disorders and neurobehavioral symptoms will exert a significant impact on daily life, and patients no longer become completely independent and need help in daily activities. This is the main feature that distinguishes dementia stages from MCI. Dementia may be further divided into mild, moderate and severe dementia. From mild to severe dementia, the activities of daily living are progressively lost until they are completely dependent on others to live [7].

1. Changes of the gut microbiota in early clinical stage of AD

SCD is the earliest stage in the pathogenesis of AD, and during this stage, changes in the gut microbiota such as significant reduction in the abundance of anti-inflammatory bacteria Faecalibacterium [8], reduction in the number of bacteria producing short-chain fatty acids (SCFAs), decline in the number of butyric acid-producing flora and SCFA-producing flora compared with normal people and patients in MCI stage [9] have taken place in the intestines of patients. Species of the reduced number of SCFA-producing flora include the Class Clostrida, Clostridiales, and Ruminococcaceae. In addition, the abundance of phylum Firmicutes and the number of the class Clostrida, order Clostridiales, family Ruminococcaceae, and genus Faecalibacterium show a gradual decline from normal cognitive stage to SCD and MCI [8].

Furthermore, the relative abundance of Bacteroides and the number of Gram-negative bacteria in the intestinal tract of SCD patients are increased. The level of lipopolysaccharide (LPS), a component of the cell membrane of Gram-negative bacteria, also elevated in patients' plasma as compared with normal subjects.

Besides, the abundance of the microflora also correlates with other indicators. The level of anti-inflammatory cytokine IL-10 in plasma is negatively correlated with the total abundance of pro-inflammatory Gram-negative bacteria, and the total level of Gram-negative bacteria is negatively correlated with auditory verbal learning test (AVLT) [9].

1.1 The mechanism of the gut microbiota affecting the development of AD in the SCD stage

Alterations in the gut microbiota of people at the SCD stage correlate with AD-related cognitive test scores, suggesting that the gut microbiota may be able to influence the progression of AD by altering the flora composition and thus the metabolites of the flora. During the SCD stage, the alterations in flora composition are mainly reflected in:

One is a decrease in the abundance of SCFA-producing microbiota, which may lead to a decrease in the content of SCFAs and thus facilitate one's progression from the cognitively normal to the SCD stage. This suggests that various functions of SCFAs may indirectly or directly slow down the progression of AD. SCFAs are the carbon sources of aerobic metabolism in the host and can promote the bacterial colonization [10]. They have the function to protect and strengthen the intestinal epithelial barrier to prevent pro-inflammatory substances such as lipopolysaccharide (LPS) from entering the bloodstream and triggering inflammation through the following pathways. First, upregulate the transcription of the tight junction protein claudin-1. Second, it acts as a substrate for oxidative synthesis in intestinal cells, synthesizing high levels of ADP and AMP, while the latter induces the expression of glycogen and epidermal growth factors [13]. SCFAs can also affect gastrointestinal physiology, liver metabolism and other peripheral immune functions and the integrity of the blood-brain barrier, and regulate the maturation of microglia, thus indirectly promoting brain function [11]. In the AD population, proliferation, activation

and concentration of microglia in the brain around amyloid plaques is a prominent feature of the disease [11]. In contrast, a mixture of short-chain fatty acids can reduce the secretion of cytotoxins from stimulated THP-1 microglia and thus may slow the progression of neurodegenerative diseases such as AD, possibly due to their ability to reduce cell viability. Low concentrations of short-chain fatty acids can regulate the secretion of the inflammatory cytokine IL-1β via short-chain fatty acid receptors such as nicotinic acid receptor-1 on microglia [12]. A typical SCFA is butyrate. Butyrate inhibits parthenogenic anaerobic Enterobacteriaceae, such as E. coli and Salmonella, by limiting nitrate production, which has been shown can promote probiotic growth, anti-inflammation and immune homeostasis [13]. Butyrate can also affect AD-related immune homeostasis by regulating microglia maturation in two ways. First, reducing inflammatory cytokines such as IL-6 and TNF-α secreted by lipopolysaccharide-stimulated primary microglia inhibits microglia nearby and avoids the excessive release of inflammatory mediators and damage to peripheral brain cells, thereby avoiding the onset of neuroinflammation, a mechanism that contributes to the pathogenesis of AD [14]. Second, butyrate promotes the production of peripheral regulatory T cells outside the thymus, with the homing capacity of peripheral-central nervous system. Regulatory T cells play useful roles in the pathophysiology of AD by slowing down the progression of the disease and regulating microglia responses to amyloid β deposition. Butyrate can also protect the blood-brain barrier. The reduction of the butyrate-producing microflora may lead to the destruction of the blood-brain barrier and thus promotes the progression of AD. Indolepropionic acid, another kind of SCFA, has been reported to protect primary neurons and neuroblastoma cells from Aβ-induced oxidative damage and death, thereby slowing down the progression of AD. In addition to the above two SCFAs, acetic acid can also slow the progression of AD. Acetic acid has been identified as an essential microbiome-derived short-chain fatty acid that drives microglia maturation and regulates homeostatic metabolic states. In AD model mice, acetic acid can modulate microglia function and also exert anti-neuroinflammatory effects by upregulating the G protein-coupled receptor GPR41 and inhibiting the ERK/JNK/NF-κB pathway. Among them, ERK and JNK are key biosynthetic regulators of pro-inflammatory cytokines, and NF-κB is a transcription factor that translocates to the nucleus and induces the expression of pro-inflammatory genes.

The second is the increased abundance of Gram-negative bacteria, which may lead to an increase in lipopolysaccharide (LPS), a characteristic component of the outer leaflet of the Gram-negative bacterial outer membranes within the plasma. In turn, when the intestinal barrier is compromised, the potential for LPS to flow into the extracellular space, blood, or directly into the brain is increased, which in turn causes systemic inflammation,

neuroinflammation, and AD pathological processes associated with amyloidosis and impaired neurocognitive function [9, 15]. Patients with SCD have elevated levels of C-reactive protein and elevated LPS levels compared to the cognitive normal population, suggesting that LPS is indeed associated with inflammation and may cause inflammation. In patients with Alzheimer's disease, enriched LPS binds to toll-like receptors, transmembrane protein receptors expressed in microglia, induces the production of IL-17A and IL-22, two cytokines associated with inflammation-mediated AD, activates the NF-KB signaling pathway to trigger inflammatory responses, and enhances protofibril formation of Aβ peptides, thereby causing neuroinflammation and promoting neurodegeneration [9,15]. LPS can also increase intracellular free iron levels triggering neuroinflammation. Iron is closely associated with immunity and it plays a key role in the proliferation and maturation of immune cells. To protect iron, innate immune cells, including microglia, sequester it within the cell and bind it to cytoplasmic and mitochondrial ferritin. However, iron becomes free when not bound to mitochondrial ferritin, generating excess reactive oxygen species to induce neuroinflammation and oxidative stress-mediated neuronal apoptosis [17, 18]. LPS can also cause defects in multiple synaptic components, synaptic dysfunction and changes in synaptogenesis. In progressive neurodevelopmental and inflammatory neurodegenerative diseases such as AD, the loss of key synaptic components and synaptic tissue disorders are highly correlated with cognitive deficits. Reasons are listed in the following. First, cells are induced to adhere protein and vascular endothelial cells become unstable. Second, it mediates the production of reactive oxygen species and induces massive oxidative stress, which directly affects the abundance and integrity of synapses, synaptogenesis and cognition function. Third, it increases the permeability of the blood-brain barrier and destroys the barrier. Fourth, the activated microglia may promote synaptic destruction and neuroinflammation by promoting a neurotoxic astrocyte phenotype called A1, thereby promoting the peripheral application of LPS to destroy synapses and trigger neuroinflammation. Cognitive and functional impairment caused by AD and related forms of progressive neuroinflammation reflects the progressive neuronal atrophy and synaptic loss.

1.2 Changes of the gut microbiota in the MCI stage

MCI includes both aMCI and na-MCI disease types, with aMCI being more closely related to the AD process. It was shown that compared to subjects with normal cognition, MCI patients have a higher abundance of phylums Bacteroidetes, Proteus, Tenericutes, Firmcutes and families Bacteroidaceae, Enterobacteriaceae and , Mogibacteriaceae. The abundance of genera Shigella, which belongs to the pro-inflammatory bacteria, Brevibacterium,

Coprococcus and Phascolarctobacterium also increased in MCI patients compared to subjects with normal cognition [16, 19]. Bacterial microbiota that were significantly reduced in abundance compared to normal individuals included anti-inflammatory bacteria (rectal fungi), Clostridium and Lachnospiraceae. In contrast, the fungal flora of the intestine of MCI patients showed a slightly lower abundance compared to normal subjects, and the composition of the fungal flora of MCI patients was significantly different from that of normal subjects at the level of the major genera [16]. For example, the fungal flora with increased abundance compared to normal individuals included the phylum Stenotrophomonas,, the families Sclerotiniaceae, Phaffomyceteceae, Trichocomaceae, Cystofilobasidiaceae and Togniniaceae,, the genera Botrytis and Kazachstania. The fungal groups with reduced abundance compared to normal individuals are the Meyerozyma. To further classify MCI patients, the intestinal flora of aMCI patients with increased abundance compared to normal subjects included Bacteroides, and the abundance ratio of Firmicute/Bacteroides (F/B) was significantly higher than in cognitively normal subjects and patients at the SCD stage. Bacterial groups with reduced abundance compared to subjects with cognitive normal are the Naticidae and the Clostridium [14]. Among these groups with changes in abundance, Bacteroides was the one positively correlated with MMSE scores, attention and computational ability test scores, and Phylum Aspergillus and Enterobacteriaceae were the ones negatively correlated with MMSE scores [20].

Compared to the healthy population, MCI patients were not identical in the association between flora and AD-related cerebrospinal fluid biomarkers. MCI patients were identical to the healthy population in that both Phylum Firmcutes and Enterobacteriaceae were positively associated with tau-181 and Enterobacteriaceae was positively associated with the tau-p181/Aβ42 ratio. Unlike the healthy population, Proteus and Lactobacillus acidophilus were positively correlated with the Aβ42/Aβ40 ratio in MCI patients; in the healthy population, Proteus was mainly negatively correlated with Aβ42. Lactobacillus acidophilus was correlated insignificantly with the Aβ42/Aβ40 ratio. In terms of fungi, MCI patients were identical to the healthy population in that Aspergillus and Saccharomyces were negatively and positively correlated with Aβ-40 and Tau protein, respectively. The difference is that in the healthy population Saccharomyces and Candida were positively and negatively correlated with Aβ-40, respectively, while in the MCI group they were not correlated [16].

In terms of metabolites of the intestinal flora, there are two metabolites of intestinal microorganisms that are altered in MCI patients compared to healthy individuals. The first one is short-chain fatty acids. While the three SCFAs, acetic acid, propionic acid and butyric acid, did not differ significantly in content levels between healthy and MCI participants, although MCI participants appeared

to have slightly lower levels of acetic acid and propionic acid than the healthy population [16]. However, the relationship between these metabolites and AD-related cerebrospinal fluid biomarkers differed in MCI patients compared to the normal healthy population. For example, propionic acid was negatively correlated with Aβ42 in the MCI group and positively correlated with tau-p181 in the normal population [19]. In contrast, five short-chain fatty acids, formic acid, acetic acid, propionic acid, butyric acid and isovaleric acid, were significantly decreased in aMCI patients compared to the cognitive normal population. This suggests a closer association with AD, with the more severe the AD process, the lower the content of short-chain fatty acids is. The second one is trimethylamine N-oxide (TMAO). Cerebrospinal fluid TMAO levels were significantly increased in MCI patients compared to healthy individuals and this was significantly and positively correlated with p-tau and p-tau/Aβ42 [21].

1.3 Effects of the gut microbiota in the MCI stage on the pathogenesis of AD

In the MCI stage, compared with the healthy subjects, there are microflorae related to the scores of AD scale and AD biomarkers in the changed microflorae of patients, and the changed flora metabolites are also related to AD biomarkers, indicating a potential relationship between the gut microbiota and the progression of AD. Among the changed microflorae, a decrease in the SCFA-producing microflora Clostridiaceae and other species and an increase in the abundance of Gram-negative bacteria such as Bacteroides have been observed. This may be one of the reasons for the severity of symptoms in the MCI stage compared to the SCD stage. Therefore, the mechanisms associated with changes in intestinal flora promoting AD progression in the SCD stage could also explain part of the mechanisms by which the intestine influences the onset of AD in the MCI stage and the degree of progression is more severe than in the SCD stage. However, unlike the SCD stage, the abundance of Bacteroides increases in the MCI stage. Bacteroides are the most abundant Gram-negative bacteria in the intestinal tract, which can release a very effective pro-inflammatory lipopolysaccharide (BF-LPS). BF-LPS is known as one of the most pro-inflammatory and neurotoxic LPS, and it can destroy the intestinal barrier by cleaving the intercellular protein between intestinal epithelial cells, allowing endotoxin to enter the systemic circulation and then enter the brain through the blood-brain barrier. Furthermore, TLR2 receptor, a transmembrane protein receptor expressed by activated microglia, induces cytokine production, inflammation, phagocytosis, and innate immune defense responses, and mediates inflammatory responses to drive the progression of AD [15, 22].

In addition, NOD-like receptor thermal protein domain associated protein 3 (NLRP3)-related mechanisms may also explain the intestinal effects on the pathogenesis of

AD in the MCI stage. In the MCI stage, the abundance of Escherichia, a pro-inflammatory bacterium, is increased. Escherichia coli (E. coli) is a microflora belonging to Escherichia, and the increase in its abundance may be related to peripheral inflammation in patients with cognitive impairment and cerebral amyloidosis. E. coli has a positive correlation with the level of IL-1β, a cytokine secreted by stimulated NLRP3 inflammasome, and can induce pro-inflammatory cytokine production through a NLRP3-dependent mechanism [23]. In mice, a Rho GTPase-activating toxin from Escherichia coli, CNF1, dependent on Rac2 and Pak serine/threonine kinase, can trigger inflammation through an NLRP3-dependent mechanism [24]. NLRP3 inflammasome is an intracellular complex composed of multiple subunits [25], which can increase the levels of pro-inflammatory factors such as IL-1, β and IL-18 in the brain by activating cysteine aspartic acid specific protease-1 (caspase-1). The accumulation of many pro-inflammatory factors can further promote the activation of microglia, induce inflammatory apoptosis of nerve cells, and ultimately destroy the memory and cognitive ability of patients and accelerate the pathological progression of AD [26]. Other studies have shown that the activated NLRP3 can accelerate the progression of AD by inducing hyperphosphorylation and aggregation of tau via IL-1β-dependent tau kinase [27].

During the MCI phase, intestinal flora may also influence the progression of AD by causing an increase in the flora metabolite TMAO. TMAO is thought to be involved in disease development through a variety of mechanisms such as altering lipid and hormone homeostasis, promoting platelet hyperreactivity, regulating cholesterol and sterol metabolism, reducing reverse cholesterol transport, and inducing endothelial dysfunction through activation of the NLRP3 inflammasome. TMAO can reach the central nervous system and be detected. In the brain, TMAO has been shown to contribute to brain aging and cognitive impairment by inducing neuronal aging, increasing oxidative stress, impairing mitochondrial function, and inhibiting mTOR signaling. Elevated cerebrospinal fluid TMAO may also exacerbate central insulin resistance and the onset of AD. In terms of association with AD biomarkers, cerebrospinal fluid TMAO was associated with cerebrospinal fluid p-tau and p-tau/Aβ42, but not Aβ42/Aβ40, which may indicate that TMAO is more closely related to tau pathology than amyloid deposition alone. In addition, cerebrospinal fluid TMAO was associated with elevated cerebrospinal fluid t-tau and neurofilament light chain (NFL), but not with elevated neurogranulin. Cerebrospinal fluid t-tau and NFL are thought to reflect axonal integrity, with higher levels indicating more severe axonal degeneration, whereas neurogranulins reflect synaptic integrity, suggesting that TMAO is associated with axonal injury but not dendritic degeneration. These suggest that although TMAO may not be a major driver of amyloid production, it may affect vulnerable neurons and contribute to neurodegeneration [27].

2. The changes of the gut microbiota in the stage of AD dementia

Compared with people with normal cognition, AD patients in the dementia stage, the gut microbiota will further vary at the level of microflora quantity, abundance and metabolites, which can affect the brain through different pathways, thus promoting the progression of AD.

2.1 Mild and moderate AD
2.1.1 Changes in abundance and quantity of the gut microbiota

Compared with people with normal cognition, patients with mild and moderate AD have different changes in the abundance of different kinds of intestinal microflorae. At the phylum level in AD patients, the abundance of Actinobacillus is slightly higher, the abundance of Bacteroides slightly decreases, and that of Firmicutes significantly decreases [28]. At the genus level, the abundance of Bifidobacterium, Viscous actinomycetes, Sphingomonas, Neorhizobium, Ruminococcus, Actinomyces, Bacillus, Flarobacteria, Bacteroides, Weissella, Bilophila, Escherichia, blautia, XLVa Clostridium, and Lysobacter increases, while that of gram-negative bacilli, bacillus, Bacteroides, Odoribacter, Eubacterium, Anaerobacter, and Papillibacter decreases [29]. At the family level, the abundance of the Ruminant Coccaceae, Enterococcaceae, and Lactobacteriaceae increases, while the microflorae of Clostridiaceae, Ruminococcaceae, Lachnospiraceae, Bacteroidaceae, and Veillonellaceae declines. Patients with mild-to-moderate AD have a high abundance of Proteus and Enterobacterium and a low abundance of Clostridiaceae, Lachnospiraceae, Ruminococcaceae, Ruminococcus, Blautia and Bacteroides compared with patients with MCI. Among the abundance-increased microflorae, Flarobacterium and Escherichia/Shigella are Gram-negative bacteria, which are a microflora capable of releasing LPS and a microflora associated with inflammation. Notably, Bacteroides can be significantly enriched in the pre-AD phase and unexpectedly decrease to normal levels in the AD group [30, 31]. Sphingomonas and Clostridium XLVa belong to the microflora which has a negative correlation with the scores of Mini Mental State Examination (MMSE) and a positive correlation with the scores of Clinical Dementia Rating Scale (CDR). Among microflorae with reduced abundance, Clostridium, Lachnospiraceae, Ruminococcus, Odoribacter, Eubacterium and Papillibacter are SCFA-producing microflorae, which are correlated positively with MMSE scores and negatively with CDR scores [29]. Clostridium, Lachnospiraceae bacterium and Ruminococcus are the microflorae related to insulin resistance, one of the risk factors for the development of AD [14].

Intestinal microflorae are quantitatively increased in

patients with mild-to-moderate AD, compared with normal subjects, including the microflora associated with inflammation—Escherichia coli, the microflora associated with medial temporal lobe atrophy—Akkermansia, the microflora inhibiting melatonin secretion—Streptococcus, and Dorea and Brucella associated with lower MMSE scores [32]. Interestingly, the number of Lactobacillus—the microflora that contains probiotics, will also increase [29].

In addition, the number of microbial communities that protect the intestinal barrier, such as Ruminococcus and Clostridium, is reduced. The number of anti-inflammatory microbial communities such as actinomycetes belonging to Bifidobacterium is also reduced. However, in the altered microbial communities of AD patients compared with cognitively normal people, the Faecalibacterium to Bifidobacterium ratio can be calculated to distinguish AD patients from healthy people for noninvasive diagnosis of AD [33].

2.1.2 Functional changes of the gut microbiota

The gut microbiota of mild-to-moderate AD patients also undergoes further functional changes. The membrane transport function of the microbial community is enhanced, whereas the immune function, protein folding or sorting and degradation functions, environmental adaptation function, and cell growth and death functions are weakened [29]. The metabolic function of the microbial community shows different changes at different levels. In terms of bile acid metabolism, compared with MCI patients, in mild-to-moderate AD patients, metabolic levels and types of bile acids associated with decreased hippocampal volume are further increased. Among them, increased GCDCA bile acids are also significantly associated with decreased glucose metabolism in AD patients. At the same time, the content of bile acid TLCA, which is associated with increased glucose metabolism in the left temporal lobe, is reduced [16]. At the level of tryptophan metabolism of the microbial community, the degree of tryptophan metabolism disorder is reduced. In AD patients, the metabolic level of the endogenous tryptophan metabolite indole-3-pyruvic acid is significantly increased compared with cognitively normal people and those patients with mild cognitive impairment, and there are significant differences in indole-3-pyruvic acid metabolism levels among these three groups, so indole-3-pyruvic acid can serve as a signal to differentiate among the three stages of the disease. In terms of SCFA metabolism in microbial communities, the metabolic level of SCFAs in AD patients is further decreased. In terms of the level of trimethylamine oxide (TMAO) metabolism in the microbial community, the amount of TMAO metabolized in AD patients is increased compared with normal people, but there is no significant difference from the metabolic level in MCI patients [21]. Furthermore, lipid metabolism of the microbial community is enhanced, while energy, cofactor and vitamin metabolism levels are decreased.

2.2 Severe AD
2.2.1 Changes in the abundance of the gut microbiota of severe AD patients in microbial communities

The changes in the abundance of the gut microbiota of severe AD patients in microbial communities are more than those in increased and decreased flora species compared with the healthy population. At the phylum level, there is a marked increase in Actinobacteria and Verrucomicrobia. At the family level, key functional bacterial families such as Bifidobacteriaceae, Verrucobacterium, Corynebacteriaceae, erysipelas Bacillus, Enterococcaceae, Corynebacteriaceae and Akkermansia muciniphila increase significantly, while Ruminococcus, Lachnospiraceae and Clostridiaceae-1 decrease sharply. At the genus level, Faecalibacterium, Roseburia, anaerobic Bacillus, Gemmobacter, Dialister, Romboutsia, Coprococcus and Butyricicoccus decrease. Among the microbial communities with increased abundance, Verrucomicrobia and Bifidobacteriaceae are lactic acid-producing microbial communities; Enterococcaceae and Corynebacteriaceae are microbial communities positively correlated with pro-inflammatory cytokines such as TNF-α, and Akkermansia muciniphila is a microbial community inversely associated with the anti-inflammatory cytokine interferon-γ. TNF-α is elevated in content in AD patients and is a key factor in promoting inflammatory response. Among the reduced microbial communities, Gemmobacter is a microbial community inversely related to inflammatory cytokines such as IP-10, and Faecalibacterium, Roseburia and Dialister are microbial communities inversely associated with pro-inflammatory cytokines such as TNF-α and chemokines such as IP-10. Among them, Dialister is also a microbial community negatively correlated with the ratio of p-tau and p-tau/Aβ42. The increased microbial communities are negatively correlated with clinical indicators such as MMSE, and the decreased ones are positively correlated with clinical indicators such as MMSE, which is the same as the characteristics of the gut microbiota in mild-to-moderate AD patients [33].

In terms of the functions of the microbial communities, carbohydrate metabolism, biodegradation and metabolism of xenobiotics, transport and catabolism are enhanced. At the same time, the synthesis of folic acid, the metabolism of fatty acids and lipoic acid are enhanced. Nevertheless, transcriptional function, immune function, environmental adaptation, and bacterial chemotaxis are significantly reduced. Among the weakened functions of the gut microbiota, the first three functions can also be observed in patients with early AD [33].

2.2.2 The mechanism of the gut microbiota affecting AD progression in the dementia stage

In general, more abundant species of microbial communities observed in AD patients are associated with a more severe disease progression; the disease progression is more optimistic in the opposite case. It can be inferred that the microbial communities with a strong correlation with AD biomarkers in the AD population may be involved in the pathogenesis and development of AD [33]. Among the genera with high abundance, there are Gram-negative bacteria such as Flavobacterium, microbial communities such as Escherichia that are related to the NLRP3 inflammatory mechanism, and microbial communities positively related to pro-inflammatory cytokines, such as Enterococcaceae. Among the less abundant genera, there are SCFA-producing microbial communities such as lachnospiraceae and Ruminococcus, and microbial communities inversely associated with pro-inflammatory cytokines, such as Faecalibacterium. Therefore, the hypothesis used to explain the link between changes in the gut microbiota during SCD and MCI stages and the pathogenesis of AD may also explain that, to a certain extent, the mechanism by which the gut microbiota affects AD progression during the dementia stage. At the same time, this indicates that the microbiota may promote AD progression through the mechanism of releasing a large number of pro-inflammatory cytokines [34]. Pro-inflammatory factors can recruit microglia and astrocytes to the inflamed site. Under normal circumstances, this process promotes the removal of irritants or pathogens, allowing the inflammatory response to subside. In AD, however, excessive Aβ production and hyperphosphorylated tau make this immune clearance mechanism ineffective, and the recruited microglia and astrocytes in the inflammatory site cannot effectively clear Aβ, resulting in the production of more pro-inflammatory cytokines and chemokines [35]. Moreover, the microbiota may influence AD progression through other mechanisms. One is to increase intestinal permeability. A microbial community, Akkermansia muciniphila, that is increased in the intestinal tract of patients with dementia can increase the permeability of the intestinal mucosa [10]. The increased permeability of the intestinal barrier can allow the passage of bacterial products, aggravate the deposition of amyloid plaques in the brain, and lead to a decline in cognitive function. This may be the reason for its association with medial temporal lobe atrophy. The second is the inhibition of melatonin. In the intestinal tract of patients with dementia, the microbial communities that inhibit melatonin secretion—Streptococcus and Enterococcus—increase [36]. Melatonin can regulate the balance between Aβ production and clearance in the brain, reduce the neurotoxicity of Aβ, and improve the cognitive function of AD patients. Therefore, the reduction of melatonin and the progression of AD are in parallel [37].

The changes in the abundance of the gut microbiota in AD patients compared to that in normal people may lead to changes in gut microbiota metabolites, resulting in partial metabolic disorders in the human body, and

the latter may be an important part of AD progression. During the dementia stage, dysregulated metabolic pathways include the SCFA pathway, the TMAO pathway, the bile acid pathway, and the tryptophan pathway [20]. Among them, SCFAs may be related to the decline in the number of bacteria producing SCFAs, and the mechanism is similar to the SCFA hypothesis in the SCD stage. Dysregulation of the TMAO pathway is similar to the mechanism by which it affects AD progression during the MCI stage. On the level of cholesterol metabolism, AD patients experience functional failure and dysregulation of bile acid levels. Primary bile acids are synthesized from cholesterol and catalyzed by 7α-dehydroxylase to form secondary bile acids. The gut microbiota participates in human co-metabolism to produce bile acids, and its changes can affect the changes of bile acid metabolites. It has been shown that Lachnospiraceae can promote colonization resistance of the microbiota to resistant pathogens by converting primary bile acids to secondary bile acids [38]. Thus reduced abundance of Lachnospiraceae in AD patients may be a cause of cholesterol metabolic failure. Additionally, the gut microbiota does affect Aβ, tau and neurodegeneration [16]. Compared with normal people, AD patients have significantly reduced cholic acid (CA) levels, while the levels of the four secondary bile acids and deoxycholic acids (DCAs) produced by bacteria—glycodeoxycholic acid (GDCA), taurodeoxycholic acid (TDCA), glucolithocholic acid (GLCA), and taurolithocholic acid (TLCA)—are significantly increased [42]. The CA has a long hydrophobic chain that prevents protein self-assembly and can stabilize protein-CA complexes. There is a study showing that the CA can effectively inhibit the formation of Aβ, and the molecular mechanism of the inhibition may be its binding to the strong hydrogen bonds of the two chains of human insulin [39]. Among the elevated bile acids, DCA and GDCA are associated with cognitive decline in AD. In addition, cerebrospinal fluid (CSF) biomarkers, the brain's structural changes, and glucose metabolism are associated with specific BAs and related ratios. GLCA and TLCA are linked to higher CSFt-tau values, higher fibrillar tau and biomarker levels of neurodegeneration/neuronal damage. Increased levels of GLCA and the ratio of GLCA to CDCA are significantly related to decreased glucose metabolism in the bilateral temporal and parietal lobes. Low levels of TLCA are correlated with increased glucose metabolism in the left temporal lobe. The six ratios of high levels of secondary to primary bile acids produced by bacteria (DCA:CA, GDCA:CA, TDCA:CA, GDCA:DCA, GLCA:CDCA, and TLCA:CDCA) are correlated to reduced hippocampal volume. High levels of GCDCA are significantly associated with decreased glucose metabolism, and especially in the bilateral hippocampus, they are positively correlated with cortical thickness. These association patterns suggest a potential linking mechanism between peripheral and central biochemical changes. Thus, dysregulation of the

bile acid metabolic pathway may affect the progression of AD. At the level of tryptophan metabolism, AD patients have abnormal serotonin synthesis [40]. Some bacteria belonging to groups with reduced abundance in AD patients, such as Clostridium, Ruminococcus, Blautia, and Lactobacillus, have been identified as capable of converting tryptophan to tryptamine. In turn, tryptamine induced the release of serotonin from intestinal chromophores by 5-hydroxytryptamine. It is suggested that the reduction in the abundance of these flora may lead to abnormal serotonin synthesis in AD patients by reducing tryptamine production. In mice with an autism model, most Clostridium spp. were negatively associated with reduced tryptophan and serotonin metabolites in the cecum [41, 42]. Although serotonin is not able to cross the blood-brain barrier, i.e., there are two distinct metabolic pools of serotonin in the brain and the gut, it is possible that the gut microbiota metabolism interacts with the brain. Consistent with changes in intestinal serotonin, AD patients' brains, particularly in the temporal and frontal cortexes, also have reduced levels of serotonin. Research has indicated that in aged rats, a diet high in tryptophan can change the expressions of serotonin and brain-derived neurotrophic factors in the frontal cortex and hippocampus, and enhance learning and cognitive abilities [43]. These indicate a potential link between tryptophan metabolism and AD pathogenesis.

Table 1. Changes in gut microbiota in patients at various stages of AD compared to cognitive normal populations and their effects on pathogenesis of AD

Disease stage	Increased abundance of gut microbiota	Decreased abundance of intestinal flora	Effects on the pathogenesis of AD
SCD	Bacteroides	phylum Firmicutes, class Clostrida, order Clostridiales, and family Ruminococcaceae	Dereased SCFAs and increased LPS→damage to intestinal barrier→Over-activation of microglia→neuroinflammation
MCI	anti-inflammatory bacteria (rectal fungi),Clostridium and Lachnospiraceae fungi：phylum Stenotrophomonas; families Sclerotiniaceae, Phaffomyceteceae, Trichocomaceae, Cystofilobasidiaceae , Togniniaceae; the genera Botrytis and Kazachstania	phylum Bacteroidetes, Proteus, Tenericutes, Firmcutes; families Bacteroidaceae, Enterobacteriaceae, Mogibacteriaceae; genera Shigella, Brevibacterium, Coprococcus and Phascolarctobacterium	Dereased SCFAs and increased LPS →damage to intestinal barrier / Increased TMAO→activated NLRP3 inflammatory vesicles } Over-activation of microglia→neuro-inflammation
Mild and Moderate AD	Phylum Actinobacillus; genera Bifidobacterium, Viscous actinomycetes, Sphingomonas, Neorhizobium, Ruminococcus, Actinomyces, Bacillus, Flarobacteria, Bacteroides, Weissella, Bilophila, Escherichia, blautia, Clostridium, Lysobacter, Akkermansia, Streptococcus, Dorea, Brucella, Lactobacillus; families Ruminant Coccaceae, Enterococcaceae and Lactobacteriaceae	Phylum Bacteroides; genera gram-negative bacilli, Bacillus, Odoribacter, Eubacterium, Anaerobacter, Papillibacter; families Clostridiaceae, Ruminococcaceae, Lachnospiraceae, Bacteroidaceae and Veillonellaceae	Dereased SCFAs and increased LPS →damage to intestinal barrier } → Over-activation of microglia→neuro-inflammation / Increased TMAO→activated NLRP3 inflammatory vesicles } → Reduced inhibition of Aβ formation / Decreased Cholic acids
Severe AD	Phylum Actinobacteria , Verrucomicrobia; genera Bifidobacteriaceae, Verrucobacterium, Corynebacteriaceae, erysipelas Bacillus, Enterococcaceae, Corynebacteriaceae and Akkermansia muciniphila	Families Ruminococcus, Lachnospiracea, Clostridiaceae-1; genera Faecalibacterium, roseburia, anaerobic Bacillus, Gemmobacter, Dialister, Romboutsia, Coprococcus and Butyricicoccus	Decrease in tryptamine-producing microbiota such as Clostridium→ Abnormalities in serotonin synthesis → AD-related cognitive impairment

3. Treatment specific to the microbiota

3.1 Probiotic therapy

Current data on the therapeutic effects of probiotics and prebiotics in Alzheimer's disease is not extensive. In a transgenic mouse model of Alzheimer's disease, SLAB 51 probiotic cocktail therapy induced changes in the microbial community that resulted in alterations in the content of gut metabolites, such as short-chain fatty acids that improve cognitive function [44]. Other studies also support the idea that gut microbiota can help prevent the development of Alzheimer's disease, in part because the gut microbiota can produce short-chain fatty acids that interfere with the formation of toxic soluble amyloid aggregates [45].

Oral administration of Bifidobacterium short A1 improves cognitive performance in mice with Alzheimer's disease [46]. Gene profiling analysis showed that ingestion of bifidobacterium brevifolium A1 inhibited the inflammation of immune response genes induced by hippocampus and amyloid proteins [46]. A study conducted by the same group showed that supplementation with Bifidobacterium short A1 could have beneficial effects on cognitive function in older adults with memory problems [47]. In 124 healthy adult volunteers who received probiotic milk drinks or placebo for 21 days, the probiotic group had slightly worse cognitive function [48]. Another study showed that intake of bioactive peptides from dairy products improved cognitive function [49]. Tryptophan-related dipeptides and novel lactopeptides in fermented dairy products inhibit microglial activation and improve memory function and cognition [50, 51]. In addition, an epidemiological study involving 1056 participants showed that dietary cheese inclusion was associated with a lower prevalence of cognitive impairment [52]. In addition, a study of 1006 Japanese people aged 60-80 years with dementia observed for 15 years showed that high consumption of milk and dairy products could reduce the risk of dementia [53].

A healthy dietary pattern with high intake of prebiotics and probiotics combined with other nutrients can delay cognitive decline and reduce the risk of Alzheimer's disease [54]. Furthermore, consumption of fermented dairy products containing probiotics not only affects normal brain activity [55], but also leads to significant cognitive improvements in patients with Alzheimer's disease [56]. These effects may be caused by restoration of the gut microbiota or by the opposite effects of other pathological events associated with alzheimer's disease, such as oxidative stress [56, 57]. Transgenic Alzheimer's disease mice treated with probiotics have been shown to have better cognitive performance and a lower number of amyloid plaques than untreated Alzheimer's disease mice [58]. In another study, similar effects on cognitive function in transgenic Alzheimer's disease mice were documented after prebiotic administration [59].

3.2 Fecal transplants

The principle of fecal transplantation is similar to that of probiotics, but the difference is that the flora in feces is more complete and complex. Compared with known single probiotics, the effect of fecal transplantation may be better. A patient infected with CDI developed symptoms of AD after ineffective treatment with multiple antibiotics, and then his spouse, as a volunteer, gave stool as a source of bacteria, which significantly improved the patient's AD symptoms [60].

However, according to the research on pathology and molecular mechanism, fecal transplantation seems to lack more powerful support and needs more extensive clinical research. In addition, the existing conclusions are often limited to animal experiments, and the therapeutic effect of fecal transplantation on humans needs to be further explored [61, 62].

3.3 Possible treatment methods: antibiotics are used to clear the gut microbiota to delay AD progression

In AD animal models, some antibiotics can eliminate the neuroinflammation caused by the dysbiosis of the gut microbiota, thereby having a beneficial effect on AD. These effects include neuroprotective and anti-inflammatory, anti-tau protein, anti-amyloid protein, and cholinergic effects. The use of rifampicin in AD animal models reduces the levels of Aβ and inflammatory cytokines in the brain [63]. Minocycline has a similar effect on Aβ and reduces microglia activation in rodent AD models [64]. Likewise, rapamycin has been proven to reduce not only Aβ and microglia activation but also tau phosphorylation [65]. d-Cycloserin, also an NMDA receptor partial agonist, ameliorates cognitive deficiencies in aged rats [66] and in AD patients [67]. All of the above antibiotics can mitigate inflammation and improve cognitive deficits only in animal experiments, while the results obtained in some clinical trials are controversial.

In 2004, a combined use of doxycycline and rifampicin resulted in a significant improvement at 6 months on the Standardized Alzheimer's Disease Assessment Scale-Cognitive (SADAScog) subscale in patients with AD and mild-to-moderate dementia [68]. Conversely, in 2013, a multicenter, blinded, randomized, 2 × 2 factorial controlled trial in patients with mild to moderate AD showed that there was no significant effect on cognitive function at 12 months of doxycycline or rifampicin treatment alone or in combination [69]. Likewise, in 1999, d-cycloserine was found to be effective in improving the cognitive deficits of AD patients [50], but the positive effect was not repeated in subsequent trials. The presence or absence of bacterial infection, such as Helicobacter pylori [70], or being affected by the action of antibodies, may account for such a large difference in the results of these experiments.

Nonetheless, these studies still provide evidence for possible effects of antibiotics on intestinal bacteria in AD.

Furthermore, antibiotics can have positive effects on AD through other mechanisms besides neuroinflammation [64]. For example, rapamycin, which is claimed to have a so-called anti-aging function [71], is in fact a natural inhibitor of the mammalian target of rapamycin (mTOR). The upregulation of the mTOR signaling pathway plays an important role in the main pathological processes of AD. The use of mTOR inhibitors, such as rapamycin, ameliorates AD-like pathological changes and cognitive defects in multiple animal models [65], suggesting their potential to treat AD.

However, the therapeutic effect of antibiotics is unclear, and more attention should be paid to their side effects and possible damage. For AD model animals, the administration of an antibiotic cocktail (ABX) in APP/PS1 transgenic mice has been shown to exacerbate the neuroinflammatory state and increase cytokine levels, thereby promoting the progression of the disease [72]. In humans, when antibiotics are used with cocktail therapy, the side effects may be associated with neurological disorders, resulting in anxiety and panic attacks, major depressive disorder, psychosis, and delirium [73]. However, normal use of antibiotics in the general population is generally not associated with neuropsychiatric side effects. There are also antibiotics that disrupt the balance of the gut microbiota, such as streptozotocin and ampicillin [74]. According to hypotheses about the gut microbiota and AD, the use of these antibiotics promotes the progression of the disease. Administration of ampicillin to rats results in elevated serum corticosterone and increased anxiety-like behavior and spatial memory disorder. Elevated glucocorticoids are linked to memory dysfunction and reduced hippocampal BDNF, two common pathological features of AD. Probiotics (Lactobacillus fermentum strain NS9) can reverse ampicillin-induced physiological and psychological abnormalities in rats [75]. In this regard, germ-free mice are also characterized by similar molecular changes, such as anxiety-like behavior [76], changes in the expressions of tight junction protein, BDNF [77], GRIN2B, serotonin transporter, the NPY system [78], and the HPA axis activity.

Antibiotics such as streptozotocin have been used to induce sporadic AD in animal models and can affect their learning and memory abilities [23, 79]. The same antibiotics are used to induce diabetes in animals [80], and this is a common AD comorbidity characterized by cognitive decline [81, 82]. Furthermore, the administration of probiotic substances as food supplements has beneficial effects on synaptic activity and cognitive function in a streptozotocin-induced diabetic rat model [83]. It has been demonstrated that the expression of NMDA receptors (a class of receptors critical for learning and memory processes) may depend on the presence of the gut microbiota. The mRNA expression of hippocampal NMDA receptor subtype 2B (NR2B) is significantly reduced in germ-free mice [76]. Disruption of the gut microbiota by ampicillin treatment also significantly reduces the level of NMDA receptor in the rat hippocampus [76].

Therefore, the use of antibiotics may be a means of treating AD in the future, but the effects vary from person to person, and a series of side effects cannot be ignored.

3.4 Improvement of gut microbiota composition through diet and exercise to prevent AD development

Multiple clinical trials targeting a single target for AD have failed, suggesting that, in the face of the complex mechanisms of AD development, a multifactorial treatment strategy may be more effective to prevent and slow down the AD progression. For instance, changing the lifestyle, including diet and exercise, is currently the an effective strategy for reducing AD risk. Also, both are optimal regulators of the gut microbiome, so they have the potential to be important AD prevention methods.

Diet is one of the most important regulators of the gut microbiome, which prevents aging-related changes in the microbiome [84]. In studies using animal models of aging in the absence of malnutrition, a low-carbohydrate diet with total calorie restriction has been reported to promote healthy aging [85], stimulate neuroprotective signaling, prevent transcriptional changes in aging-related genes in the hippocampus [86], regulate the expression of Aβ precursor protein [87] to prevent the accumulation of Aβ plaques, and develop specific gut microbes associated with healthy aging [88].

In the complex human gut microbial ecosystem, diet-microbe associations may not be limited to a specific microbiota, but may also include interactions with other microbes, such as fungi (fungal communities), which play common roles in various environments for host's health and disease. However, whether and how the gut fungal communities differ between MCI patients and healthy controls, and whether and how specific dietary interventions affect the characteristics of these fungal communities, remain unclear. Fungi are ubiquitous in our environment, so fungi are also part of normal microbial communities. Although fungal communities are only a small fraction of normal microbial communities, their roles in human health and diseases have recently been investigated. Studies have pointed to the role of intestinal fungi in several human diseases, including inflammatory bowel disease [89, 90], enterocolitis [91, 92], colorectal cancer [93], and graft-versus-host disease [94], alcoholic liver disease [95], asthma [96] and hepatitis B virus infection [97]. Therefore, the characteristics of intestinal fungal communities in MCI patients may interact with the host's diet, gut bacteria and cerebrospinal fluid AD biomarkers. At the same time, research has also found specific gut microbiota signatures in MCI patients, in

which Mediterranean-ketogenic diet (MMKD) modulates the gut microbiota in MCI patients and improves cerebrospinal fluid markers in AD patients.

Despite emerging evidence that the gut microbiome is involved in AD, the relationship among specific dietary patterns, gut microbes, and AD pathophysiology remains unclear. In a recent MCI and CN study, a modified MMKD was found to have good applicability in those older adults at risk for AD. In addition, compared with the control group—a 6-week intervention on the low-fat American Heart Association Diet, the MMKD group had the following changes:
(a) improved biomarkers in the CSF of AD patients,
(b) increased cerebral perfusion and cerebral ketone body uptake and metabolism,
(c) improved metabolic health of MCI patients.

Therefore, improving diet and regular exercise to modulate gut microbiota health may also become an important approach for AD prevention [19].

4. Summary and limitations of research

All in all, current reviewed evidence suggests that the intestinal flora may increase the abundance of pro-inflammatory-associated bacteria and decrease the abundance of anti-inflammatory-associated bacteria by changing the composition, which in turn increases the secretion of pro-inflammatory-capable metabolites and decreases anti-inflammatory-capable metabolites by the flora as a whole and increases the intestinal barrier permeability, thus allowing the accumulation of intestinal or extraintestinal pro-inflammatory substances in the brain and continuously activating immune cells such as microglia in the brain, leading to progressively severe neuroinflammation in the brain, exacerbating the symptoms of cognitive decline and progression of the SCD stage to the MCI stage and then to the AD stage. Although there are many studies on the changes of the gut microbiota at various stages of AD progression in recent years, there are still quite a few limitations. First, some studies are cross-sectional ones which are unable to establish a causal relationship between the gut microbiota and stages of AD development. Longitudinal studies will be needed in the future, with follow-up surveys of patients, focusing on changes in the gut microbiota and its metabolite content during different periods to determine the relationship between the gut microbiota and AD progression [14, 20, 21, 33, 98]. Second, in follow-up studies, attention needs to be paid to the effects of drugs taken by AD patients on the gut microbiome. For example, acetylcholinesterase inhibitor drugs may cause side effects such as gastrointestinal discomfort, nausea and diarrhea in patients, and affect the composition of the gut microbiota [31]. Third, many studies have used the most common method for analyzing the microbiota—16srDNA amplified sequences can only reach the genus level [8], which limits

research on finding specific bacteria associated with AD at the species level. Fourth, follow-up research requires AD-related bacteria through culture to conduct animal experiments to determine the specific causal link between these bacteria and AD pathogenesis [33].

Abbreviations

Abbreviations	Full name
AD	Alzheimer's disease
SCD	subjective cognitive decline
MCI	mild cognitive impairment
SCFAs	short-chain fatty acids
LPS	lipopolysaccharide
AVLT	auditory verbal learning test
MMSE	Mini-mental State Examination
CDR	Clinical Dementia Rating
CSF	cerebrospinal fluid
NLRP3	NOD-like receptor thermal protein domain associated protein 3
TMAO	trimethylamine N-oxide
NFL	neurofilament light chain
F/B	abundance ratio of Firmicute/ Bacteroides
DCAs	deoxycholic acids
GDCA	glycodeoxycholic acid
TDCA	taurodeoxycholic acid
GLCA	glucolithocholic acid
TLCA	taurolithocholic acid
CDI	clostridium difficile
MMKD	Mediterranean-ketogenic diet

Disclosure

The authors declare no conflict of interest.

Authors' contributions

Ye Zhiwei: Conceptualization, Investigation, Data Curation, Writing - Original Draft, Editing
Li Ruohu: Conceptualization, Investigation, Data Curation, Writing - Original Draft, Editing
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Besides, Ye Zhiwei and Li Ruohu have made the same contribution to the article and should be considered as co-first author.

References

[1] C.A. Lane, J. Hardy, J.M. Schott. Alzheimer's disease. *European Journal of Neurology*. 2018;25(1): 59-70. doi:https://doi.org/10.1111/ene.13439.

[2] Lin L., Zheng L.J., Zhang L.J. Neuroinflammation, Gut Microbiome, and Alzheimer's Disease. *Molecular neurobiology*. 2018;55(11): 8243-8250. doi:https://doi.org/10.1007/s12035-018-0983-2.

[3] Minter M.R., Hinterleitner R., Meisel M., et al. Antibiotic-induced perturbations in microbial diversity during post-natal development alters amyloid pathology in an aged APPSWE/PS1DeltaE9 murine model of Alzheimer's disease. *Scientific reports*. 2017; 7(1): 1-8. doi:https://doi.org/10.1038/s41598-017-11047-w.

[4] Jessen F., Amariglio R.E., Buckley R.F., et al. The characterisation of subjective cognitive decline. *The Lancet Neurology*. 2020;19(3): 271-278. doi:https://doi.org/10.1016/S1474-4422(19)30368-0.

[5] Rabin L.A., Smart C.M., Amariglio R.E. Subjective Cognitive Decline in Preclinical Alzheimer's Disease. *Annual review of clinical psychology*. 2017;13: 369-396. doi:https://doi.org/10.1146/annurev-clinpsy-032816-045136.

[6] Petersen R.C., Caracciolo B., Brayne C., et al. Mild cognitive impairment: a concept in evolution. *Journal of internal medicine*. 2014;275(3): 214-228. doi:https://doi.org/10.1111/joim.12190.

[7] Jack Jr C.R., Bennett D.A., Blennow K., et al. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimer's & Dementia*. 2018;14(4): 535-562. doi:https://doi.org/10.1016/j.jalz.2018.02.018.

[8] Sheng C., Lin L., Lin H., et al., Altered Gut Microbiota in Adults with Subjective Cognitive Decline: The SILCODE Study. *Journal of Alzheimer's Disease*. 2021;82(2): 513-526. doi:10.3233/JAD-210259.

[9] Duan M., Liu F., Fu H., et al. Preoperative Microbiomes and Intestinal Barrier Function Can Differentiate Prodromal Alzheimer's Disease From Normal Neurocognition in Elderly Patients Scheduled to Undergo Orthopedic Surgery. *Frontiers in cellular and infection microbiology*. 2021;11: 592842. doi:https://doi.org/10.3389/fcimb.2021.592842.

[10] Zhang L., Wang Y., Xiayu X., et al. Altered Gut Microbiota in a Mouse Model of Alzheimer's Disease. *Journal of Alzheimer's Disease*. 2017;60(4): 1241-1257. doi:10.3233/JAD-170020.

[11] Hansen DV, Hanson JE, Sheng M. Microglia in Alzheimer's disease. *Journal of Cell Biology*. 2018; 217(2): 459-472. doi:https://doi.org/10.1083/jcb.201709069.

[12] Wenzel T.J., Gates E.J., Ranger A.L., et al. Short-chain fatty acids (SCFAs) alone or in combination regulate select immune functions of microglia-like cells. Molecular and Cellular Neuroscience. 2020;105: 103493. doi:https://doi.org/10.1016/j.mcn.2020.103493.

[13] Li Q., Chen H., Zhang M., et al. Altered short chain fatty acid profiles induced by dietary fiber intervention regulate AMPK levels and intestinal homeostasis. Food & function. 2019;10(11): 7174-7187. doi:https://doi.org/10.1039/C9FO01465A.

[14] Liu P., Wu L., Peng G., et al. Altered microbiomes distinguish Alzheimer's disease from amnestic mild cognitive impairment and health in a Chinese cohort. *Brain, behavior, and immunity*. 2019;80: 633-643. doi:https://doi.org/10.1016/j.bbi.2019.05.008.

[15] Lukiw W.J. Bacteroides fragilis Lipopolysaccharide and Inflammatory Signaling in Alzheimer's Disease. *Frontiers in microbiology*. 2016;7: 1544. doi:https://doi.org/10.3389/fmicb.2016.01544

[16] Nho K., Kueider-Paisley A., MahmoudianDehkordi S., et al. Altered bile acid profile in mild cognitive impairment and Alzheimer's disease: Relationship to neuroimaging and CSF biomarkers. *Alzheimer's & Dementia*. 2019;15(2): 232-244. doi:https://doi.org/10.1016/j.jalz.2018.08.012.

[17] Muhammad T., Ikram M., Ullah R., et al. Hesperetin, a Citrus Flavonoid, Attenuates LPS-Induced Neuroinflammation, Apoptosis and Memory Impairments by Modulating TLR4/NF-kappaB Signaling. *Nutrients*. 2019;11(3): 648. doi:https://doi.org/10.3390/nu11030648.

[18] Sfera A., Gradini R., Cummings M., et al. Rusty Microglia: Trainers of Innate Immunity in Alzheimer's Disease. *Frontiers in neurology*. 2018;9: 1062. doi:https://doi.org/10.3389/fneur.2018.01062.

[19] Nagpal R., Neth B.J., Wang S., et al. 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: 529-542. doi:https://doi.org/10.1016/j.ebiom.2019.08.032

[20] Wu L., Han Y., Zheng Z., et al. Altered Gut Microbial Metabolites in Amnestic Mild Cognitive Impairment and Alzheimer's Disease: Signals in Host-Microbe Interplay. *Nutrients*. 2021;13(1): 228. doi:https://doi.org/10.3390/nu13010228.

[21] Vogt N.M., Romano K.A., Darst B.F., et al. The gut microbiota-derived metabolite trimethylamine N-oxide is elevated in Alzheimer's disease. *Alzheimer's research & therapy*. 2018;10(1): 1-8. doi:https://doi.org/10.1186/s13195-018-0451-2.

[22] Lukiw W.J. Gastrointestinal (GI) Tract Microbiome-Derived Neurotoxins-Potent Neuro- Inflammatory Signals From the GI Tract via the Systemic Circulation Into the Brain. *Frontiers in cellular and infection microbiology*. 2020;10: 22. doi:https://doi.org/10.3389/fcimb.2020.00022.

[23] Cattaneo A., Cattane N., Galluzzi S., et al. Association of brain amyloidosis with pro-inflammatory gut bacterial taxa and peripheral inflammation markers

in cognitively impaired elderly. *Neurobiology of aging*. 2017;49: 60-68. doi:https://doi.org/10.1016/j.neurobiolaging.2016.08.019.

[24] Dufies O., Doye A., Courjon J., et al. Escherichia coli Rho GTPase-activating toxin CNF1 mediates NLRP3 inflammasome activation via p21-activated kinases-1/2 during bacteraemia in mice. *Nature microbiology.* 2021;6(3): 401-412. doi:https://doi.org/10.1038/s41564-020-00832-5.

[25] Saresella M., La Rosa F., Piancone F., et al. The NLRP3 and NLRP1 inflammasomes are activated in Alzheimer's disease. *Molecular neurodegeneration*. 2016;11(1): 1-14. doi:https://doi.org/10.1186/s13024-016-0088-1.

[26] Shen H., Guan Q., Zhang X., et al. New mechanism of neuroinflammation in Alzheimer's disease: The activation of NLRP3 inflammasome mediated by gut microbiota. *Progress in Neuro-Psychopharmacology and Biological Psychiatry.* 2020;100: 109884. doi:https://doi.org/10.1016/j.pnpbp.2020.109884.

[27] Ising C., Venegas C., Zhang S., et al. NLRP3 inflammasome activation drives tau pathology. *Nature*. 2019;575(7784): 669-673. doi:https://doi.org/10.1038/s41586-019-1769-z.

[28] Zhuang Z.Q., Shen L.L., Li W.W., et al. Gut Microbiota is Altered in Patients with Alzheimer's Disease. *Journal of Alzheimer's disease*. 2018;63(4): 1337-1346. doi:10.3233/JAD-180176.

[29] Zhou Y., Wang Y., Quan M., et al. Gut Microbiota Changes and Their Correlation with Cognitive and Neuropsychiatric Symptoms in Alzheimer's Disease. *Journal of Alzheimer's Disease*. 2021;81(2): 583-595. doi:10.3233/JAD-201497.

[30] Giordano N.P., Cian M.B., Dalebroux Z.D. Outer Membrane Lipid Secretion and the Innate Immune Response to Gram- Negative Bacteria. *Infection and Immunity.* 2020;88(7): e00920-19. doi:https://doi.org/10.1128/IAI.00920-19.

[31] Vogt N.M., Kerby R.L., Dill-McFarland K.A., et al. Gut microbiome alterations in Alzheimer's disease. *Scientific reports.* 2017;7(1): 1-11. doi:https://doi.org/10.1038/s41598-017-13601-y.

[32] Li B., He Y., Ma J., et al. Mild cognitive impairment has similar alterations as Alzheimer's disease in gut microbiota. *Alzheimer's & Dementia.* 2019;15(10): 1357-1366. doi:https://doi.org/10.1016/j.jalz.2019.07.002.

[33] Ling Z., Zhu M., Yan X., et al. Structural and Functional Dysbiosis of Fecal Microbiota in Chinese Patients With Alzheimer's Disease. *Frontiers in Cell and Developmental Biology.* 2020;8: 634069. doi:https://doi.org/10.3389/fcell.2020.634069.

[34] Calsolaro V., Edison P. Neuroinflammation in Alzheimer's disease: Current evidence and future directions. *Alzheimer's & dementia.* 2016;12(6): 719-732. doi:https://doi.org/10.1016/j.jalz.2016.02.010.

[35] Minter M.R., Taylor J.M., Crack P.J. The contribution of neuroinflammation to amyloid toxicity in Alzheimer's disease. *Journal of neurochemistry.* 2016;136(3): 457-474. doi:https://doi.org/10.1111/jnc.13411.

[36] Lv W.J., Liu C., Yu L.Z., et al., Melatonin Alleviates Neuroinflammation and Metabolic Disorder in DSS-Induced Depression Rats. *Oxidative medicine and cellular longevity.* 2020: 1241894. doi:https://doi.org/10.1155/2020/1241894.

[37] Shukla M., Govitrapong P., Boontem P., et al. Mechanisms of Melatonin in Alleviating Alzheimer's Disease. *Current neuropharmacology.* 2017;15(7): 1010-1031. doi:https://doi.org/10.2174/1570159X15666170313123454.

[38] Sorbara M.T., Littmann E.R., Fontana E., et al. Functional and Genomic Variation between Human-Derived Isolates of Lachnospiraceae Reveals Inter- and Intra-Species Diversity. *Cell host & microbe.* 2020;28(1): 134-146. doi:https://doi.org/10.1016/j.chom.2020.05.005.

[39] Majid N., Siddiqi M.K., Khan A.N., et al. Biophysical Elucidation of Amyloid Fibrillation Inhibition and Prevention of Secondary Nucleation by Cholic Acid: An Unexplored Function of Cholic Acid. *ACS Chemical Neuroscience*. 2019;10(11): 4704-4715. doi:https://doi.org/10.1021/acschemneuro.9b00482.

[40] Gao K., Mu C.L., Farzi A., et al. Tryptophan Metabolism: A Link Between the Gut Microbiota and Brain. *Advances in Nutrition.* 2020;11(3): 709-723. doi:https://doi.org/10.1093/advances/nmz127.

[41] Williams B.B., Van Benschoten A.H., Cimermancic P., et al. Discovery and characterization of gut microbiota decarboxylases that can produce the neurotransmitter tryptamine. *Cell host & microbe.* 2014;16(4): 495-503. doi:https://doi.org/10.1016/j.chom.2014.09.001.

[42] Xiao L., Yan J., Yang T., et al. Fecal Microbiome Transplantation from Children with Autism Spectrum Disorder Modulates Tryptophan and Serotonergic Synapse Metabolism and Induces Altered Behaviors in Germ-Free Mice. *MSystems*. 2021;6(2): 1343-20. doi:https://doi.org/10.1128/mSystems.01343-20.

[43] Aaldijk E., Vermeiren Y. The role of serotonin within the microbiota-gut-brain axis in the development of Alzheimer's disease: A narrative review. *Ageing Research Reviews*. 2022;75: 101556. doi:https://doi.org/10.1016/j.arr.2021.101556.

[44] Bonfili L., Cecarini V., Berardi S., et al. Microbiota modulation counteracts Alzheimer's disease progression influencing neuronal proteolysis and gut hormones plasma levels. *Scientific reports.* 2017; 7(1): 1-21. doi:https://doi.org/10.1038/s41598-017-02587-2.

[45] Ho L., Ono K., Tsuji M., et al. Protective roles of intestinal microbiota derived short chain fatty acids in Alzheimer's disease-type beta-amyloid neuropathological mechanisms. *Expert review of neurotherapeutics*. 2018;18(1): 83-90. doi:https://doi.

org/10.1080/14737175.2018.1400909.

[46] Kobayashi Y., Sugahara H., Shimada K., et al. Therapeutic potential of Bifidobacterium breve strain A1 for preventing cognitive impairment in Alzheimer's disease. *Scientific reports*. 2017;7(1): 1-10. doi:https://doi.org/10.1038/s41598-017-13368-2.

[47] Kobayashi Y., Kuhara T., Oki M., et al. Effects of Bifidobacterium breve A1 on the cognitive function of older adults with memory complaints: a randomised, double-blind, placebo- controlled trial. *Beneficial microbes*. 2019;10(5): 511-520. doi:https://doi.org/10.3920/BM2018.0170.

[48] Benton D., Williams C., Brown A. Impact of consuming a milk drink containing a probiotic on mood and cognition. *European journal of clinical nutrition*. 2007;61(3): 355-361. doi:https://doi.org/10.1038/sj.ejcn.1602546.

[49] Camfield D.A., Owen L., Scholey A.B., et al. Dairy constituents and neurocognitive health in ageing. *British journal of nutrition*. 2011;106(2): p. 159-174. doi:https://doi.org/10.1017/S0007114511000158.

[50] Ano Y., Ayabe T., Kutsukake T., et al. Novel lactopeptides in fermented dairy products improve memory function and cognitive decline. *Neurobiology of Aging*. 2018;72: 23-31. doi:https://doi.org/10.1016/j.neurobiolaging.2018.07.016.

[51] Ano Y., Yoshino Y., Kutsukake T., et al. Tryptophan-related dipeptides in fermented dairy products suppress microglial activation and prevent cognitive decline. *Aging (Albany NY)*. 2019;11(10): 2949-2967. doi:https://doi.org/10.18632%2Faging.101909.

[52] Rahman A., Baker P.S., Allman R.M., et al. Dietary factors and cognitive impairment in community-dwelling elderly. *The Journal of Nutrition, Health & Aging*. 2007;11(1): 49-54. Available from:https://www.proquest.com/openview/b4ee6457333248029849799 63315dca3/1?pq-origsite=gscholar&cbl=28850.

[53] Ozawa M., Ninomiya T., Ohara T., et al. Dietary patterns and risk of dementia in an elderly Japanese population: the Hisayama Study. *The American of Clinical Nutrition*. 2013;97(5): 1076-1082. doi:https://doi.org/10.3945/ajcn.112.045575.

[54] Pistollato F., Iglesias R.C., Ruiz R., et al. Nutritional patterns associated with the maintenance of neurocognitive functions and the risk of dementia and Alzheimer's disease: A focus on human studies. *Pharmacological research*. 2018;131: 32-43. doi:https://doi.org/10.1016/j.phrs.2018.03.012.

[55] Tillisch K., Labus J., Kilpatrick L., et al. Consumption of fermented milk product with probiotic modulates brain activity. *Gastroenterology*. 2013;144(7): 1394-1401. doi:https://doi.org/10.1053/j.gastro.2013.02.043.

[56] Akbari E., Asemi Z., Daneshvar Kakhaki R., et al. 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*. 2016;8: 256. doi:https://doi.org/10.3389/fnagi.2016.00256.

[57] Athari Nik Azm S., Djazayeri A., Safa M., et al. Lactobacilli and bifidobacteria ameliorate memory and learning deficits and oxidative stress in beta-amyloid (1-42) injected rats. *Applied Physiology, Nutrition, and Metabolism*. 2018;43(7): 718-726. doi:https://doi.org/10.1139/apnm-2017-0648.

[58] Abraham D., Feher J., Scuderi G.L., et al. Exercise and probiotics attenuate the development of Alzheimer's disease in transgenic mice: Role of microbiome. *Experimental gerontology*. 2019;115: 122-131. doi:https://doi.org/10.1016/j.exger.2018.12.005.

[59] Chen D., Yang X., Yang J., et al. Prebiotic Effect of Fructooligosaccharides from Morinda officinalis on Alzheimer's Disease in Rodent Models by Targeting the Microbiota-Gut-Brain Axis. *Frontiers in aging neuroscience*. 2017;9: 403. doi:https://doi.org/10.3389/fnagi.2017.00403.

[60] Hazan S. Rapid improvement in Alzheimer's disease symptoms following fecal microbiota transplantation: a case report. *Journal of International Medical Research*. 2020;48(6): 300060520925930. doi:https://doi.org/10.1177/0300060520925930.

[61] Sun J., Xu J., Ling Y., et al. Fecal microbiota transplantation alleviated Alzheimer's disease-like pathogenesis in APP/PS1 transgenic mice. *Translational psychiatry*. 2019;9(1): 1-13. doi:https://doi.org/10.1038/s41398-019-0525-3.

[62] Vendrik K.E., Ooijevaar R.E., De Jong P.R., et al. Fecal Microbiota Transplantation in Neurological Disorders. *Frontiers in cellular and infection microbiology*. 2020;10: 98. doi:https://doi.org/10.3389/fcimb.2020.00098.

[63] Yulug B., Hanoglu L., Ozansoy M., et al. Therapeutic role of rifampicin in Alzheimer's disease. *Psychiatry and Clinical Neurosciences*. 2018;72(3): 152-159. doi:https://doi.org/10.1111/pcn.12637.

[64] Budni J., L. Garcez M., Medeiros J.D., et al. The Anti-Inflammatory Role of Minocycline in Alzheimer's Disease. *Current Alzheimer Research*. 2016;13(12): 1319-1329. Available from:http://www.ingentaconnect.com/content/ben/car/2016/00000013/00000012/art00003#expand/collapse.

[65] Wang C., Yu J.T., Miao D., et al. Targeting the mTOR signaling network for Alzheimer's disease therapy. *Molecular Neurobiology*. 2014;49(1): 120-135. doi:https://doi.org/10.1007/s12035-013-8505-8.

[66] Portero-Tresserra M., Martí-Nicolovius M., Tarrés-Gatius M., et al. Intra-hippocampal D-cyloserine rescues decreased social memory, spatial learning reversal, and synaptophysin levels in aged rats. *Psychopharmacology*. 2018;235(5): 1463-1477. doi:https://doi.org/10.1007/s00213-018-4858-z.

[67] Tsai G.E., Falk W.E., Gunther J., et al. Improved

cognition in Alzheimer's disease with short-term D-cycloserine treatment. *American journal of Psychiatry.* 1999;156(3): 467-469. doi:https://doi.org/10.1176/ajp.156.3.467.

[68] Loeb M.B., Molloy D.W., Smieja M., et al. A randomized, controlled trial of doxycycline and rifampin for patients with Alzheimer's disease. *Journal of the American Geriatrics Society.* 2004; 52(3): 381-387. doi:https://doi.org/10.1111/j.1532-5415.2004.52109.x.

[69] Molloy D.W., Standish T.I., Zhou Q., et al. A multicenter, blinded, randomized, factorial controlled trial of doxycycline and rifampin for treatment of Alzheimer's disease: the DARAD trial. *International journal of geriatric psychiatry.* 2013;28(5): 463-470. doi:https://doi.org/10.1002/gps.3846.

[70] Kountouras J., Boziki M., Gavalas E., et al. Eradication of Helicobacter pylori may be beneficial in the management of Alzheimer's disease. *Journal of neurology.* 2009;256(5): 758-767. doi:https://doi.org/10.1007/s00415-009-5011-z.

[71] Richardson A., Galvan V., Lin A.L., et al. How longevity research can lead to therapies for Alzheimer's disease: The rapamycin story. *Experimental gerontology.* 2015;68: 51-58. doi:https://doi.org/10.1016/j.exger.2014.12.002.

[72] Minter M.R., Zhang C., Leone V., et al. Antibiotic-induced perturbations in gut microbial diversity influences neuro-inflammation and amyloidosis in a murine model of Alzheimer's disease. *Scientific reports.* 2016;6(1): 1-12. doi:https://doi.org/10.1038/srep30028.

[73] Neufeld N.H., Mohamed N.S., Grujich N., et al. Acute Neuropsychiatric Symptoms Associated With Antibiotic Treatment of Helicobacter Pylori Infections: A Review. *Journal of Psychiatric Practice.* 2017;23(1): 25-35. doi:https://doi.org/10.1097/PRA.0000000000000205.

[74] Zarrinpar A., Chaix A., Xu Z.Z., et al. Antibiotic-induced microbiome depletion alters metabolic homeostasis by affecting gut signaling and colonic metabolism. *Nature communications.* 2018;9(1): 1-13. doi:https://doi.org/10.1038/s41467-018-05336-9.

[75] Wang T., Hu X., Liang S., et al. Lactobacillus fermentum NS9 restores the antibiotic induced physiological and psychological abnormalities in rats. *Beneficial microbes.* 2015;6(5): 707-717. doi:https://doi.org/10.3920/BM2014.0177.

[76] Neufeld K.M., Kang N., Bienenstock J., et al. Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterology & Motility.* 2011;23(3): 255-264. doi:https://doi.org/10.1111/j.1365-2982.2010.01620.x.

[77] Bercik P., Denou E., Collins J., et al. The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. *Gastroenterology* 2011;141(2): 599-609. doi:https://doi.org/10.1053/j.gastro.2011.04.052.

[78] Fröhlich E.E., Farzi A., Mayerhofer R., et al. Cognitive impairment by antibiotic-induced gut dysbiosis: Analysis of gut microbiota-brain communication. *Brain, Behavior, and Immunity.* 2016;56: 140-155. doi:https://doi.org/10.1016/j.bbi.2016.02.020.

[79] Ravelli K.G., Rosário B.D., Camarini R., et al. Intracerebroventricular Streptozotocin as a Model of Alzheimer's Disease: Neurochemical and Behavioral Characterization in Mice. *Neurotoxicity research.* 2017;31(3): 327-333. doi:https://doi.org/10.1007/s12640-016-9684-7.

[80] Itzhaki R.F. Herpes simplex virus type 1 and Alzheimer's disease: possible mechanisms and signposts. *The FASEB Journal.* 2017;31(8): 3216-3226. doi:https://doi.org/10.1096/fj.201700360.

[81] Barnes L.L., Capuano A.W., Aiello A.E., et al. Cytomegalovirus infection and risk of Alzheimer disease in older black and white individuals. *The Journal of infectious diseases.* 2015;211(2): 230-237. doi:https://doi.org/10.1093/infdis/jiu437.

[82] Walker J.M., Harrison F.E. Shared Neuropathological Characteristics of Obesity, Type 2 Diabetes and Alzheimer's Disease: Impacts on Cognitive Decline. *Nutrients.* 2015;7(9): 7332-7357. doi:https://doi.org/10.3390/nu7095341.

[83] Davari S., Talaei S.A., Alaei H. Probiotics treatment improves diabetes-induced impairment of synaptic activity and cognitive function: behavioral and electrophysiological proofs for microbiome-gut-brain axis. *Neuroscience.* 2013;240: 287-296. doi:https://doi.org/10.1016/j.neuroscience.2013.02.055.

[84] Claesson M.J., Jeffery I.B., Conde S., et al. Gut microbiota composition correlates with diet and health in the elderly. *Nature.* 2012;488(7410): 178-184. doi:https://doi.org/10.1038/nature11319.

[85] Fontana L., Partridge L., Longo V.D. Extending healthy life span--from yeast to humans. *Science.* 2010;328(5976): 321-326. doi:https://doi.org/10.1126/science.1172539.

[86] Schafer M.J., Dolgalev I., Alldred M.J., et al. Calorie Restriction Suppresses Age-Dependent Hippocampal Transcriptional Signatures. *PLoS One.* 2015;10(7): e0133923. doi:https://doi.org/10.1371/journal.pone.0133923.

[87] Schafer M.J., Alldred M.J., Lee S.H., et al. Reduction of beta-amyloid and gamma-secretase by calorie restriction in female Tg2576 mice. *Neurobiology of Aging.* 2015;36(3): 1293-1302. doi:https://doi.org/10.1016/j.neurobiolaging.2014.10.043.

[88] Zhang C., Li S., Yang L., et al. Structural modulation of gut microbiota in life-long calorie-restricted mice. *Nature communications.* 2013;4(1): 1-10. doi:https://doi.org/10.1038/ncomms3163.

[89] Hoarau G., Mukherjee P.K., Gower-Rousseau C., et al. Bacteriome and Mycobiome Interactions Underscore Microbial Dysbiosis in Familial Crohn's Disease. *MBio*. 2016;7(5): e01250-16. doi:https://doi.org/10.1128/mBio.01250-16.

[90] Sokol H., Leducq V., Aschard H., et al. Fungal microbiota dysbiosis in IBD. *Gut*. 2017;66(6): 1039-1048. doi:http://dx.doi.org/10.1136/gutjnl-2015-310746.

[91] Frykman P.K., Nordenskjöld A., Kawaguchi A., et al. Characterization of Bacterial and Fungal Microbiome in Children with Hirschsprung Disease with and without a History of Enterocolitis: A Multicenter Study. *PLoS One*. 2015;10(4): e0124172. doi:https://doi.org/10.1371/journal.pone.0124172.

[92] Iliev I.D., Funari V.A., Taylor K.D., et al. Interactions between commensal fungi and the C-type lectin receptor Dectin-1 influence colitis. *Science*. 2012; 336(6086): 1314-1317. doi:https://doi.org/10.1126/science.1221789.

[93] Luan C., Xie L., Yang X., et al. Dysbiosis of fungal microbiota in the intestinal mucosa of patients with colorectal adenomas. *Scientific reports*. 2015;5(1): 1-9. doi:https://doi.org/10.1038/srep07980.

[94] van der Velden W.J., Netea M.G., de Haan A.F., et al. Role of the mycobiome in human acute graft-versus-host disease. *Biology of Blood and Marrow Transplantation*. 2013;19(2): 329-332. doi:https://doi.org/10.1016/j.bbmt.2012.11.008.

[95] Lemoinne S., Kemgang A., Belkacem K.B., et al. Fungi participate in the dysbiosis of gut microbiota in patients with primary sclerosing cholangitis. *Gut*. 2020;69(1): 92-102. doi:http://dx.doi.org/10.1136/gutjnl-2018-317791.

[96] Sharma A., Laxman B., Naureckas E.T., et al. Associations between fungal and bacterial microbiota of airways and asthma endotypes. *Journal of Allergy and Clinical immunology*. 2019;144(5): 1214-1227. doi:https://doi.org/10.1016/j.jaci.2019.06.025.

[97] Chen Y., Chen Z., Guo R., et al. Correlation between gastrointestinal fungi and varying degrees of chronic hepatitis B virus infection. *Diagnostic microbiology and infectious disease*. 2011;70(4): 492-498. doi:https://doi.org/10.1016/j.diagmicrobio.2010.04.005.

[98] Saji N., Murotani K., Hisada T., et al. The relationship between the gut microbiome and mild cognitive impairment in patients without dementia: a cross-sectional study conducted in Japan. *Scientific reports*. 2019;9(1): 1-10. doi:https://doi.org/10.1038/s41598-019-55851-y.