Gut dysbiosis as an early biomarker of amyotrophic lateral sclerosis

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder affecting both upper and lower motor neurons, typically leading to death within 3-5 years after symptom onset (Hardiman et al., 2017). Though historically ALS was considered to affect exclusively the motor system, it is now well established that it is a multi-system disorder with many extra-motor features, ranging from cognitive-behavioural symptoms, extrapyramidal, cerebellar, and sensory nerve abnormalities (Volonté & Amadio, 2023) to gastrointestinal involvement, which in animal models has been shown to even precede motor neuron degeneration (Zhang et al., 2021). While such evidence in human ALS is limited, a study reported gastrointestinal symptoms in all participants before neurological symptoms emerged, hinting at early gut involvement (Rowin et al., 2017).

The incidence of ALS vary based on geographical location and ancestral origin, with the overall crude worldwide incidence estimated to be 1.59 / 100,000 persons (Xu et al., 2019). The differences between epidemiological studies suggest that other factors such as the environment and lifestyle (Luna et al., 2017), and possibly the gut microbiome, may also play a role. In most cases, ALS is sporadic (sALS), while 10–15% accounts for a familial form of the disease, caused by mutations in more than 30 different known genes (Logan et al., 2022).

While the pathophysiological mechanisms of ALS remain poorly understood, evidence points to neuroinflammation, glutamate excitotoxicity, axonal transport defects, endosomal and vesicular transport impairment, dysregulated protein homeostasis, disruptions in DNA repair, RNA integrity, nuclear export, and axonal excitability (Mejzini et al., 2019). Furthermore, an increasing amount of evidence has

linked the enteric microbiota and the gut-brain axis to the pathogenesis of ALS (Noor Eddin et al., 2024).

Various biomarkers of ALS are being investigated, including neurofilament and p75 neurotrophin receptor (Banack et al., 2024). Biomarkers based on RNA, in particular mRNA and miRNA, emerged as candidate diagnostic and prognostic tools, with a recent study demonstrating an eight-microRNA diagnostic signature that shows high sensitivity, specificity, and 98% accuracy (Banack et al., 2024).

Biomarkers are crucial not only for a more in-depth understanding of the disease but also for therapeutic development. For example, neurofilament light chain (NfL) served as a key pharmacodynamic biomarker in the tofersen clinical trials, where its reduction provided critical evidence to support the United States Food and Drug Administration (FDA) approval under the accelerated approval pathway (Miller et al., 2023).

However, although promising, no single biomarker for clinical use in ALS has yet been definitively established, so the search for others is still relevant. One potential source of alternative biomarkers is the gut microbiome.

1.1. Microbiome and gut dysbiosis

The term human microbiome refers to the collective genome of all the different microbes present in the human body, with most of the microbiota – i.e. the community of the microbes themselves – residing within the intestine (Boddy et al., 2021). The gut microbiota composition is shaped by a variety of factors, including gender, genetics, age, socio-economic status, stress levels, diet, and environmental influences such as exposure to pollutants, antibiotics, and other elements that form part of the exposome (Vineis et al., 2020).

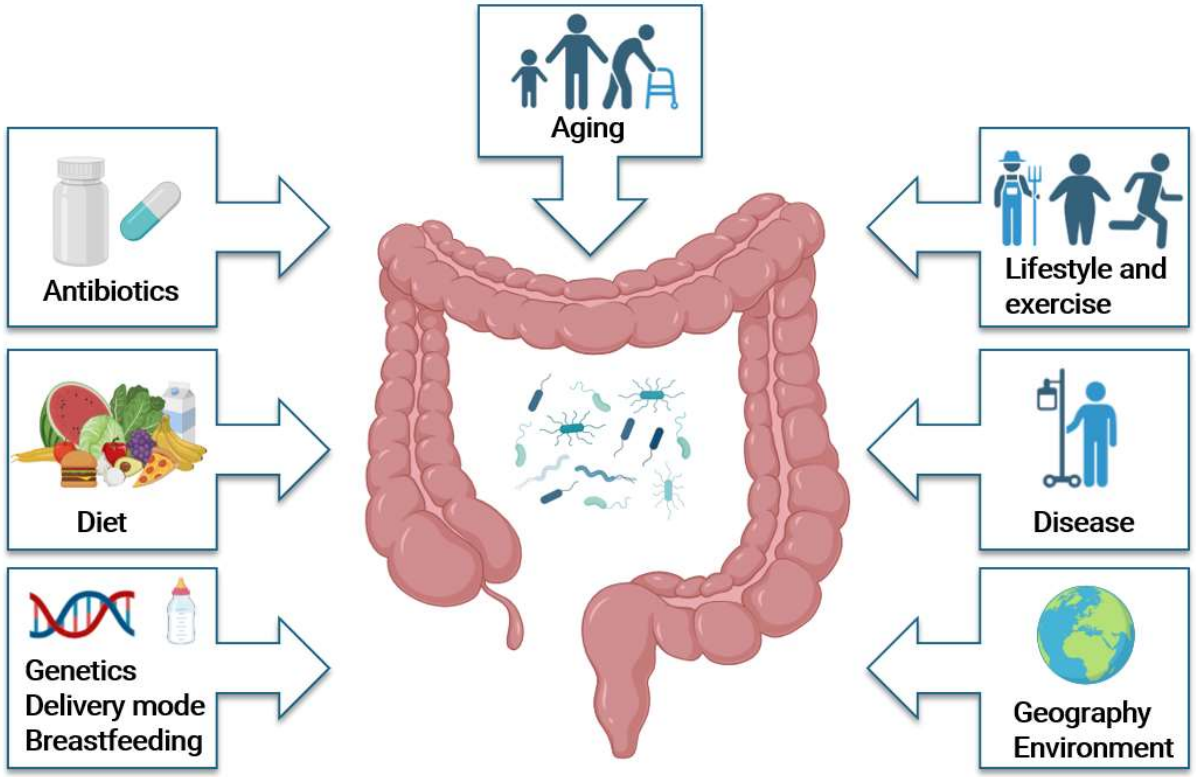

Figure 1 Gut microbiome and its main determinants. Created with BioRender.com.

Over the past decade, research has revealed the central role of the gut microbiome in human health and disease, particularly in relation to the development and function of systemic immune elements (Choden & Cohen, 2022). Imbalances or changes in the composition of the gut microbiome are commonly referred to as gut dysbiosis, and there has been steadily accumulating evidence for their association with both gastrointestinal and extra-intestinal diseases such as diabetes, malignancy, autoimmune disorders, and neurodegenerative diseases (Zheng et al., 2020).

1.2. Gut-brain axis and neurodegeneration

The gut microbiota is considered essential for brain physiological processes such as myelination, neurogenesis, and maturation and activation of microglia, which play key roles in neuroinflammation and other mechanisms underlying neurodegeneration (Zhang et al., 2022).

The gut-brain axis (GBA), a key concept linking the gut microbiome with the central nervous system (CNS), emerged already in 1980 (Track, 1980). The GBA is a complex bidirectional communication network connecting the gastrointestinal tract and the brain via neuroanatomical, immunological and neuroendocrine signalling pathways (Wang & Wang, 2016).

Disturbances of the GBA, often through the gut dysbiosis, have been implicated in the pathogenesis of several neurological disorders, including Parkinson's disease (PD), Alzheimer's disease (AD), multiple sclerosis (MS), Huntington's disease (HD) (Zhang et al., 2022), and ALS (Boddy et al., 2021).

Generally, two main mechanisms of how the microbiome acts are proposed: via gut microbiota-driven inflammation or via its released metabolites. In the former, increased gut permeability allows microbial components to activate systemic immune responses, promoting chronic neuroinflammation and neuronal damage. In the latter, metabolites produced by gut microbiota influence the CNS via the bloodstream. For instance, tryptophan derivatives and transformed bile acids can affect brain function by modulating neurotransmitter synthesis, neuronal signalling, and inflammatory pathways (Sabahat et al., 2024).

1.3. Objective of the review

The objective of this review is to critically evaluate the potential of gut microbiome dysbiosis as a biomarker for the early detection of ALS. Specifically, this work aims to determine the validity and reliability of microbiome alterations as a diagnostic and prognostic tool by synthesizing current research findings from animal and human studies of gut dysbiosis in ALS and other neurodegenerative diseases.

This focus on early detection is especially important considering recent evidence showing that gut microbiome modulation, such as through fecal microbiota transplantation (FMT), can yield clinical improvements in ALS patients (Yan et al., 2024). Identification of specific microbiome changes in preclinical ALS could lay the foundations for early interventions, including microbiome-targeted therapies, early initiation of neuroprotective treatments, and enrolment in clinical trials. In individuals with known risk factors for ALS, detecting early changes by screening for these biomarkers could allow early implementation of personalized prevention strategies.

2. Gut-brain axis

2.1. Gut-brain axis communication pathways

In the GBA, the gut influences CNS functions through various microbiota-derived metabolites, gut hormones, and neuroactive compounds, which reach the brain via the enteric nervous system and vagus nerve, circulatory system, and immune system (Loh et al., 2024).

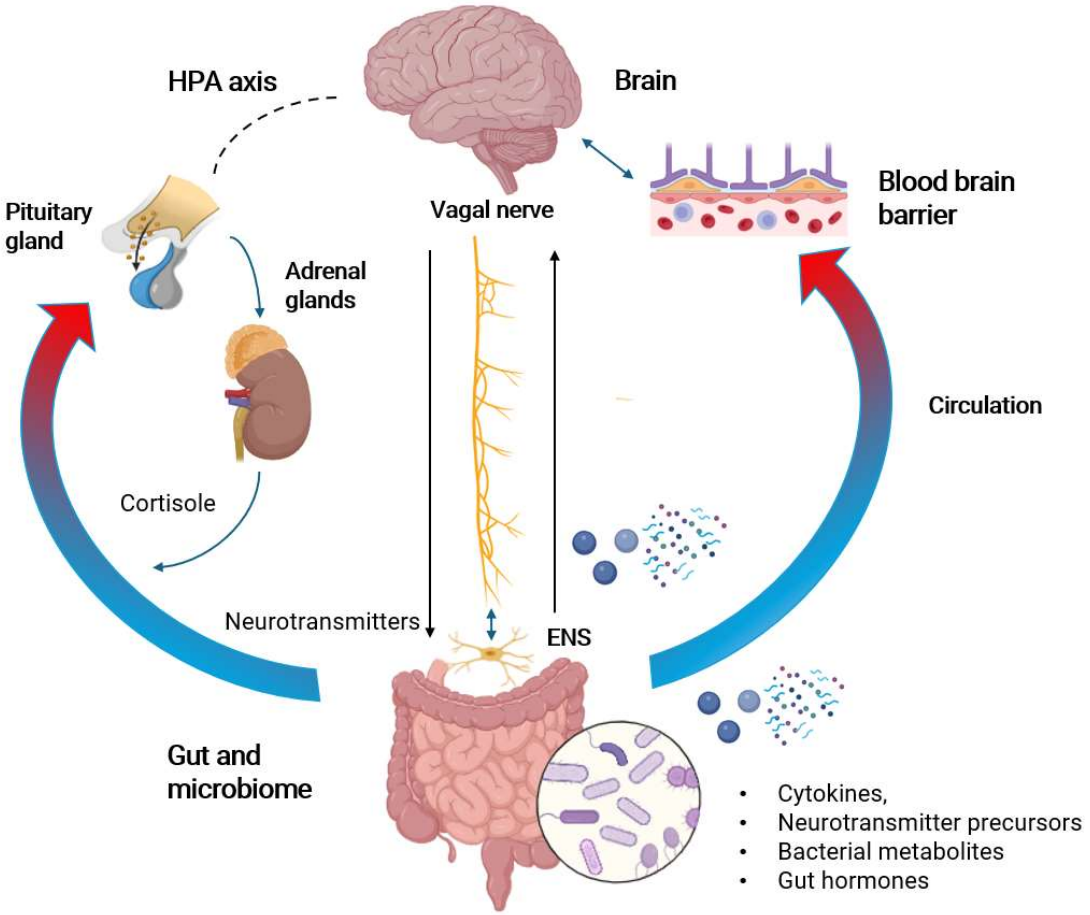

Figure 2: Main elements of the GBA pathways. HPA - hypothalamic-pituitary-adrenal, *ENS – enteric nervous system. Created with BioRender.com.*

2.1.1. Vagal pathway

The fastest and most direct route (Ni et al., 2022) in the GBA is the vagus nerve, which transmits signals from the luminal environment to the CNS and serves as a bidirectional communication pathway (Rodrigues, 2024). The vagal afferents can detect various intestinal molecules like bacterial by-products, neurotransmitters or gut hormones, and therefore are considered polymodal, responding to mechanical, hormonal or chemical signals (Berthoud et al., 2004). The ability to detect bacterial metabolites is mediated by the so-called neuropods, specialized enteroendrocrine cells within the gut that can form synapses with afferent nerves (Bohórquez et al.,

2015), thus connecting the gut lumen with the brainstem (Kaelberer et al., 2018). Therefore, any metabolite derived from microbes interacting with the enteric nervous system can potentially reach and affect the CNS (Boddy et al., 2021).

The vagus nerve controls the cholinergic anti-inflammatory pathway (CAP), which decreases intestinal permeability and inflammation (Bonaz et al., 2018) through the reduction of proinflammatory cytokines and modulation of microglia and macrophages, thus influencing neuroinflammation (Fang et al., 2023). This immunomodulatory effect has been investigated in animal studies involving vagus nerve stimulation (VNS) showing a reduction in both systemic and brain inflammation (Huffman et al., 2019), with one paper demonstrating attenuation of neuroinflammation via VNS-mediated shift of microglia toward an anti-inflammatory state (Chen et al., 2022). However, a systematic review of the anti-inflammatory effects of VNS found no consistent evidence of its efficacy in humans (Schiweck et al., 2024).

2.1.2. Immune and neuroendocrine pathways

The gut microbiota plays a central role in both the development and priming of the immune system, helping to shape immune responses in the host (Fujimura et al., 2016). Its communication with the brain is mediated by immune-signalling molecules, including cytokines, chemokines and molecular patterns associated with microbes, which enables it to modulate immune responses and neuroinflammation and at the same time the brain to influence the immune functions in the gut (Rojas-Valverde et al., 2023). In the state of gut eubiosis, immune mechanisms are anti-inflammatory, but in dysbiosis and local inflammation, inflammatory mediators, bacteria, metabolites etc. enter the systemic circulation and cause peripheral inflammation (Lobionda et al., 2019).

Inflammatory processes lead to activation of the hypothalamic-pituitary-adrenal (HPA) axis, an essential neuroendrocrine communication pathway within the GBA that plays a key role in the stress response (Kasarello et al., 2023). Activated HPA releases glucocorticoids, whose appropriate levels are crucial to development and normal function of the CNS, and it has been shown that they are influenced by microbial modulation of the HPA axis (Rusch et al., 2023). This is further supported by the findings that the administration of probiotics ameliorates the stress-dependent increase in corticosterone levels in rats (Mindus et al., 2021) and chronic stress-induced anxiety in mice (Tian et al., 2021).

The HPA axis also regulates the intestinal permeability, which has been demonstrated to increase during acute stress, thereby stimulating pro-inflammatory pathways (de Punder & Pruimboom, 2015).

2.1.3. Circulatory and lymphatic system pathways

In addition to the specific pathways discussed above, the systemic circulation offers a general route for gut-derived metabolites to reach the CNS by crossing the gut-vascular barrier (GVB), a selective membrane regulating the passage of molecules from the intestine into the bloodstream (Deng et al., 2021). Disruption of the GVB can therefore result in the systemic translocation of bacteria or bacterial products (Mouries et al., 2019).

Moreover, recent research suggests the lymphatic system as an additional pathway connecting the gut and brain (Zhuang et al., 2024), potentially contributing to neuroinflammation and disease development. This raises the possibility of undiscovered routes in the gut-brain axis, indicating further complexity.

2.2. CNS dysfunction and gut dysbiosis

Building on the complex communication pathways within the GBA, it becomes evident that disruptions—whether through immune, neural, or hormonal routes—can significantly impact both gut and brain health.

Gut dysbiosis can alter gut physiology, leading to abnormal GBA signalling that affects CNS function and contributes to disease states, while stress originating in the CNS can likewise disrupt gut function and destabilize the microbiota (Cryan & Dinan, 2012).

Research in animal models has shown that gut microbiome alterations can drive neurodegenerative disease pathogenesis, particularly by influencing microglia function and activation (Dodiya et al., 2021; Sampson et al., 2016). This is an important clue because among neuronal and glial cells, microglia appear particularly susceptible to the influence of the gut microbiome (Huang et al., 2023). Furthermore, the microbiota-gut-brain axis has been identified as an important regulator of glial functions (Erny et al., 2015). Chronic activation of microglia leads to sustained low-grade neuroinflammation, a common pathological feature of both aging and neurodegenerative diseases (Wang et al., 2022), exerting harmful effects on neurons and synapses and contributing to neurodegeneration (Loh et al., 2024).

In animal models, gut microbiome has been shown to influence integrity of the blood-brain barrier (BBB), the interface between the circulatory system and the brain parenchyma. In their work, Braniste et al. (2014) reported germ-free mice displaying increased BBB permeability, which was restored after monocolonization by specific bacteria strains. Another study demonstrated an increase of both BBB and intestinal permeability following low dose of penicillin administered in early life (Leclercq et al., 2017). In addition to the identified link between the gut and the BBB, further research

provided evidence for a specific part of the BBB reacting independently to gut inflammation (Carloni et al., 2021).

Enhanced intestinal and BBB permeability – both of which increase with aging - allow the bacterial products and microbial-associated molecules to enter the systemic circulation and reach the CNS (Zhao et al., 2017). Among these, lipopolysaccharide (LPS), a pro-inflammatory component of the outer wall of Gram-negative bacteria, is especially concerning due to its central role in inducing low-grade systemic inflammation (Cao et al., 2018). The scheme of the proposed mechanism is shown in Figure 3.

Studies have shown that LPS administration increases neuroinflammation, further disrupts the BBB, triggers neuronal loss and activates microglia, resulting in the release of neurotoxic factors (Zhao et al., 2019). The pathogenic role of LPS has been implicated in neurodegenerative diseases such as sALS, AD (Zhang et al., 2009), PD (Zhao et al., 2023; Gorecki et al., 2019) and MS (Escribano et al., 2017; Teixeira et al., 2013), and its production resulting from gut dysbiosis contributes to inducing neuroinflammation and brain damage (Li et al., 2023).

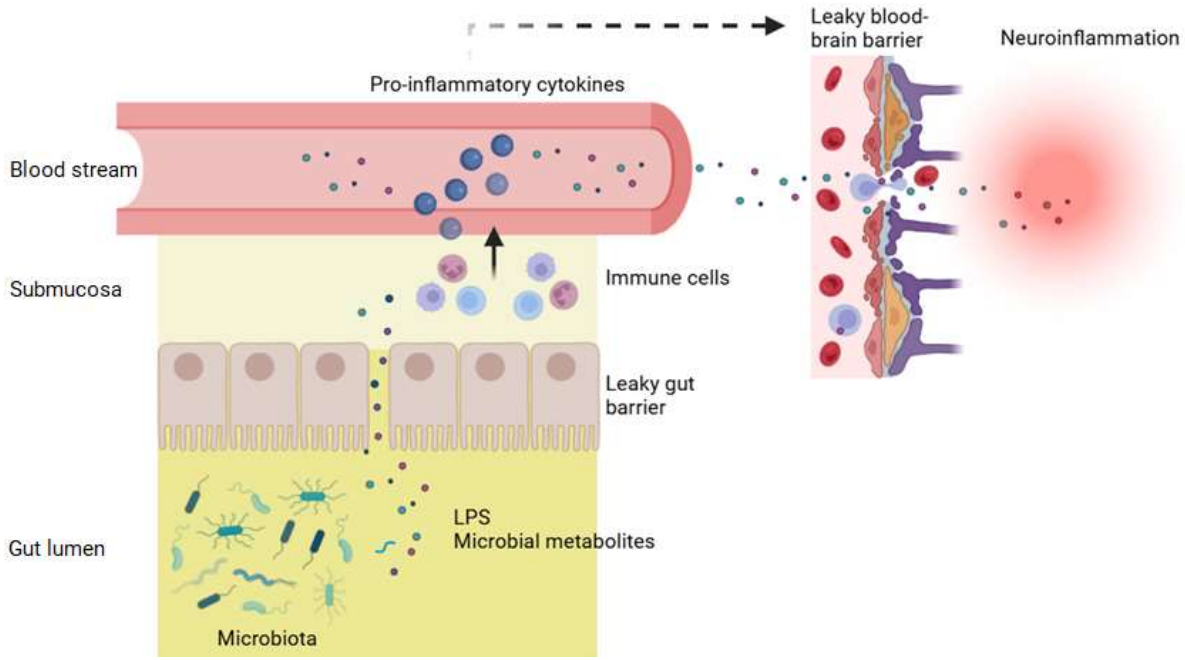

Figure 3: A scheme of the proposed role of lipopolysaccharide (LPS) in neuroinflammation. Created with BioRender.com.

Gut dysbiosis also impairs production of short-chain fatty acids (SCFAs), such as acetate, propionate and butyrate (Liu et al., 2024). These possess anti-oxidative, anti-carcinogenic and anti-inflammatory properties and play a central role in gastrointestinal and immune homeostasis (Ciarlo et al., 2016). Evidence suggests that SCFAs regulate microglial maturation and function (Erny et al., 2015), signalling via neurotransmitters (Magzal et al., 2021) and neuroinflammation (Liu et al., 2021) and also influence the integrity of the BBB (Fock & Parnova, 2023). Butyrate, propionate and acetate have been demonstrated to have promising effects in animal models of various CNS disorders, including AD (Fernando et al., 2020; Gibbs et al., 2009). Additionally, a work by Hoyles et al. (2018) demonstrated in an in vitro model using human cerebromicrovascular endothelial cells that propionate may have protective effects on the BBB against bacterial LPS.

Gut microbiota has been also shown to influence brain function by modulating the production of neurotransmitters and neuromodulators (Chen et al., 2021), which is particularly important given that many neurodegenerative diseases are associated with neurotransmitter system dysfunction (Loh et al., 2024).

Despite the compelling evidence linking gut dysbiosis to CNS pathologies, there are several limitations and challenges. Primarily, most studies rely on animal models, such as monocolonized or germ-free mice, which cannot capture the full the complexity of the human gut microbiota and its interactions with the CNS. This is further complicated by differences in human and animal gut microbiota composition, neurophysiology, and immune system responses. Additionally, many interventions, such as those conducted by Braniste et al. (2014) and Leclercq et al. (2017), are performed at specific time points during early life under controlled conditions, which contrasts with the gradual and dynamic changes in the human microbiome influenced by factors like aging, diet, environmental exposures, lifestyle, and comorbidities. Another problem is to determine whether the observed changes in the microbiome are causal or consequential. Specifically, causal claims in rodent models investigating the effects of human-derived microbiota require further validation, especially given the remarkably high rate of positive results (95%) reported in these studies (Loh et al., 2024).

3. Gut dysbiosis in neurodegenerative diseases

Increasing evidence suggests that gut dysbiosis plays a major role in the onset and progression of neurodegenerative diseases (Rojas-Valverde et al., 2023). Early alterations of the gut microbiome and impaired intestinal barrier integrity have been reported in prodromal PD, preclinical AD and HD patients (Loh et al., 2024), MS (Zhang et al., 2022) and ALS patients (Zhang et al., 2021). The relevance of these

associations is further underscored by reports showing that patients receiving FMT in various studies have demonstrated clinical improvements in AD (Park et al., 2022; Hazan, 2020), mild improvements of motor symptoms in early PD (Bruggeman et al., 2024) and – in two cases - of advanced ALS (Yan et al., 2024). However, a recent study found no effect in PD patients compared to placebo (Scheperjans et al., 2024), while others report lasting subjective but only temporary objective improvement in motor symptoms (DuPont et al., 2023; Bruggeman et al., 2024). These mixed results, together with small sample sizes and donor variability, demonstrate the need for standardized protocols.

3.1. Alzheimer's disease

Given the evidence of the gut microbiota's role in neuroinflammation, research has increasingly focused on its link to AD development and progression. Studies have shown that the presence or absence of certain gut microbes influences microglial activation, a process that likely contributes to hallmarks of AD: amyloid-beta plaque formation (Aβ), tau pathology, and synaptic dysfunction. Moreover, cerebrospinal fluid (CSF) markers of the disease – such as phosphorylated tau-181 and Aβ - have been found to correlate (uncorrected $p < 0.05$) with the gut microbiome changes in AD patients (Vogt et al., 2017), though the sample size of 40 participants with 9 AD cases limits the statistical power of the results. Of note, a study by Zhang et al. (2009) have shown increased concentrations of LSP in the circulation of 18/18 AD patients.

Animal models, such as the triple transgenic (3xTg-AD) mouse model, provide strong evidence of this relationship (Chen et al., 2022). When 3xTg-AD mice are raised in a germ-free environment, they exhibit reduced AD pathology compared to those raised in specific pathogen-free conditions, which allow for a diverse gut microbiota. Notably, FMT from AD patients into germ-free mice can restore these AD pathologies,

indicating the microbiome's significant influence on disease mechanisms (Chen et al., 2022). This pattern holds in amyloidogenic APP/PS1 mice, where antibiotic treatment to reduce gut microbiota decreases Aβ deposition and microglial activation in males, though less so in females (Dodiya et al., 2021), but the underlying mechanisms remain unknown. These findings suggest that microbiome manipulation could potentially modulate AD progression, though the effects vary by sex and specific treatment windows.

The influence of the gut microbiome extends to tau-mediated neurodegeneration in AD. In studies on genetically engineered tau transgenic mice with the human APOE4 gene, those raised in germ-free conditions displayed less neurodegeneration and reduced microglial and astrocytic activation than the conventionally raised ones (Seo et al., 2023).

Furthermore, early-life antibiotic treatments in these models have shown sustained protective effects against tau pathology, with these effects also displaying sex-dependence and APOE-isoform specificity - being limited to males and more pronounced in the presence of APOE3, stressing the need for further research.

3.1.1. Gut dysbiosis as biomarker

Evidence indicates that Aβ and tau protein deposits may begin accumulating 10–20 years before clinical symptoms of AD appear (Zhang et al., 2022). The progression from a cognitively normal state to preclinical AD and eventually to symptomatic AD is characterized by specific biomarkers that can be detected by positron emission tomography (PET), magnetic resonance imaging (MRI), and CSF assays (Ferreiro et al., 2023). As the gut microbiome is increasingly recognized as a key factor in the

pathogenesis of AD, the identification of related biomarkers has become a focus of active research.

In a study comparing the gut microbiota of APP/PS1 transgenic mice and their wild-type littermates, significant changes in microbiota composition were observed as early as 1–3 months of age, before the onset of amyloid deposition and microglial activation in the cerebral cortex. By 6–9 months, inflammation-related bacterial taxa, such as *Escherichia-Shigella* and *Desulfovibrio*, showed distinct shifts, indicating that AD pathologies are preceded by microbiota alterations (Chen et al., 2020). Another study reported gut dysbiosis in 3xTg-AD mice during the presymptomatic stages at 3 and 5 months of age (C. Bello Medina, 2021).

In a study involving 164 participants aged 68-94 years from the Knight Alzheimer Disease Research Center cohort, gut microbiome profiles were examined in relation to preclinical Alzheimer's disease (Ferreiro et al., 2023). While there were no significant differences in total caloric intake or nutrient composition between the healthy and preclinical AD groups, the gut microbiome profiles showed striking differences. Principal coordinates analysis (PCoA) and canonical analysis (CAP) found significant differences in gut microbial composition between healthy and preclinical AD individuals, providing further evidence that gut microbiome changes may occur in early stages of AD, prior to cognitive symptoms. The authors suggested that gut microbiome differences in preclinical AD could serve as biomarkers, improving early screening accuracy and enabling gut-focused interventions to slow disease progression (Ferreiro et al., 2023).

3.2. Parkinson’s disease

Patients with PD frequently report gastrointestinal symptoms, but the exact mechanisms linking the gut microbiome to the disease have only recently become clearer. Evidence shows that the gut microbiome impacts microglial activation, development of α-synuclein pathology, and motor deficits in α-synuclein-overexpressing (ASO) mice, where specific pathogen-free ASO mice exhibited greater PD pathology than GF or antibiotic-treated mice (Sampson et al., 2016). Notably, the authors reported that following FMT from PD patients to GF ASO mice, the disease features were restored, including α-synuclein-mediated motor dysfunction.

In another study, FMT from 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treated mice, a neurotoxin inducing PD-like symptoms by damaging dopaminergic neurons, led to neurotransmitter loss and motor impairments in healthy mice, while FMT from healthy mice to MPTP-treated mice alleviated gut dysbiosis and gut inflammation, glial activation, neurotransmitter disturbances, and motor impairments (Sun et al., 2018).

In humans, multiple studies reported gut dysbiosis in PD patients compared to healthy controls, though the described bacterial taxa vary (Scheperjans et al., 2014; Qian et al., 2018).

3.2.1. Gut dysbiosis as biomarker

Hyposmia and gastrointestinal symptoms, such as constipation, often precede PD by more than a decade (Abbott et al., 2003), and growing evidence implicating the GBA in PD pathogenesis provides further rationale for investigating gut dysbiosis biomarkers as potential early indicators.

A large meta-analysis on the gut microbiome in PD, involving 2269 samples via 16S rRNA gene sequencing and 236 by shotgun metagenomics, highlighted significant microbial shifts associated with PD (Nie et al., 2022). Pro-inflammatory bacteria, genes, and pathways were found to be elevated, while anti-inflammatory ones were reduced, likely decreasing levels of SCFAs and increasing potential pro-inflammatory substances such as LSP. Both methods identified five genera that decreased and five that increased in PD. Additionally, for PD prediction, a random forest model using 11 genera achieved over 80 % accuracy (Nie et al., 2022).

Using machine learning, Huang et al. (2023) identified 12 microbial markers that are effective in differentiating individuals with REM sleep behaviour disorder (RBD) from healthy controls. Since RBD is regarded as one of the most specific prodromal markers of PD, these findings indicate that the gut dysbiosis may begin in the early stages of the disease, as RBD develops. Notably, similar microbial patterns were also observed in younger first-degree relatives of RBD patients.

However, the reported changes in microbiota often vary considerably between studies and therefore the batch effect needs to be removed. This has been addressed by a recent meta-analysis by Zhao et al. (2024), where 3 analytical approaches identified 5 out of 35 bacterial genera as potential microbial biomarkers for PD. Furthermore, the authors developed and optimized a classification model that achieved high accuracy using 11 specific genera.

4. Gut dysbiosis in ALS

Gastrointestinal symptoms are commonly reported in patients with ALS, and even those without overt problems may exhibit gastrointestinal motor dysfunction such as delayed colonic transit time and gastric emptying (Toepfer et al, 2000). A study examining gastrointestinal symptoms in ALS patients identified constipation (60.5%),

rectal tenesmus (57.5%), hard stools (55.0%) and borborygmus (42.5%) as the most common complaints. Additionally, symptoms such as constipation, acid regurgitation, eructation and rectal tenesmus were more common in intermediate to advanced stages of the disease (Parra-Cantu et al, 2021). However, the study only divided patients into two groups according to disease stage (mild to moderate and moderate to advanced), without further differentiation or exclusion, for example, of patients on a modified diet or percutaneous endoscopic gastrostomy (PEG). In addition, no information was provided regarding the time points at which symptoms were recorded.

In another study, Rowin et al. (2017) evaluated gastrointestinal health and stool microbiome profiles in a small cohort of ALS patients, and found altered gut microbiomes in all patients, marked by reduced microbial diversity compared to healthy individuals, despite relatively intact overall abundance. Additionally, most patients showed signs of intestinal inflammation.

Considering the complex pathophysiology of ALS and the systemic changes it causes, several factors must be taken into account as potentially contributing to or influencing the observed alterations. These include dietary changes, decreased fluid intake resulting from dysphagia, progressively reduced motor activity, effects of medication and supplements, and the impact of anxiety and depression. Each of these factors could individually or in combination contribute to or exacerbate the changes in gastrointestinal health and microbiome composition observed in ALS patients. Nevertheless, when combined with observations of GBA involvement in other neurodegenerative diseases, the current findings suggest that gut dysbiosis may be involved in the pathogenesis of the disease and may even play a central role.

In a large study by Zhang et al. (2022), a two-sample Mendelian randomization approach was utilized to investigate the causal link between gut microbiota and ALS in 20,806 patients and 59,804 controls. It identified certain bacterial taxa, including OTU10032 (unclassified *Enterobacteriaceae*), unclassified *Acidaminococcaceae*, OTU4607 (*Sutterella*), and the *Lactobacillales* order, as being associated with an increased risk of ALS. These taxa were linked to Gamma-glutamyl-related metabolites, suggesting a possible role for glutaminergic excitotoxicity in ALS pathogenesis, supporting a potential causal link between the microbiome and the disease. However, the authors did not state whether the results were corrected for multiple comparisons, which might have affected the reliability of the reported associations, and the robustness of the analysis may be affected by the limited number of genetic variants used as instrumental variables for gut microbiota composition and ALS risk, though instruments were checked for sufficient strength (F-statistic>10). In addition, the study focused on people of European descent, which limits the generalisability of the results. Also, it should be noted that the use of 16S rRNA sequencing has relatively low resolution and advanced techniques such as metagenomics could improve the accuracy of species-specific linkage detection.

While studies in presymptomatic individuals are constrained by practical challenges, the study by Rowin et al. (2017) stated that all participants reported gastrointestinal symptoms preceding the onset of neurological symptoms. However, the generalizability of these findings is limited due to the study's small sample size, reliance on retrospective patient recollections without objective measurements and the fact that gastrointestinal issues (not including cancers and inflammatory bowel disease) are very common in middle-aged and older patients, with reported pooled prevalence rates between 30 and 40% worldwide (Sperber et al., 2021). A more

detailed investigation of the time of onset of gastrointestinal problems, their course and duration before the onset of ALS symptoms would have been valuable.

Currently, rodent models of ALS provide strong evidence that the gut dysbiosis occurs early in the disease process, potentially even before the onset of symptoms (Wu et al., 2015; Figueroa-Romero et al., 2019). This is further supported by the observation that in SOD1^{G93A} mice, the gut microbiome alterations precede the aggregation of human-SOD1^{G93A} protein in the colon and intestine (Zhang et al., 2021). These findings together suggest that the gut dysbiosis is a contributing factor rather than a secondary consequence of the disease, but these findings are only as valid in the animal model.

The gut microbiome is closely associated with the function of the C9orf72 gene (Burberry et al., 2020), which is a major genetic cause of ALS and frontotemporal dementia (FTD). Studies in C9orf72-mutant mice have shown that the microbiota can significantly influence disease severity and microglial activation. Mice raised in pro-inflammatory environments exhibited altered gut microbiota, more pronounced autoimmune and inflammatory responses, increased microglial activation, and shorter lifespans compared to those raised in pro-survival conditions, characterized by the absence of certain microbial strains (e.g. *Helicobacter spp*) and increased relative abundance of others, such as *Akkermansia muciniphila* (Burberry et al., 2020). Interestingly, FMT from the pro-survival environment helped alleviate the inflammatory phenotypes in the pro-inflammatory environment mice, suggesting the potential impact of gut microbiota on ALS progression (Burberry et al., 2020). Interestingly, experiments with C9orf72 transgenic mice carrying 500 GGGGCC repeats (C9-500) have produced contradictory results across different labs regarding the observed phenotypic outcomes (Liu et al., 2016; Mordes et al., 2020). Potential

reasons for these discrepancies include variations in the housing conditions and the gut microbiome, both of which can influence the manifestation of the disease traits in these models.

Further evidence from studies involving antibiotics – the effect of which on the gut microbiome is well known - supports the potential role of gut microbiota in ALS pathology, although findings remain conflicting. Antibiotic treatment effectively reduced $SOD1^{G93A}$ aggregation in the intestines of $SOD1^{G93A}$ mice and improved enteric neuromuscular function (Zhang et al., 2021), with similar benefits observed in C9orf72-mutant mice. In $C9orf72^{-/-}$ mice, antibiotic administration suppressed inflammatory and autoimmune phenotypes, and lifelong antibiotic use prevented the infiltration of myeloid cells into the spinal cord and inhibited microglial activation (Burberry et al., 2020). On the other hand, antibiotic treatment has been also reported to have negative effects in $SOD1^{G93A}$ mice, where its long-term use resulted in motor neuron death, brain atrophy, and worsened motor impairment (Blacher et al., 2019). Furthermore, antibiotic-induced dysbiosis accelerated disease progression, and the analysis of the microbiome changes revealed reduced *Akkermansia* and butyrate-producing bacteria (Cox et al., 2022). Notably, the changes in microglia induced by antibiotics preceded the changes in motor function.

Interestingly, in human case-study involving 2,484 ALS patients and 12,420 age-matched controls, Sun et al. (2019) reported that any, but especially repeated antibiotic use might be associated with an increased risk of ALS, with odds ratios of 1.06, 1.13 and 1.18 for 1, 2–3 and ≥4 prescriptions, respectively (Sun et al., 2019).

4.1. Evidence for gut dysbiosis in human ALS

Human studies frequently report marked differences in the composition and diversity of the gut microbiome in ALS patients compared to controls. For example, multiple

studies found the Firmicutes/Bacteroidetes (F/B) ratio, which is considered a sign of intestinal homestatis (Noor Eddin et al., 2024), to be decreased in ALS patients (Fang et al., 2016; Rowin et al., 2017; Zeng et al., 2020). A further study reported contrasting results (Zhai et al., 2019) whilst another failed to find any difference in F/B ratio (Brenner et al., 2018). Interestingly, Ngo et al. (2020) reported only non-significant differences between ALS patients and controls, but did observe that patients with increased F/B had a greater risk for earlier death.

Fang et al. (2016) found increased relative abundance of Bacteroidetes (phylum), *Bacteroidia* (class), *Bacteroidales* (order), and *Dorea* (genus) in ALS patients, while Firmicutes (phylum), Clostridia (class), *Lachnospiraceae* (family), and the genera *Oscillibacter* and *Anaerostipes* were reduced. The authors reasoned that the imbalance in gut microbiota of ALS patients, characterized by an overgrowth of pathogens and a reduction in beneficial probiotic organisms, may lead to altered production of key molecules such as nitric oxide (NO), gamma-aminobutyric acid (GABA), SCFAs, and LPS, which eventually contributes to the pathogenesis of ALS. However, the sample size was small, including only 5 healthy controls and 6 patients with no disease subtype or duration information, which – together with a single sample collection – limits the strength of the study.

In another study on ALS patients, Di Gioia et al. (2020) found that certain microbial populations were significantly altered. Escherichia coli, *Clostridiales Family XI* (family), *Gastranaerophilales* (family), and Cyanobacteria (phylum) were markedly elevated, while *Clostridiaceae 1* (family) was found to be reduced compared to controls. The study included 50 controls and 50 ALS patients, excluding those with PEG, nasogastric tube, concomitant diseases and medications that could affect the gut microbiota.

Recently, a large human study longitudinally investigated gut microbiome changes in ALS patients and correlated them to plasma metabolome (Guo et al., 2023). The fecal samples of 75 ALS patients and 110 healthy controls were profiled at three time points, and plasma metabolites were profiled at the first time point. The study reported reduced microbial diversity and altered relative abundance of specific genera, such as increased *Bacteroides* and decreased *Faecalibacterium*, which was noted also by Fang et al. (2016) and Nicholson et al. (2020). Integration of microbiome data with plasma metabolomics profiles revelated that specific bacterial species correlate with alterations in lipid-related metabolites, and interestingly, Mendelian randomization described potential causality from particular lipid species, especially fatty acid, bile acid and acylcarnitine metabolism (Guo et al., 2023). Advanced statistical methods further identified *Lachnospiraceae UCG-010*, *Erysipelotrichaceae UCG-003*, and *Anaerofustis stercorihominis* – along with a few highly interrelated metabolites - as microbial signatures differentiating ALS patients from controls. However, the study, relying on 16S rRNA sequencing, lacked dietary and environmental data and sample collection times were not standardized and relative to disease onset.

One of the few studies utilizing shotgun metagenomics to investigate both the taxonomic and functional profiles of the gut microbiome examined 66 ALS patients and 61 healthy controls, reporting a significant reduction in the relative abundance of eight prominent butyrate-producing bacteria, including two dominant species *Roseburia intestinalis* and *Eubacterium rectale* (now called *Agathobacter rectalis*) in ALS patients (Nicholson et al.,2020). The authors used 16S rRNA sequencing in a subset of participants, revealing that the relative abundance of the family *Lachnospiraceae* (which includes also the two aforementioned species) was lower in

ALS patients, though the difference did not reach statistical significance. Nevertheless, the reduced abundance of *Lachnospiraceae* is a fairly consistent finding across 16S rRNA studies (Fang et al., 2016; Niccolai et al., 2021; Özaydin et al., 2024).

Recently, direct clinical evidence supporting the central role of gut dysbiosis in ALS emerged from experiments with FMT. In the first case report by Lu et al. (2023), a 48-year-old ALS patient received washing microbiota transplantation (WMT), following which her muscle tone improved, and functional scores stabilized. A subsequent relapse following antibiotic treatment was mitigated by rescue WMT, leading to renewed improvement. Microbial analysis indicated a shift in her gut microbiota toward that of healthy donors (Lu et al., 2023).

In another study with 2 patients (Yan et al., 2024), FMT led to considerable improvements in respiratory function, allowing weaning off mechanical ventilation, and increased strength, enabling the patients to stand or move with assistance. Analysis revealed increased beneficial microbes (*Bacteroides spp*., *Faecalibacterium prausnitzii*), elevated arginine biosynthesis metabolites, and reduced branched-chain amino acid metabolites (Yan et al., 2024).

However, the promising results of these studies need to be interpreted in the context of their significant limitations, i.e. very small sample sizes, absence of controls and lack of long-term follow-up. More comprehensive assessment is needed, such as the randomized, double-blind, multicenter study *Fecal Microbiota Transplantation Effect on Amyotrophic Lateral Sclerosis Patients (FETR-ALS)* by Mandrioli et al. (2019). The study is underway and should conclude in February 2025.

In December 2024, the only double-blind, placebo-controlled, randomized clinical trial of ALS-FMT published the results (Feng et al., 2024). The study included 27 patients with sALS, of whom 14 received healthy donor FMT. Although no significant improvements in the ALS Functional Rating Scale-Revised (ALSFRS-R) were reported, the FMT group demonstrated improvements in constipation, depression, and anxiety. The study had several limitations: a small sample size, not accounting for pre-baseline disease progression and employing a multi-donor approach, thus limiting insights into individual microbial contributions.

4.2. Gut dysbiosis as biomarker

Insights from AD and PD underline the potential of gut dysbiosis as a biomarker in neurodegenerative diseases, with parallels that justify its investigation in ALS. In both AD and PD, gut microbiota composition shifts are observed in preclinical stages, often preceding the onset of cognitive or motor symptoms. Not only are these dysbiotic profiles detectable, but they have also been found to correlate with disease-specific pathological and metabolic changes, supporting their utility as potential early non-invasive biomarkers.

While the gut microbiome in ALS is less studied compared to AD or PD, mounting evidence suggests that its alterations occur early and reflects both taxonomic and functional shifts that may correlate with disease onset and progression. Though not consistently found, the decreased F/B ratio and reduced SCFA-producing taxa align with findings in PD and AD, where relative abundances of potential pro-inflammatory bacteria are significantly increased, while potential anti-inflammatory bacteria are significantly decreased (Nie et al., 2022; Ferreiro et al., 2023). The functional impact of the gut microbiome alterations —such as impaired SCFAs and systemic inflammation—reflects shared mechanisms across neurodegenerative diseases,

further justifying their investigation as biomarkers. Shared features of findings in human AD, PD and ALS are summarized in Table 1.

Finding	AD	PD	ALS
Pro-inflammatory taxa	Increased, e.g. *Bacteroides, Escherichia-Shigella* (Cattaneo et al., 2017; Cammann et al., 2023)	Increased, e.g. *Collinsella*, *Desulfovibrio*, and *Oscillospiraceae UCG-005* (Huang et al., 2023)	Increased, e.g. *Bacteroides, Lactococcus* and *Escherichia coli* (Guo et al., 2023; Blacher et al., 2019)
SCFA producers	Decreased, e.g. *Lachnospiraceae spp*., *Lachnoclostridium spp*. and *Roseburia hominis* (Loh et al., 2024; Verhaar et al., 2022).	Decreased, e.g. Lachnospiraceae family and the *Faecalibacterium* genus (Romano et al., 2021)	Decreased, e.g. *Fusicatenibacter* and *Catenibacterium*; (Ning et al., 2022)
LPS levels in plasma	Increased (Zhang et al., 2009)	Increased (Zhao et al., 2023)	Increased (Zhang et al., 2009)
Recent FMT studies	1 case: improvement (Hazan, 2020) 10 cases: improvement (Park et al., 2022)	46 cases: no effect (Scheperjans et al., 2024) 46 cases: limited and temporary effect (Bruggeman et al., 2024) 12 cases: limited and temporary effect (DuPont et al., 2023).	1 case: improvement Lu et al. (2023) 2 cases: improvement (Yan et al., 2024) 27 cases: no effect (Feng et al., 2024).
Evidence of prodromal gut dysbiosis	Reported (Palacios et al., 2023)	Reported (Ferreiro et al., 2023)	Only limited and subjective data (Rowin et al., 2017)

Table 1: Overlapping or shared findings in human studies of gut microbiome in Alzheimer's disease (AD), Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS). SCFAs - short-chain fatty acids, LPS – lipopolysaccharide, FMT – fecal microbiota transplantation.

Integrative analyses linking the gut microbiome to plasma metabolomics have provided some of the most promising leads. As discussed above, Guo et al. (2023) reported specific bacterial taxa and lipid-related plasma metabolites, which not only distinguished ALS from controls but also aligned with observed metabolic dysfunctions, such as disruptions in fatty acid and bile acid pathways. Of note, primary and secondary bile acids have been reported to correlate with body mass index (BMI) in ALS and could represent maladaptation of intestinal lipid absorption

and dysregulation of cholesterol metabolism (Guo et al.,2023), which some studies suggest may be causal in ALS (Zeng & Zhou, 2018; Xia et al., 2023).

Early changes in SCFA levels or shifts in metabolite-associated pathways may be found in asymptomatic carriers of ALS-associated mutations, similarly to prodromal markers in AD and PD. However, reduced F/B ratios and depletion of SCFA-producing taxa (*Lachnospiraceae* and *Faecalibacterium*) are hallmark alterations in a variety of inflammatory and metabolic diseases, so their specificity would need to be improved, for example by integration with ALS-specific metabolomic findings, thus creating a composite biomarker.

Such a biomarker could combine – optimally leveraging machine learning models - reduced abundance of *Lachnospiraceae* and *Faecalibacterium prausnitzii* with increased *Bacteroides* and *Prevotella copri*, and ALS-specific metabolomic alterations discussed earlier, such as disrupted lipid and bile acid metabolism.

While such an approach is feasible, it also has significant limitations. Firstly, the mechanistic links of gut dysbiosis, metabolomic shifts, and ALS pathology, though promising, remain incompletely understood, which complicates the clinical interpretation. Secondly, variations across studies demonstrate the inconsistency in observed alterations and metabolomic profiles, caused by factors such as small sample sizes, heterogeneity in patient cohorts, and methodological differences in microbiome analysis (Zhao et al., 2024; Fang et al., 2016). Shotgun metagenomics provides more extensive data compared to 16S rRNA sequencing, but its use is limited by cost and the need for standardized protocols. In addition, environmental, dietary and regional factors have a major influence on gut microbiota and need to be accounted for.

5. Conclusion

Increasing evidence of the contribution of gut dysbiosis to the pathogenesis of neurodegenerative diseases points to the importance of GBA and its role in these processes, and changes in the composition of the gut microbiota have been consistently reported also in ALS patients. These include reduced microbial diversity, depletion of SCFA-producing taxa, and increased abundance of pro-inflammatory taxa such as *Bacteroides*. Metabolomic studies have furthermore shown disruptions in lipid and bile acid metabolism, possibly reflecting disease-specific alterations promoted or caused by the gut dysbiosis, given the intimate link between the gut microbiome and host metabolism. Although studies in rodent models and early evidence from FMT suggest that gut dysbiosis may be a contributing rather than a consequential factor in the pathogenesis of ALS, definitive conclusions are prevented by various limitations such as small cohorts, reliance on 16S rRNA sequencing, and lack of longitudinal data.

Given the parallels with AD and PD, where gut dysbiosis has been observed early in the disease process, the development of composite biomarkers integrating microbial taxa and metabolomic profiles may be beneficial. Such biomarkers – if validated - could provide a foundation for a microbiome-screening system targeted at early detection of the disease, especially in individuals with known genetic mutations or those with vague symptoms and inconclusive diagnostic tests. Identifying at-risk individuals before the onset of symptoms or at a very early stage of the disease would allow early therapeutic interventions that could delay disease progression or reduce its severity. Also, early detection of certain dysbiosis patterns could serve to optimize personalized microbiome-based treatment strategies.

However, issues related to demographic heterogeneity, methodological differences, and the low specificity of certain gut microbial changes must be resolved. Most importantly, larger, longitudinal studies using advanced technologies like shotgun metagenomics, combined with robust statistical approaches, are critical for refining these findings. In conclusion, although evidence for the contribution of gut dysbiosis to ALS pathogenesis is increasing, its clinical translation into a novel biomarker will necessitate thorough validation, standardization and deeper mechanistic understanding.

6. References

1. Abbott, R.D. *et al.* (2003) ‘Environmental, life-style, and physical precursors of clinical parkinson?s disease: Recent findings from the honolulu-asia aging study’, *Journal of Neurology*, 250(0), pp. 1–1. doi:10.1007/s00415-003-1306-7.
2. Banack, S.A. *et al.* (2024) ‘A microrna diagnostic biomarker for amyotrophic lateral sclerosis’, *Brain Communications*, 6(5). doi:10.1093/braincomms/fcae268.
3. Bello-Medina, P. (2021) *Spatial memory and gut microbiota alterations are already present in early-adulthood in a preclinical transgenic model of alzheimer´s disease* [Preprint]. doi:10.26226/morressier.6076f2e66e987178c2dbdf4d.
4. Berthoud, H.R. *et al.* (2004) ‘Neuroanatomy of extrinsic afferents supplying the gastrointestinal tract’, *Neurogastroenterology & Motility*, 16(s1), pp. 28–33. doi:10.1111/j.1743-3150.2004.00471.x.
5. Blacher, E. *et al.* (2019) ‘Potential roles of gut microbiome and metabolites in modulating ALS in mice’, *Nature*, 572(7770), pp. 474–480. doi:10.1038/s41586-019-1443-5.
6. Braniste, V. *et al.* (2014) ‘The gut microbiota influences blood-brain barrier permeability in mice’, *Science Translational Medicine*, 6(263). doi:10.1126/scitranslmed.3009759.
7. Brenner, D. *et al.* (2018) ‘The fecal microbiome of ALS patients’, *Neurobiology of Aging*, 61, pp. 132–137. doi:10.1016/j.neurobiolaging.2017.09.023.
8. Bruggeman, A. *et al.* (2024) ‘Safety and efficacy of faecal microbiota transplantation in patients with mild to moderate parkinson’s disease (gut-PARFECT): A double-blind, placebo-controlled, randomised, phase 2 trial’, *eClinicalMedicine*, 71, p. 102563. doi:10.1016/j.eclinm.2024.102563.
9. Bohórquez, D.V. *et al.* (2015) ‘Neuroepithelial circuit formed by innervation of sensory enteroendocrine cells’, *Journal of Clinical Investigation*, 125(2), pp. 782–786. doi:10.1172/jci78361.
10. Bonaz, B., Bazin, T. and Pellissier, S. (2018) ‘The vagus nerve at the interface of the microbiota-gut-brain axis’, *Frontiers in Neuroscience*, 12. doi:10.3389/fnins.2018.00049.

11. Boddy, S. *et al.* (2021) ‘The gut microbiome: A key player in the complexity of amyotrophic lateral sclerosis (ALS)’, *BMC Medicine*, 19(1). doi:10.1186/s12916-020-01885-3.
12. Cammann, D. *et al.* (2023) ‘Genetic correlations between alzheimer’s disease and gut microbiome genera’, *Scientific Reports*, 13(1). doi:10.1038/s41598-023-31730-5.
13. Cantoni, C. *et al.* (2022) ‘Alterations of host-gut microbiome interactions in multiple sclerosis’, *eBioMedicine*, 76, p. 103798. doi:10.1016/j.ebiom.2021.103798.
14. Cao, S. *et al.* (2018a) ‘LPS challenge increased intestinal permeability, disrupted mitochondrial function and triggered mitophagy of piglets’, *Innate Immunity*, 24(4), pp. 221–230. doi:10.1177/1753425918769372.
15. Carloni, S. *et al.* (2021) ‘Identification of a choroid plexus vascular barrier closing during intestinal inflammation’, *Science*, 374(6566), pp. 439–448. doi:10.1126/science.abc6108.
16. Cattaneo, A. *et al.* (2017) ‘Association of brain amyloidosis with pro-inflammatory gut bacterial taxa and peripheral inflammation markers in cognitively impaired elderly’, *Neurobiology of Aging*, 49, pp. 60–68. doi:10.1016/j.neurobiolaging.2016.08.019.
17. Ciarlo, E. *et al.* (2016) ‘Impact of the microbial derived short chain fatty acid propionate on host susceptibility to bacterial and fungal infections in vivo’, *Scientific Reports*, 6(1). doi:10.1038/srep37944.
18. Cox, L.M. *et al.* (2022) ‘The microbiota restrains neurodegenerative microglia in a model of amyotrophic lateral sclerosis’, *Microbiome*, 10(1). doi:10.1186/s40168-022-01232-z.
19. Cryan, J.F. and Dinan, T.G. (2012) ‘Mind-altering microorganisms: The impact of the gut microbiota on brain and behaviour’, *Nature Reviews Neuroscience*, 13(10), pp. 701–712. doi:10.1038/nrn3346.
20. Deng, Y. *et al.* (2021) ‘Involvement of the microbiota-gut-brain axis in chronic restraint stress: Disturbances of the kynurenine metabolic pathway in both the gut and brain’, *Gut Microbes*, 13(1). doi:10.1080/19490976.2020.1869501.
21. de Punder, K. and Pruimboom, L. (2015) ‘Stress induces endotoxemia and low-grade inflammation by increasing barrier permeability’, *Frontiers in Immunology*, 6. doi:10.3389/fimmu.2015.00223.
22. Dodiya, H.B. *et al.* (2021) ‘Gut microbiota–driven brain AB amyloidosis in mice requires microglia’, *Journal of Experimental Medicine*, 219(1). doi:10.1084/jem.20200895.
23. DuPont, H.L. *et al.* (2023) ‘Fecal microbiota transplantation in parkinson’s disease—a randomized repeat-dose, placebo-controlled clinical pilot study’, *Frontiers in Neurology*, 14. doi:10.3389/fneur.2023.1104759.
24. Erny, D. *et al.* (2015) ‘Host microbiota constantly control maturation and function of microglia in the CNS’, *Nature Neuroscience*, 18(7), pp. 965–977. doi:10.1038/nn.4030.
25. Escribano, B.M. *et al.* (2017) ‘Lipopolysaccharide binding protein and oxidative stress in a multiple sclerosis model’, *Neurotherapeutics*, 14(1), pp. 199–211. doi:10.1007/s13311-016-0480-0.
26. Fang, Y.-T. *et al.* (2023a) ‘Neuroimmunomodulation of vagus nerve stimulation and the therapeutic implications’, *Frontiers in Aging Neuroscience*, 15. doi:10.3389/fnagi.2023.1173987.

27. Fang, X. *et al.* (2016) ‘Evaluation of the microbial diversity in amyotrophic lateral sclerosis using high-throughput sequencing’, *Frontiers in Microbiology*, 7. doi:10.3389/fmicb.2016.01479.
28. Feng, R. *et al.* (2024) ‘Effect of fecal microbiota transplantation on patients with sporadic amyotrophic lateral sclerosis: A randomized, double-blind, placebo-controlled trial’, *BMC Medicine*, 22(1). doi:10.1186/s12916-024-03781-6.
29. Fernando, W.M.A.D.B. *et al.* (2020) ‘Sodium butyrate reduces brain amyloid-β levels and improves cognitive memory performance in an alzheimer’s disease transgenic mouse model at an early disease stage’, *Journal of Alzheimer’s Disease*, 74(1), pp. 91–99. doi:10.3233/jad-190120.
30. Ferreiro, A.L. *et al.* (2023) ‘Gut microbiome composition may be an indicator of preclinical alzheimer’s disease’, *Science Translational Medicine*, 15(700). doi:10.1126/scitranslmed.abo2984.
31. Figueroa-Romero, C. *et al.* (2019) ‘Temporal evolution of the microbiome, immune system, and epigenome with disease progression in ALS mice’, *Disease Models & Mechanisms* [Preprint]. doi:10.1242/dmm.041947.
32. Fock, E. and Parnova, R. (2023a) ‘Mechanisms of blood–brain barrier protection by microbiota-derived short-chain fatty acids’, *Cells*, 12(4), p. 657. doi:10.3390/cells12040657.
33. Fujimura, K.E. *et al.* (2016) ‘Neonatal gut microbiota associates with childhood multisensitized atopy and T cell differentiation’, *Nature Medicine*, 22(10), pp. 1187–1191. doi:10.1038/nm.4176.
34. Gibbs, M.E., Gibbs, Z. and Hertz, L. (2009) ‘Rescue of AB1–42-induced memory impairment in day-old chick by facilitation of astrocytic oxidative metabolism: Implications for alzheimer’s disease’, *Journal of Neurochemistry*, 109(s1), pp. 230–236. doi:10.1111/j.1471-4159.2009.05800.x.
35. Guo, K. *et al.* (2023) ‘Gut microbiome correlates with plasma lipids in amyotrophic lateral sclerosis’, *Brain* [Preprint]. doi:10.1093/brain/awad306.
36. Chen, C. *et al.* (2022) ‘Gut microbiota regulate alzheimer’s disease pathologies and cognitive disorders via PUFA-associated neuroinflammation’, *Gut*, 71(11), pp. 2233–2252. doi:10.1136/gutjnl-2021-326269.
37. Chen, H. *et al.* (2022) ‘Vagus nerve stimulation reduces neuroinflammation through microglia polarization regulation to improve functional recovery after spinal cord injury’, *Frontiers in Neuroscience*, 16. doi:10.3389/fnins.2022.813472.
38. Chen, Y. *et al.* (2020) ‘Gut microbiome alterations precede cerebral amyloidosis and microglial pathology in a mouse model of alzheimer’s disease’, *BioMed Research International*, 2020, pp. 1–15. doi:10.1155/2020/8456596.
39. Chen, Yijing, Xu, J. and Chen, Yu (2021) ‘Regulation of neurotransmitters by the gut microbiota and effects on cognition in neurological disorders’, *Nutrients*, 13(6), p. 2099. doi:10.3390/nu13062099.
40. Choden, T. and Cohen, N.A. (2022) ‘The gut microbiome and the immune system’, *Exploration of Medicine*, pp. 219–233. doi:10.37349/emed.2022.00087.
41. Hardiman, O. *et al.* (2017) ‘Amyotrophic lateral sclerosis’, *Nature Reviews Disease Primers*, 3(1). doi:10.1038/nrdp.2017.71.

42. Hazan, S. (2020) ‘Rapid improvement in alzheimer’s disease symptoms following fecal microbiota transplantation: A case report’, *Journal of International Medical Research*, 48(6). doi:10.1177/0300060520925930.
43. Hoyles, L. *et al.* (2018) ‘Microbiome–host systems interactions: Protective effects of propionate upon the blood–brain barrier’, *Microbiome*, 6(1). doi:10.1186/s40168-018-0439-y.
44. Huang, B. *et al.* (2023) ‘Gut microbiome dysbiosis across early parkinson’s disease, REM sleep behavior disorder and their first-degree relatives’, *Nature Communications*, 14(1). doi:10.1038/s41467-023-38248-4.
45. Huang, Y. et al. (2023) ‘The gut microbiome modulates the transformation of microglial subtypes’, Molecular Psychiatry, 28(4), pp. 1611–1621. doi:10.1038/s41380-023-02017-y.
46. Huffman, W.J. *et al.* (2019) ‘Modulation of neuroinflammation and memory dysfunction using percutaneous vagus nerve stimulation in mice’, *Brain Stimulation*, 12(1), pp. 19–29. doi:10.1016/j.brs.2018.10.005.
47. Kaelberer, M.M. *et al.* (2018) ‘A gut-brain neural circuit for nutrient sensory transduction’, *Science*, 361(6408). doi:10.1126/science.aat5236.
48. Kasarello, K., Cudnoch-Jedrzejewska, A. and Czarzasta, K. (2023) ‘Communication of gut microbiota and brain via immune and neuroendocrine signaling’, *Frontiers in Microbiology*, 14. doi:10.3389/fmicb.2023.1118529.
49. Leclercq, S. *et al.* (2017) ‘Low-dose penicillin in early life induces long-term changes in murine gut microbiota, brain cytokines and behavior’, *Nature Communications*, 8(1). doi:10.1038/ncomms15062.
50. Li, T. *et al.* (2023) ‘Deciphering the interplay between LPS/TLR4 pathways, neurotransmitter, and deltamethrin-induced depressive-like behavior: Perspectives from the Gut-Brain Axis’, *Pesticide Biochemistry and Physiology*, 197, p. 105697. doi:10.1016/j.pestbp.2023.105697.
51. Liu, L. *et al.* (2024) ‘Impact of age-related gut microbiota dysbiosis and reduced short-chain fatty acids on the autonomic nervous system and atrial fibrillation in rats’, *Frontiers in Cardiovascular Medicine*, 11. doi:10.3389/fcvm.2024.1394929.
52. Liu, J. *et al.* (2021) ‘The neuroprotective effect of short chain fatty acids against sepsis-associated encephalopathy in mice’, *Frontiers in Immunology*, 12. doi:10.3389/fimmu.2021.626894.
53. Liu, Y. *et al.* (2016) ‘C9orf72 BAC mouse model with motor deficits and neurodegenerative features of ALS/FTD’, *Neuron*, 90(3), pp. 521–534. doi:10.1016/j.neuron.2016.04.005.
54. Logan, R. *et al.* (2022) ‘Novel genetic signatures associated with sporadic amyotrophic lateral sclerosis’, *Frontiers in Genetics*, 13. doi:10.3389/fgene.2022.851496.
55. Lobionda, S. *et al.* (2019) ‘The role of gut microbiota in intestinal inflammation with respect to diet and extrinsic stressors’, *Microorganisms*, 7(8), p. 271. doi:10.3390/microorganisms7080271.
56. Loh, J.S. *et al.* (2024) ‘Microbiota–gut–brain axis and its therapeutic applications in neurodegenerative diseases’, *Signal Transduction and Targeted Therapy*, 9(1). doi:10.1038/s41392-024-01743-1.
57. Lu, G. *et al.* (2023) ‘Washed microbiota transplantation stopped the deterioration of amyotrophic lateral sclerosis: The first Case report and narrative review’, *The Journal of Biomedical Research*, 37(1), p. 69. doi:10.7555/jbr.36.20220088.

58. Luna, J. *et al.* (2017) ‘Current issues in ALS Epidemiology: Variation of als occurrence between populations and physical activity as a risk factor’, *Revue Neurologique*, 173(5), pp. 244–253. doi:10.1016/j.neurol.2017.03.035.
59. Magzal, F. *et al.* (2021) ‘Associations between fecal short-chain fatty acids and sleep continuity in older adults with insomnia symptoms’, *Scientific Reports*, 11(1). doi:10.1038/s41598-021-83389-5.
60. Mandrioli, J. *et al.* (2019) ‘FETR-ALS study protocol: A randomized clinical trial of fecal microbiota transplantation in amyotrophic lateral sclerosis’, *Frontiers in Neurology*, 10. doi:10.3389/fneur.2019.01021.
61. Mejzini, R. *et al.* (2019) ‘Als genetics, mechanisms, and Therapeutics: Where are we now?’, *Frontiers in Neuroscience*, 13. doi:10.3389/fnins.2019.01310.
62. Miller, T.M. *et al.* (2022) ‘Trial of antisense oligonucleotide tofersen for *sod1* ALS’, *New England Journal of Medicine*, 387(12), pp. 1099–1110. doi:10.1056/nejmoa2204705.
63. Mindus, C. *et al.* (2021) ‘Lactobacillus-based probiotics reduce the adverse effects of stress in rodents: A meta-analysis’, *Frontiers in Behavioral Neuroscience*, 15. doi:10.3389/fnbeh.2021.642757.
64. Mouries, J. *et al.* (2019) ‘Microbiota-driven gut vascular barrier disruption is a prerequisite for non-alcoholic steatohepatitis development’, *Journal of Hepatology*, 71(6), pp. 1216–1228. doi:10.1016/j.jhep.2019.08.005.
65. Mordes, D.A. *et al.* (2020) ‘Absence of survival and motor deficits in 500 repeat c9orf72 bac mice’, *Neuron*, 108(4). doi:10.1016/j.neuron.2020.08.009.
66. Ngo, S.T. *et al.* (2020) ‘Progression and survival of patients with motor neuron disease relative to their fecal microbiota’, *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration*, 21(7–8), pp. 549–562. doi:10.1080/21678421.2020.1772825.
67. Ni, S. *et al.* (2022) ‘Vagus nerve stimulated by microbiota-derived hydrogen sulfide mediates the regulation of berberine on microglia in transient middle cerebral artery occlusion rats’, *Phytotherapy Research*, 36(7), pp. 2964–2981. doi:10.1002/ptr.7490.
68. Nie, S. *et al.* (2022) ‘Inflammatory microbes and genes as potential biomarkers of parkinson’s disease’, *npj Biofilms and Microbiomes*, 8(1). doi:10.1038/s41522-022-00367-z.
69. Niccolai, E. *et al.* (2021) ‘The gut microbiota-immunity axis in ALS: A role in deciphering disease heterogeneity?’, *Biomedicines*, 9(7), p. 753. doi:10.3390/biomedicines9070753.
70. Nicholson, K. *et al.* (2020) ‘The human gut microbiota in people with amyotrophic lateral sclerosis’, *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration*, 22(3–4), pp. 186–194. doi:10.1080/21678421.2020.1828475.
71. Ning, J. *et al.* (2022) ‘Investigating casual associations among gut microbiota, metabolites, and neurodegenerative diseases: A mendelian randomization study’, *Advances in Alzheimer’s Disease* [Preprint]. doi:10.3233/aiad220023.
72. Noor Eddin, A. *et al.* (2024) ‘Gut-modulating agents and amyotrophic lateral sclerosis: Current evidence and Future Perspectives’, *Nutrients*, 16(5), p. 590. doi:10.3390/nu16050590.
73. Özaydin Aksun, Z. *et al.* (2024) ‘Is gut microbiota of patients with ALS different from that of healthy individuals?’, *Turkish Journal of Medical Sciences*, 54(3), pp. 579–587. doi:10.55730/1300-0144.5825.

74. Park, S.-H. *et al.* (2022) ‘Fecal microbiota transplantation can improve cognition in patients with cognitive decline and clostridioides difficile infection’, *Aging*, 14(16), pp. 6449–6466. doi:10.18632/aging.204230.
75. Parra-Cantu, C. *et al.* (2021) ‘Prevalence of gastrointestinal symptoms, severity of dysphagia, and their correlation with severity of amyotrophic lateral sclerosis in a Mexican cohort’, *Neurodegenerative Diseases*, 21(1–2), pp. 42–47. doi:10.1159/000517613.
76. Qian, Y. *et al.* (2018) ‘Alteration of the fecal microbiota in Chinese patients with parkinson’s disease’, *Brain, Behavior, and Immunity*, 70, pp. 194–202. doi:10.1016/j.bbi.2018.02.016.
77. Rodrigues, G. (2024) ‘Is the vagus nerve the “MVP” in the brain–body axis?’, *Clinical Autonomic Research*, 34(3), pp. 383–384. doi:10.1007/s10286-024-01042-3.
78. Rojas-Valverde, D. *et al.* (2023) ‘Examining the interaction between exercise, gut microbiota, and neurodegeneration: Future research directions’, *Biomedicines*, 11(8), p. 2267. doi:10.3390/biomedicines11082267.
79. Romano, S. *et al.* (2021) ‘Meta-analysis of the Parkinson’s Disease Gut Microbiome suggests alterations linked to intestinal inflammation’, *npj Parkinson’s Disease*, 7(1). doi:10.1038/s41531-021-00156-z.
80. Rowin, J. *et al.* (2017) ‘Gut inflammation and dysbiosis in human motor neuron disease’, *Physiological Reports*, 5(18). doi:10.14814/phy2.13443.
81. Rusch, J.A., Layden, B.T. and Dugas, L.R. (2023) ‘Signalling cognition: The gut microbiota and hypothalamic-pituitary-adrenal axis’, *Frontiers in Endocrinology*, 14. doi:10.3389/fendo.2023.1130689.
82. Sabahat, S.E. *et al.* (2024) ‘Bile acid modulation by gut microbiota: A bridge to understanding cognitive health’, *Annals of Medicine & Surgery*, 86(9), pp. 5410–5415. doi:10.1097/ms9.0000000000002433.
83. Sampson, T.R. *et al.* (2016) ‘Gut microbiota regulate motor deficits and neuroinflammation in a model of parkinson’s disease’, *Cell*, 167(6). doi:10.1016/j.cell.2016.11.018.
84. Seo, D. *et al.* (2023) ‘ApoE isoform– and microbiota-dependent progression of neurodegeneration in a mouse model of tauopathy’, *Science*, 379(6628). doi:10.1126/science.add1236.
85. Schiweck, C. *et al.* (2024) ‘No consistent evidence for the anti-inflammatory effect of vagus nerve stimulation in humans: A systematic review and meta-analysis’, *Brain, Behavior, and Immunity*, 116, pp. 237–258. doi:10.1016/j.bbi.2023.12.008.
86. Scheperjans, F. *et al.* (2014) ‘Gut microbiota are related to parkinson’s disease and clinical phenotype’, *Movement Disorders*, 30(3), pp. 350–358. doi:10.1002/mds.26069.
87. Scheperjans, F. *et al.* (2024) ‘Fecal microbiota transplantation for treatment of parkinson disease’, *JAMA Neurology*, 81(9), p. 925. doi:10.1001/jamaneurol.2024.2305.
88. Sampson, T.R. *et al.* (2016) ‘Gut microbiota regulate motor deficits and neuroinflammation in a model of parkinson’s disease’, *Cell*, 167(6). doi:10.1016/j.cell.2016.11.018.
89. Sun, J. *et al.* (2019) ‘Antibiotics use and risk of amyotrophic lateral sclerosis in Sweden’, *European Journal of Neurology*, 26(11), pp. 1355–1361. doi:10.1111/ene.13986.

90. Sperber, A.D. *et al.* (2021) ‘Worldwide prevalence and burden of functional gastrointestinal disorders, results of Rome Foundation Global Study’, *Gastroenterology*, 160(1). doi:10.1053/j.gastro.2020.04.014.
91. Teixeira, B. *et al.* (2013) ‘Low sensitivity to glucocorticoid inhibition of in vitro th17-related cytokine production in multiple sclerosis patients is related to elevated plasma lipopolysaccharide levels’, *Clinical Immunology*, 148(2), pp. 209–218. doi:10.1016/j.clim.2013.05.012.
92. Tian, P. *et al.* (2021) ‘*pediococcus acidilactici*CCFM6432 mitigates chronic stress-induced anxiety and gut microbial abnormalities’, *Food & Function*, 12(22), pp. 11241–11249. doi:10.1039/d1fo01608c.
93. Toepfer, M. *et al.* (2000) ‘Gastrointestinal dysfunction in amyotrophic lateral sclerosis’, *Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders*, 1(1), pp. 15–19. doi:10.1080/146608299300079484.
94. Track, N. S. (1980). The gastrointestinal endocrine system. *Canadian Medical Association Journal*, *122*(3), 287.
95. Verhaar, B.J. *et al.* (2022) ‘Gut microbiota composition is related to ad pathology’, *Frontiers in Immunology*, 12. doi:10.3389/fimmu.2021.794519.
96. Vineis, P. *et al.* (2020) ‘What is new in the exposome?’, *Environment International*, 143, p. 105887. doi:10.1016/j.envint.2020.105887.
97. Vogt, N.M. *et al.* (2017) ‘Gut microbiome alterations in alzheimer’s disease’, *Scientific Reports*, 7(1). doi:10.1038/s41598-017-13601-y.
98. Volonté, C. and Amadio, S. (2023) ‘Amyotrophic lateral sclerosis disease burden: Doing better at getting better’, *Neural Regeneration Research*, 18(8), p. 0. doi:10.4103/1673-5374.363193.
99. Xia, K. *et al.* (2023) ‘Lipids and amyotrophic lateral sclerosis: A two-sample Mendelian Randomization Study’, *European Journal of Neurology*, 30(7), pp. 1899–1906. doi:10.1111/ene.15810.
100. Xu, L. *et al.* (2019) ‘Global variation in prevalence and incidence of amyotrophic lateral sclerosis: A systematic review and meta-analysis’, *Journal of Neurology*, 267(4), pp. 944–953. doi:10.1007/s00415-019-09652-y.
101. Yan, J. *et al.* (2024) ‘Fecal microbiota transplantation significantly improved respiratory failure of amyotrophic lateral sclerosis’, *Gut Microbes*, 16(1). doi:10.1080/19490976.2024.2353396.
102. Wang, H.-X. and Wang, Y.-P. (2016) ‘Gut microbiota-brain axis’, *Chinese Medical Journal*, 129(19), pp. 2373–2380. doi:10.4103/0366-6999.190667.
103. Wang, Y. *et al.* (2022) ‘Chronic microglial inflammation promotes neuronal lactate supply but impairs its utilization in primary rat astrocyte-neuron co-cultures’, *Biochemical and Biophysical Research Communications*, 607, pp. 28–35. doi:10.1016/j.bbrc.2022.03.122.
104. Wu, S. *et al.* (2015) ‘Leaky intestine and impaired microbiome in an amyotrophic lateral sclerosis mouse model’, *Physiological Reports*, 3(4). doi:10.14814/phy2.12356.
105. Zeng, P. and Zhou, X. (2018) ‘Causal effects of blood lipids on amyotrophic lateral sclerosis: A mendelian randomization study’, *Human Molecular Genetics*, 28(4), pp. 688–697. doi:10.1093/hmg/ddy384.
106. Zeng, Q. *et al.* (2020) ‘The alteration of gut microbiome and metabolism in amyotrophic lateral sclerosis patients’, *Scientific Reports*, 10(1). doi:10.1038/s41598-020-69845-8.

107. Zhai, C.-D. *et al.* (2019) ‘Intestinal microbiota composition in patients with amyotrophic lateral sclerosis’, *Chinese Medical Journal*, 132(15), pp. 1815–1822. doi:10.1097/cm9.0000000000000351.
108. Zhang, H. *et al.* (2022) ‘Implications of gut microbiota in Neurodegenerative Diseases’, *Frontiers in Immunology*, 13. doi:10.3389/fimmu.2022.785644.
109. Zhang, L. *et al.* (2022) ‘Assessment of bidirectional relationships between 98 genera of the human gut microbiota and amyotrophic lateral sclerosis: A 2-sample mendelian randomization study’, *BMC Neurology*, 22(1). doi:10.1186/s12883-021-02522-z.
110. Zhang, R. *et al.* (2009) ‘Circulating endotoxin and systemic immune activation in sporadic amyotrophic lateral sclerosis (SALS)’, *Journal of Neuroimmunology*, 206(1–2), pp. 121–124. doi:10.1016/j.jneuroim.2008.09.017.
111. Zhang, Y. *et al.* (2021) ‘Aberrant enteric neuromuscular system and dysbiosis in amyotrophic lateral sclerosis’, *Gut Microbes*, 13(1). doi:10.1080/19490976.2021.1996848.
112. Zhao, J. *et al.* (2019) ‘Neuroinflammation induced by lipopolysaccharide causes cognitive impairment in mice’, *Scientific Reports*, 9(1). doi:10.1038/s41598-019-42286-8.
113. Zhao, Y. *et al.* (2017) ‘Microbiome-derived lipopolysaccharide enriched in the perinuclear region of alzheimer’s disease brain’, *Frontiers in Immunology*, 8. doi:10.3389/fimmu.2017.01064.
114. Zhao, Z. *et al.* (2024) ‘Microbial Biomarker discovery in parkinson’s disease through a network-based approach’, *npj Parkinson’s Disease*, 10(1). doi:10.1038/s41531-024-00802-2.
115. Zheng, D., Liwinski, T. and Elinav, E. (2020) ‘Interaction between microbiota and immunity in health and disease’, *Cell Research*, 30(6), pp. 492–506. doi:10.1038/s41422-020-0332-7.
116. Zhou, X. *et al.* (2022) ‘Gut microbiome of multiple sclerosis patients and paired household healthy controls reveal associations with disease risk and course’, *Cell*, 185(19). doi:10.1016/j.cell.2022.08.021.
117. Zhuang, M., Zhang, X. and Cai, J. (2024) ‘Microbiota–gut–brain axis: Interplay between microbiota, barrier function and lymphatic system’, *Gut Microbes*, 16(1). doi:10.1080/19490976.2024.2387800.