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# Analysis methods for the gut microbiome in neuropsychiatric and neurodegenerative disorders

Jae Gwang Song [a,1], Myeong-Sang Yu [b,1], Bomi Lee [a], Jingyu Lee [b], Su-Hee Hwang [b], Dokyun Na [b,*], Hyung Wook Kim [a,*]

[a] Department of Bio-integrated Science and Technology, College of Life Sciences, Sejong University, Seoul 05006, Republic of Korea
[b] Department of Biomedical Engineering, Department of Biomedical Engineering, Chung-Ang University, 84 Heukseok-ro, Dongjak-gu, Seoul 06974, Republic of Korea

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ABSTRACT

For a long time, the central nervous system was believed to be the only regulator of cognitive functions. However, accumulating evidence suggests that the composition of the microbiome is strongly associated with brain functions and diseases. Indeed, the gut microbiome is involved in neuropsychiatric diseases (e.g., depression, autism spectrum disorder, and anxiety) and neurodegenerative diseases (e.g., Parkinson's disease and Alzheimer's disease). In this review, we provide an overview of the link between the gut microbiome and neuropsychiatric or neurodegenerative disorders. We also introduce analytical methods used to assess the connection between the gut microbiome and the brain. The limitations of the methods used at present are also discussed. The accurate translation of the microbiome information to brain disorder could promote better understanding of neuronal diseases and aid in finding alternative and novel therapies.



**Contents**



* Corresponding authors.
*E-mail addresses:* blisszen@lile.cau.ac.kr (D. Na), kimhyung@sejong.ac.kr (H.W. Kim).
[1] These authors contributed equally to this work.

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## 1. Introduction

Over the past decades, a considerable amount of research has shown the gut microbiome to be associated with brain functions. Despite Hippocrates' insightful claims, "All diseases begin in the gut," the interaction between the gut and brain is not fully understood yet. For a long time, neuropsychiatric and neurodegenerative diseases have been investigated by solely focusing on the central nervous system. However, recent groundbreaking studies have indicated that microorganisms living in the gut could influence the development of brain disorders by changing metabolites and neurochemicals [1,2]. The chemicals produced by intestinal microorganisms can be transported across the intestinal epithelium [3]. The gut microbiome communicates with the brain via different pathways such as the neurological pathways [4,5], endocrine pathways [6–8], and immune pathways [9–12]. The vagus nerve is also a connection that transmits information about the inner organs from the gastrointestinal tract to the brain [13]. Under stress conditions, the hypothalamic-pituitaryadrenal (HPA) axis responds by inducing hormone secretion, such as cortisol, through the release of adrenocorticotropic hormone. Cortisol regulates immune signaling responses, which affect the intestinal barrier [14]. Short-chain fatty acids (SCFAs), one of the metabolites produced by bacteria, can be transported across the intestinal epithelium and reduce the gene expression related to tight junction formation in the brain microvascular endothelial cells, which makes the blood–brain barrier more permeable and could consequently regulate microglia homeostasis [12].

This interesting association between the gut microbiome and the brain has drawn researchers and encouraged them to unravel this association, particularly in terms of neurological diseases. Despite their limitations, most studies have relied on animal models to mimic the details of human diseases. Advanced analysis methods have been established to study the relationship between the gut and the brain and confirm the underlying biological mechanisms to develop treatments against brain disorders. In this review, the current state of the gut microbiome–brain research was discussed, and the evidence was summarized in favor of the involvement of the gut microbiota in neuropsychiatric and neurodegenerative disorders (Fig. 1).

## 2. Analysis methods and bioinformatics tools for analyzing the microbiome

Various techniques for analyzing the microbiome are available. Here we discuss the basic analysis methods, the primary tools available for each method (Table 1), and previous studies about the relationship between the gut microbiome and neurodegenerative or neuropsychiatric diseases.

### 2.1. Marker gene analysis

Marker gene analysis is the most popular bioanalytical method to survey microbial populations and phylogenies. The 16S ribosomal RNA (rRNA) is globally found in bacteria. It contains a highly conserved domain and a species-dependent hypervariable sequence region [28]. Owing to these qualities, 16S rRNA can be easily targeted for amplification using polymerase chain reaction (PCR) using primers that bind to the conserved domain. 18S rRNA or internal transcribed spacers are used in fungi and yeasts as marker genes [29]. The microbial phylogeny and relative abundance of certain microbes in samples are determined from the amplified marker gene proportion [30]. Preparing the sample for cloning, sequencing the marker gene, and analyzing the resulting amplicon are tasks that are relatively inexpensive and straightforward. Well-known bioinformatics tools, such as QIIME [31], and large-scale public datasets, such as the Ribosomal Database Project [32], can be used to enhance the analysis. However, the results are subjected to PCR-related biases [33]. Indeed, biases may occur during the amplification process because the primer binding affinity for the conserved regions is not equal for the 16S rRNA component from various species. Some PCR parameters, including variable region selection, amplicon size, and the number of PCR cycles, may be further sources of biases [34]. These biases can be decreased by

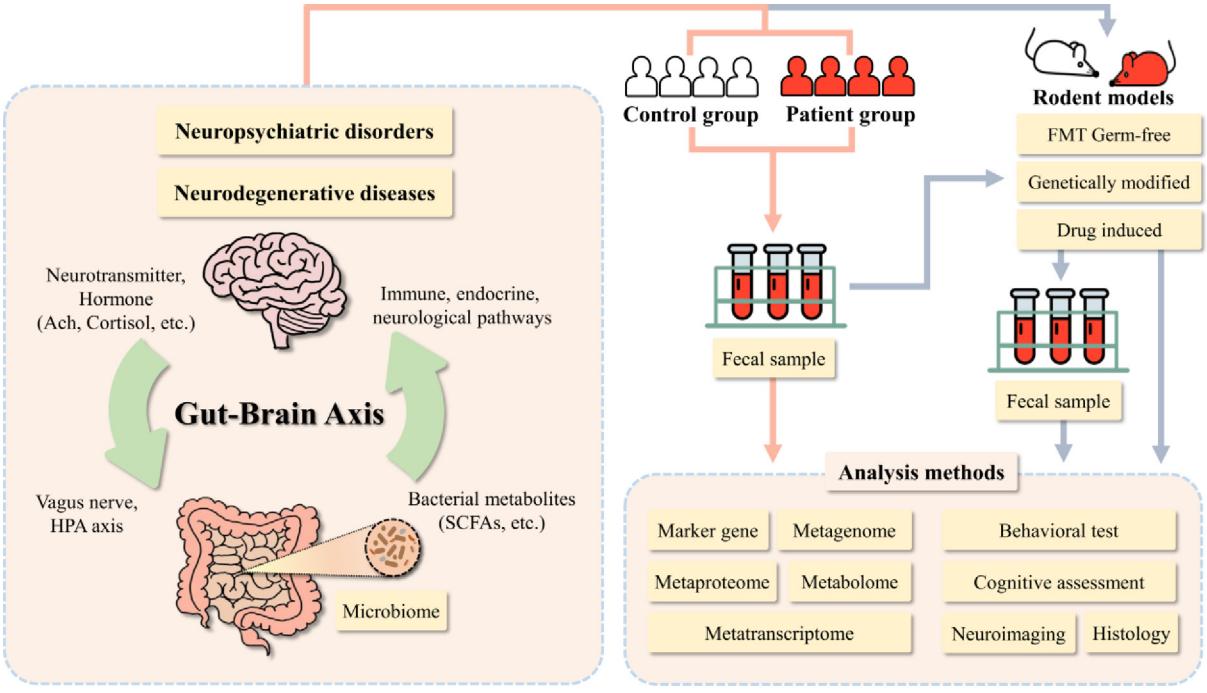


**Fig. 1.** Overall analysis process of the microbiome and neuronal diseases.

**Table 1**
Tools for microbiome analysis.

| Name | Description | Analysis | Link |
|---|---|---|---|
| Mothur [15] | Includes useful tools such as SONS, DOTOUR, TreeClimber, LIBSHUFF, ∫-LIBSHUFF, and UniFrac. Implemented over 25 calculators for quantifying parameters to estimate α and β diversity. | Marker gene analysis | https://mothur.org/ |
| QIIME 2 [16] | Plugin containing q2-cscs, q2-metabolomics, q2-shotgun, q2-metaphlan2, and q2-picrust methods. Provides various interactive visualization tools. | Marker gene analysis | http://qiime2.org/ |
| DADA 2 [17] | Performs the full amplicon process: filtering, dereplication, sample inference, chimera-filtered, and merging paired reads. | Marker gene analysis | https://benjjneb.github.io/dada2/index.html |
| Phyloseq [18] | Converts data output of OUT clustering pipelines into a suitable form amenable to modern analysis methods such as discriminant analysis, and canonical correspondence analysis. | Marker gene analysis | http://www.bioconductor.org/packages/release/bioc/html/phyloseq.html |
| VEGAN [19] | Provides basic functions of diversity analysis and multivariate analysis. | Marker gene analysis | https://github.com/vegandevs/vegan |
| DESeq 2 [20] | Focused on quantitative analysis rather than on differential expression. Uses the negative binomial distribution, the Wald, and the Likelihood Ratio Tests. | Marker gene analysis | https://bioconductor.org/packages/release/bioc/html/DESeq2.html |
| IDBA-UD [21] | Based on the iterative de Bruijn graph assembler for standard metagenomics and single-cell analysis. | Shotgun Metagenomics | http://www.cs.hku.hk/~alse/idba_ud |
| SPAdes [22] | Uses k-mers to construct the de Bruijn graph for mate-pair, pair-end reads, and unpaired reads. SPAdes pipeline with two separate modules: 1) BayesHammer and 2) SPAdes. | Shotgun Metagenomics | http://cab.spbu.ru/software/spades/ |
| MEGAHIT [23] | Based on the construction of succinct de Bruijn graphs with CPU-favored graph module. | Shotgun Metagenomics | https://www.metagenomics.wiki/tools/assembly/megahit |
| MetaPhlAn 3 [24] | Performs unambiguous taxonomic assignment by MetaPhlAn markers of clade-specific. Possibility to get additional information on identifying metagenomics data using StrainPhlAn 3, PanPhlAn 3, PhyloPhlAn 3, and HUMAnM 3. | Shotgun Metagenomics | https://huttenhower.sph.harvard.edu/metaphlan/ |
| MG-RAST [25] | Web application server based on SEED framework for metagenomics data. Performs five pipelines: 1) data hygiene, 2) feature extraction, 3) feature annotation, 4) profile generation, 5) data loading. | Shotgun Metagenomics | https://www.mg-rast.org/ |
| PICRUSt 2.0 [26] | Enables output of MetaCyc predictions that will be linked with shotgun metagenomics results. | Shotgun Metagenomics | https://github.com/picrust/picrust2/wiki |
| SOAP denovo 2 [27] | Consists of six modules: 1) handle read error correction, 2) de Bruijn graph construction, 3) contig assembly, 4) paired-end reads mapping, 5) scaffold construction, and 6) gap closure. | Metatranscriptomics | https://sourceforge.net/projects/soapdenovo2/ |

optimizing primers and PCR parameters [35]. After proper optimization, marker gene analysis can be applied to different research pipelines and sample types. However, the methodology only offers limited and low-resolution information about the microbial community, without microbial genome expression.

### 2.2. Whole genome analysis

Whole genome analysis is a sequencing method that detects whole genomic contents in a sample and detects bacteria, fungi, yeasts, and viral DNAs [36]. With dramatic advances in sequencing technologies, parallel and rapid total DNA sequencing can be performed with next-generation sequencing (NGS), also known as high-throughput sequencing (HTS). Various commercial NGS platforms based on different sequencing technologies are available [37]. Additionally, shotgun sequencing allows profiling functional representations or metabolic pathways in the microbiota community [38]. *In silico* alignment tools have been introduced to analyze sequencing data, which consist of billions of short sequence fragments called reads [39]. Read profiling techniques provide taxonomy information and annotate genes from the sample by comparing unassembled DNA reads to reference data. Many read-based profiling tools extract all short sequences with length k from the sequencing result (k-mer) and build a de Bruijn graph to reconstruct long DNA sequences [40]. Burrows–Wheeler transform algorithm can also be used in read-based profiling [41]. Rapid and accurate mapping for entire reads are achieved by compressing large-scale genomic sequence databases with the algorithm. Another method for reading alignment consist in assembling reads into longer sequences also called contigs with the overlap-layout-consensus (ORC) algorithm. The algorithm function in three steps: 1) search overlapping sequences between the read sequences, 2) layout and assemble the overlapping reads, and 3) search for the most reliable sequences from the assembled contigs [42]. Although processes including sample preparation, whole-genome sequencing, and analysis are relatively complex and expensive, whole metagenome analysis offers detailed information about the microbial community [43].

### 2.3. Meta-transcriptome analysis

Metagenomic analysis provides information about the microbial populations or the microbial genomic contents; however, it cannot identify transcriptionally active microbial genes or functions. In metatranscriptome analysis, a whole-genome sequencing technique is applied to conduct RNA sequencing. Microbial transcription, gene expression, and functional outputs are profiled from the sequencing data [44]. Metatranscriptome analysis allows to estimate the transcription activity and identify active metabolic pathways of species from the microbiome community [45]. However, because of the instability of RNA molecules, sample preparation, amplification, and analysis are the most expensive and complex among the microbial analyse techniques. Despite the cost, metatranscriptome analysis offers unique information to the microbiome research community.

### 2.4. Metabolome and meta-proteome analyses

Metabolome analysis is an emerging tool that can be used to investigate the status of the microbial community by focusing on functional dysregulation of the gut microbiome because small metabolites are the actual players of the gut–brain communication [46]. The extraction and quantification of metabolites enables the

study of active metabolic pathways expressed by the gut microbiome and investigation of the interactions of these metabolites with host metabolism. Of the metabolites produced by gut microorganisms, SCFAs are the main bacterial metabolites associated with gut physiology. Of SCFAs, acetate (C1), propionate (C2), and butyrate (C3) are the most abundant ones [47]. Acetate is generally produced from acetyl-CoA, a metabolite produced *via* glycolysis. Propionate and butyrate are the products of metabolites produced via glycolysis or amino acid metabolism [47].

SCFAs can be separated and quantified using different methods such as nuclear magnetic resonance, mass spectroscopy (MS), liquid chromatography (LC), and gas chromatography (GC) [48]. It is difficult to identify the source of the detected metabolites or to differentiate the microbial metabolites from host-derived products based solely on metabolome analysis. However, the gut microbial phylogeny or functional alterations can be established by measuring relative amounts of metabolites in the samples and comparing this profile to reference libraries [49].

As in metabolome analysis, functional changes in the microbiome can be characterized by focusing on proteins as markers. The metaproteomic analysis pipeline consists of extracting proteins from microbiome samples, digesting the proteins into peptides, separating these peptides using LC-MS, and identifying and quantifying proteins by searching and comparing data to metagenome databases [50]. After annotating proteins from the samples using various protein databases, such as UniProt [51] or BRENDA [52], functional analysis or taxonomic assignment can be conducted. Moreover, phylogenetic or metagenomic information about the microbial community in the samples can be linked to the proteomic data for further analysis. Proteomic analysis provides information about real activities of the microbiome or allows to identify potential drug targets for certain pathways or diseases [53]. However, the sample preparation is very complex and the computational analysis is also difficult due to the large and complex proteomics datasets [54].

## 3. Gut-brain axis (GBA)

The gut-brain axis (GBA) consists of the biochemical signaling between the gut microbiome and the brain resulting in changes in the central nervous system state [55]. In recent years, the research focus was on the gut microbiome functions influencing the GBA bidirectional communication [6,56–58], which is important to maintain homeostasis of the gastrointestinal and central nervous systems of animals [59–61]. It is likely that the GBA mediates various aspects of the pathogenesis of brain diseases, and intensive research is necessary to clarify the underlying mechanisms. Various studies have shown the potential relationships between the gut microbiome and neurodegenerative or neuropsychiatric diseases (Table 2).

### 3.1. Gut microbiome and neuropsychiatric disorders

Many studies in animal models and human patients have shown an association between the gut microbiome and the development of various neuropsychiatric disorders. Most studies reported changes in the bacterial community but lacked explaining how these changes affected brain physiology. Although many mediators, such as SCFAs, have been identified recently, the bacterial species that produce such mediators and how they influence brain functions needs further clarification.

#### 3.1.1. Schizophrenia (SCZ)

SCZ is a psychiatric disorder characterized by positive symptoms such as delusions, hallucinations, and thought abnormalities. Patients with SCZ also exhibit negative symptoms such as anhedonia, abolition, social withdrawal, and poverty of thought [103]. SCZ is associated with dysregulation of the dopaminergic, glutamatergic, γ-aminobutyric acid (GABA)ergic, and cholinergic neurotransmitters [104].

The comparison of differences in fecal microbiota between SCZ patients and healthy controls was performed using 16S rRNA sequencing and diversity analyses [83]. In the study, a lower microbiome diversity in patients with SCZ patients. At the phylum level, *Proteobacteria* were considerably enriched in patients with SCZ. At the genus level, *Blautia*, *Coprococcus,* and *Roseburia* were found less often in patients with SCZ, whereas *Succinivibrio*, *Megasphaera*, *Collinsella*, *Clostridium*, *Klebsiella,* and *Methanobrevibacter* were present to a larger extent. In another study, 16S rRNA gene sequence analysis and diversity analysis were performed [84], and a decreased microbiome alpha-diversity, that was assessed using the species richness indices and species diversity indices, and disturbance of gut microbial composition in SCZ patients were observed. Specifically, the abundance of *Lachnospiraceae* and *Ruminococcaceae,* which are the most abundant in the order *Clostridiales*, was reduced in the gut microbiome of SCZ patients. In addition, significant deviations in *Aerococcaceae*, *Bifidobacteriaceae*, *Brucellaceae*, *Pasteurellaceae*, and *Rikenellaceae* between SCZ patients and healthy controls were observed. Based on the deviations, a stepwise regression analysis was conducted and showed the potential of SCZ diagnosis by gut microbiome analysis. This finding suggests that patients with SCZ have abnormal microbial status. Furthermore, the changes in the gut microbiota, resulting from the transfer of human SCZ fecal microbiome to mice, led to SCZ-relevant hyperactive behavior in mice who received SCZ microbiota, relative to healthy control microbiota mice [84,105]. The study indicates that glycerophospholipids, which are key regulators of synaptic function are reduced in the hippocampus and SCZ mice serum, but increased in fecal samples of SCZ mice. This suggests that lower levels of glycerophospholipids are consistent with symptoms of patients with SCZ having synaptic deficits and disconnectivity.

There have been attempts to identify specific bacterial metabolites that are associated with SCZ symptoms [106]. SCFA-producing bacteria, such as *Blautia*, *Coprococcus*, and *Roseburia*, were reduced in patients with SCZ [83]. Butyrate, an SCFA, can cross the BBB and inhibit histone deacetylase 1 (HDAC1) [107,108]. Because HDAC1 level is elevated in the PFC and hippocampus of patients with SCZ, the elevation might be due to the reduced butyrate level produced by the bacterial species [109,110]. A study hypothesized that the increased populations of *Clostridiales*, *Prevotella*, and *Lactobacillus ruminis* increased SCFA levels that stimulated TNF production, and the increased SCFA activated microglia and disrupted membrane metabolism in patients with SCZ [111]. However, specific SCFAs were not measured in the study.

Germ-free (GF) mice transplanted with the microbiome of SCZ patients have low glutamate and high GABA levels in the hippocampus and display SCZ-relevant behaviors [84]. Dickerson *et al*. showed that SCZ subjects treated with probiotics containing *L. rhamnosus* strain GG and *Bifidobacterium lactis* strain Bb12 had less severe bowel difficulties, although there was no difference in the psychiatric symptoms compared with those of the placebo group [112]. Using the same strain as Dickerson *et al*., Tomasik *et al*. showed a decrease in the von Willebrand factor (vWF) amounts and an increase in monocyte chemotactic protein-1 (MCP-1) and brain-derived neurotrophic factor (BDNF) levels in probiotic-treated SCZ patients. It also modulated immune and intestinal epithelial cells through the IL-17 cytokine family. They hypothesized that this controlled gastrointestinal leakage [113].

Severance *et al*. conducted a longitudinal, double-blind, and placebo-controlled pilot study on 56 schizophrenia subjects to

**Table 2**
Studies showing a potential link between gut microbiome and neurodegenerative or neuropsychiatric diseases.

| Disease | Number of subjects | Sample | Analysis method | Alterations of microbiota |
|---|---|---|---|---|
| AD [62] | AD patients = 61, healthy controls = 30 | Human | Gastrointestinal endoscopy | The elimination of pathogenic bacteria such as *Helicobacter pylori* by triple eradication antibiotic regimen (omeprazole,clarithromycin, and amoxicillin) led to improved cognitive and functional status parameters in AD patients. The eradication of *Helicobacter pylori* influenced AD manifestations positively. |
| AD [63] | AD patients = 60 | Human blood | ELISA, cognitive test | Probiotic consumption (*Lactobacillusacidophilus, Lactobacilluscasei, Bifidobacteriumbifidum,* and *Lactobacillusfermentum*) induced significant cognitive improvements and affected metabolic pathways in AD patients. |
| AD [64] | AD patients = 25, non-AD = 94 | Human fecal samples | 16S rRNA gene sequencing | Differences in bacterial abundance, including decreased *Firmicutes and Bifidobacterium levels* as well as increased *Bacteroidetes* amounts, in the microbiome of AD participants. |
| AD [65] | AD patients = 43, healthy controls = 43 | Human fecal samples | 16S rRNA gene sequencing | Several bacteria taxa were different in AD patients than those in controls, such as *Bacteroides, Actinobacteria, Ruminococcus, Lachnospiraceae*, and *Selenomonadales*. |
| AD [66] | n = 32 | Mice fecal samples | 16S rRNA gene sequencing | The composition and diversity of gut microbiota changed with aging in a tauopathy mice model. In detail, at the phylum level, the relative abundance of *Bacteroidetes* was increased, whereas that of *Firmicutes* was decreased in 3-month-old P301L mice compared with that in aged-matched wild-type mice. *Actinobacteria* levels were decreased in 3 to 6-month-old P301L mice. Less *Tenericutes* was found in 10-month-old P301L mice. Several specific macrobiota were highly associated with the levels of AT8-tau or pT231-tau protein in the brain. |
| AD [67] | n = 24 | Mice fecal samples | 16S rRNA gene sequencing | At the phylum level, *Proteobacteria* and *Verrucomicrobia* levels were increased in AD mice. At the genus level, *Ruminococcus* and *Butricicocus* were less abundant in AD mice. |
| AD [68] | n = 18 | Mice fecal samples | 16S rRNA gene sequencing (qPCR) | At the phylum level, *Firmicutes* levels were increased in AD mice. *Bacteriodetes* were less abundant in AD mice. |
| PD [65] | PD patients = 75, healthy controls = 45 | Human fecal samples | 16S rRNA gene sequencing | Increase in the abundance of four bacterial families (*Eubacteriaceae, Bifidobacteriaceae, Aerococcaceae, and Desulfovibrionaceae*) and significant decrease in the abundance of 17 bacterial families (*Lachnospiraceae*, etc.) in PD patients compared to the levels found in controls. |
| PD [69] | PD patients = 76, healthy controls = 78 | Human fecal samples | 16S/18S rRNA gene sequencing | Increased *Akkermansia* levels in PD patients. |
| PD [70] | PD patients = 64, healthy controls = 64 | Human fecal samples | 16S rRNA gene sequencing | Increased *Bifidobacterium* levels and decreased amounts of *Prevotellaceae* and *Roseburia* in PD patients. |
| PD [71] | PD patients = 52, healthy controls = 36 | Human fecal samples | 16S rRNA gene sequencing (with qRT-PCR) | The amounts of *Lactobacillus* were increased, whereas the *Clostridium coccoides* and *Bacteroides fragilis* groups were less abundant in PD patients. |
| PD [72] | PD patients = 31, healthy controls = 28 | Human fecal samples | Shotgun metagenomic sequencing | Increased amounts of *Verrucomicrobiaceae* (*Akkermansia muciniphila*) and unclassified *Firmicutes* in PD patients. *Prevotellaceae* (*Prevotella copri*) and *Erysipelotrichaceae* (*Eubacterium biforme*) were markedly less abundant in PD patients than they were in controls. |
| PD [73] | PD patients = 197, healthy controls = 130 | Human fecal samples | 16S rRNA gene sequencing | Increased *Bifidobacteriaceae, Lactobacillaceae, Tissierellaceae, Christensenellaceae, and Verrucomicrobiaceae* levels and decreased amounts of *Lachnospiraceae, Pasteurellaceae* in PD patients. |
| PD [74] | PD patients = 89, healthy controls = 66 | Human fecal samples | 16S rRNA gene sequencing | Increased abundance of *Christensenella, Catabacter, Lactobacillus, Oscillospira, Bifidobacterium, Christensenella minuta, Catabacter hongkongensis, Lactobacillus mucosae, Ruminococcus bromii,* and *Papillibacter cinnamivorans*, and decreased levels of *Dorea, Bacteroides, Prevotella, Faecalibacterium, Bacteroides massiliensis, Stoquefichus massiliensis, Bacteroides coprocola, Blautia glucerasea, Dorea longicatena, Bacteroides dorei, Bacteroides plebeus, Prevotella copri, Coprococcus eutactus,* and *Ruminococcus callidus* in PD patients. |

(*continued on next page*)

**Table 2** (*continued*)

| Disease | Number of subjects | Sample | Analysis method | Alterations of microbiota |
|---|---|---|---|---|
| PD [75] | PD patients = 9, healthy controls = 13 | Human fecal samples | 16S rRNA gene sequencing | Increased *Akkermansia* and *Bifidobacterium levels* and decreased *Prevotella* amounts in PD patients. |
| PD [76] | PD patients = 10, healthy controls = 10 | Human fecal samples | 16S rRNA gene sequencing | Increased abundance of *Ruminococcaceae, Verrucomicrobiaceae, Porphyromondaceae, Hydrogenoanaerobacterium*, and *Lachnospiraceae* and decreased amounts of *Bacteroides* and *Prevotellaceae* in PD patients. |
| PD [77] | PD-MCI (mild cognitive impairment) = 13, PD-NC (normal cognition) = 14, healthy controls = 13 | Human fecal samples | 16 s rRNA gene sequencing, gas chromatography-mass spectrometry | The fecal microbial diversities of PD-MCI and PD-NC were higher than that of healthy controls. In PD-MCI, *Alistipes, Barnesiella, Butyricimonas*, and *Odoribacter* levels were higher than those in the other two groups. Compared with the PD-NC group, the genus *Blautia* and *Ruminococcus* were less abundant in the PD-MCI group. |
| PD [78] | PD patients = 38, healthy controls = 34 | Human mucosal and fecal samples | 16S rRNA gene sequencing | Increased abundance of *Faecalibacterium* and *Ralstonia* in mucosal samples. Increased levels of *Blautia, Coprococcus,* and *Roseburia* in fecal samples. |
| PD [79] | PD patients = 29, healthy controls = 29 | Human fecal samples | 16S rRNA gene sequencing (next-generation-sequencing) | Increased abundance of *Lactobacillaceae, Barnesiellaceae*, and *Enterococcaceae* in PD patients. |
| PD [80] | PD patients = 24, healthy controls = 14 | Human fecal samples | 16S rRNA gene sequencing | More *Escherichia-Shigella, Streptococcus, Proteus*, and *Enterococcus* were found in PD patients than in healthy controls. *Blautia, Faecalibacterium*, and *Ruminococcus* levels were decreased in PD patients. |
| PD [81] | PD patients = 45, healthy controls = 45 | Human fecal samples | 16S rRNA gene sequencing | *Clostridium IV, Aquabacterium, Holdemania, Sphingomonas, Clostridium XVIII, Butyricicoccus*, and *Anaerotruncus* levels were increased in PD patients. |
| PD [82] | PD patients = 34, healthy controls = 34 | Human fecal samples | 16 s rRNA gene sequencing (RT-qPCR) | *Enterobacteriaceae Phylum* was more abundant in PD patients, whereas the levels of *Bacteroidetes* and *Prevotellaceae* were decreased. |
| Schizophrenia [83] | SCZ patients = 64, healthy controls = 53 | Human fecal samples | 16S rRNA gene sequencing (metagenomes, PICRUSt analysis) | *Proteobacteria* levels were significantly increased in SCZ patients. At the genus level, *Succinivibrio, Megasphaera, Collinsella, Clostridium, Klebsiella,* and *Methanobrevibacter* were more abundant in SCZ patients than they were in healthy controls, whereas *Blautia, Coprococcus*, and *Roseburia* levels were decreased in SCZ patients. |
| Schizophrenia [84] | SCZ patients = 63, healthy controls = 69 | Human fecal samples | 16S rRNA gene sequence analysis | Unmedicated and medicated SCZ patients had a decreased microbiome alpha-diversity index and marked disturbances of gut microbial composition compared to those of healthy controls. Several unique bacterial taxa like *Veillonellaceae* and *Lachnospiraceae* were linked to SCZ severity. *Aerococcaceae, Bifidobacteriaceae, Brucellaceae, Pasteurellaceae*, and *Rikenellaceae* were different in SCZ patients than those found in healthy controls. |
| ALS [85] | ALS patients = 50, healthy controls = 50 | Human fecal samples | 16S rRNA gene sequencing with qPCR | *Escherichia coli* and *Enterobacteriaceae* levels were higher in ALS patients than those of healthy controls, whereas the amounts of *Clostridium* were lower in ALS patients. |
| ALS [86] | ALS patients = 6, healthy controls = 5 | Mice fecal samples | 16S rRNA gene sequencing, Shotgun metagenomic sequencing | Decreased levels of *Akkermansia muciniphila, Parabaceroides distasonis, Rikenellaceae, Prevotella, Lactobacillus murinus, Alistipes* unclassified, and *Eggertella* unclassified as well as increased *Sutterella, Allobaculum, Desulfovibrionaceae, Coprococcus, Oscillospira, Bifidobacterium, Helicobacter hepaticus, Lactobacillus johnsonii,* and *Lactobacillus reuteri* in ALS mice. |
| | ALS patients = 37, healthy controls = 29 | Human fecal samples | Shotgun metagenomic sequencing | The amounts of *Anaerostipes hadrus* and *Bacteroidales bacterium* were increased marginally in ALS patients and *Bifidobacterium pseudocatenulatum, Clostridium leptum*, and *Escherichia coli* levels were decreased. |
| ALS [87] | ALS patients = 6, healthy controls = 5 | Human fecal samples | 16S rRNA gene sequencing | *Dorea* levels were increased and the amounts of *Oscillibacter, Anaerostipes, and Lachnospiraceae* were decreased in ALS patients compared with those of healthy controls. |
| ALS [88] | not mentioned (454 16rRNA sequencing data) | Mice fecal samples | 16S rDNA qRT-PCR with pyrosequencing | A gut dysbiosis was evidenced in ALS mice, particularly in terms of reduced levels of butyrate-producing bacteria, including *Butyrivibriofibrisolvens* and *E. coli.* |
| ALS [89] | ALS patients = 8, healthy controls = 8 | Human fecal samples | 16S rRNA gene sequencing | Increased amounts of *Methanobrevibacter* and decreased levels of *Faecalibacterium* and *Bacteroides* in ALS patients. |

**Table 2** (*continued*)

| Disease | Number of subjects | Sample | Analysis method | Alterations of microbiota |
|---|---|---|---|---|
| Depression [90] | Depressed patients = 5, healthy controls = 5 | Mice fecal samples | 16S rRNA genes pyrosequencing | Stressed and depressed mice showed changes in microbial diversity with more *Desulfovibrionaceae, Rikenellaceae*, and *Lachnospiraceae* families and less *Allobaculum* and *Mucispirillum.* |
| Depression [91] | Depressed patients = 13, healthy controls = 15 | Rats fecal samples | 16 s rRNA sequencing | At the phylum level, the relative abundances of *Actinobacteria* and Candidate Division TM7 were decreased in rats that received the depression fecal microbiota transplantation (FMT rats).<br>At the family level, the relative proportions of *Bifidobacteriaceae*, *Coriobacteriaceae*, *Porphyromonadaceae*, Candidate Division TM7 uncultured bacterium, *Caldicoprobacteraceae*, *Alcaligenaceae* were decreased in FMT rats. *Propionibacteriaceae* levels were increased in FMT rats.<br>At the genus level, the relative abundances of *Bifidobacterium* uncultured, *Coriobacteriaceae* uncultured, *Caldicoprobacter*, *Roseburia, Allobaculum, Burkholderiales* were decreased and *Freudenreichii, Staphyloccus, Peptococcus* levels were increased in FMT rats. |
| | depressed patients = 34, healthy controls = 33 | Human fecal samples | 16 s rRNA sequencing | At the family level, the relative proportions of *Prevotellaceae* were decreased, whereas those of *Thermoanaerobacteriaceae* were increased in depressed patients. At the genus level, there was an increase of the relative proportions of *Eggerthella, Holdemania, Gelria, Turicibacter, Paraprevotella* and *Anaerofilum*, and decreased amounts of *Prevotella* and *Dialister* in depressed patients. |
| Depression [92] | depressed patients = 58, healthy controls = 63 | Human fecal samples | 16S rRNA gene sequencing | *Actinobacteria* levels were increased, whereas *Bacteroidetes* amounts were decreased in depressed patients compared with the levels in healthy controls. |
| Depression [93] | Active-Major Depressive Disorder (A-MDD) patients = 29, Responded-Major Depressive Disorder (R-MDD) patients = 17, healthy controls = 30 | Human fecal samples | 16S rRNA gene pyrosequencing | The fecal bacterial α-diversity was increased in A-MDD patients compared with that in healthy controls, but there was no difference between R-MDD patients and the HC group.<br>The levels of *Bacteroidetes, Proteobacteria,* and *Actinobacteria* were strongly increased, whereas those of *Firmicutes* were significantly reduced in the A-MDD and R-MDD patients compared with the levels in healthy controls.<br>Depressed patients had increased *Enterobacteriaceae* and *Alistipes* levels and decreased amounts of *Faecalibacterium.* |
| Autism/ASD [94] | Autism patients = 30, healthy controls = 24 | Human fecal samples | 16S rRNA gene amplicon in NGS | ASD patients have a higher relative abundance of the families *Lactobacillaceae, Bifidobacteraceae,* and *Veillonellaceae,* whereas healthy controls have higher levels of the *Prevotellaceae* family. |
| Autism/ASD [95] | Autism patients = 20, healthy controls = 10 | Human fecal samples | 16S rDNA and 16S rRNA sequencing (by using Bacterial tag-encoded FLX-titanium amplicon pyrosequencing [bTEFAP]) | Higher microbial diversity in autism patients. Autism patients have higher levels of the genera *Caloramator, Sarcina* and *Clostridium*, and higher amounts of the species of *Alistipes* and *Akkermansia.* |
| Autism/ASD [96] | Autism patients = 13, healthy controls = 8 | Human fecal samples | 16S rRNA gene sequencing | Autism patients have higher amounts of *clostridial* species. The abundance and type of *Clostridium* and *Ruminococcus* species in autism patients are differ from those of healthy controls. Nine species of *Clostridium* were found in autism patients but not in controls. In controls, there were three species not found in children with autism. |
| Autism/ASD [97] | Autism patients = 21, healthy controls = 19 | Human fecal samples | 16S rRNA gene pyrosequencing | The abundance of the genus *Burkholderia* was higher and that of the genus *Neisseria* was lower in autism patients. At the species level, the amounts of two *Bacteroides* species and *Escherichia coli* were decreased in autism patients. |
| Autism/ASD [98] | Autism patients = 40, healthy controls = 40 | Human fecal samples | 16S rRNA gene pyrosequencing | Significant increase of the *Firmicutes*/*Bacteroidetes* ratio in autistic patients due to a reduction of the *Bacteroidetes* relative abundance. At the genus level, decreased levels of *Alistipes, Bilophila, Dialister, Parabacteroides*, and *Veillonella*, and increased amounts of *Collinsella, Corynebacterium, Dorea, and Lactobacillus* in autistic patients. |
| Autism/ASD [99] | Autism patients = 58, healthy controls = 39 | Human fecal samples | 16S rDNA sequencing | Much lower levels of *Bifidobacterium*, slightly lower levels of *Enterococcus*, and the much higher levels of *Lactobacillus* in autism patients.<br>Autism patients were likely to have *Bacillus* spp. and less likely to have *Klebsiella oxytoca.* |

(*continued on next page*)

**Table 2** (*continued*)

| Disease | Number of subjects | Sample | Analysis method | Alterations of microbiota |
|---|---|---|---|---|
| Autism/ASD [100] | Autism patients = 33, healthy controls = 7 | Human fecal samples | DNA pyrosequencing | At the phylum level, autistic patients have higher *Bacteroidetes* and lower *Firmicutes* levels compared with those of healthy controls. Amounts of *Desulfovibrio* species and *Bacteroides vulgatus* were increased in autistic patients compared with those of healthy controls. |
| Autism/ASD [101] | Autism patients = 58, healthy controls = 22 | Human fecal samples | FISH analysis using a collection of 59 Cy3-labeled 16S rRNA oligonucleotide probes | ASD patients have the higher amounts of the *Clostridium histolyticum* group (*Clostridium clusters I and II*) compared with the levels in healthy controls. |
| Huntington's disease (HD) [102] | HD patients = 18, healthy controls = 17 | Mice fecal samples | 16S rRNA gene sequencing | Sex differences: in males, *Bacteroidales* levels were increased and *Clostridiales* amounts were decreased. In females, *Coriobacteriales*, *Erysipelotrichales*, *Bacteroidales*, and *Burkholderiale* levels were increased and *Clostridiales* amounts were decreased. |

explore the effects of probiotic treatment on antibodies against *Candida albicans*. Probiotic administration significantly reduced *Candida albicans* antibody levels, *Candida albicans*-associated gut discomfort, and positive psychiatric symptoms in male individuals [114]. Okubo *et al*. investigated the effects of *Bifidobacterium breve* A-1 administration in 29 SCZ patients with anxiety and depression symptoms. Their HADS total score and PANSS anxiety/depression score were improved and their interleukin 22 (IL-22) and tumor necrosis factor related activation-induced cytokine (TRANCE) levels were increased after treatment with the probiotics [115].

### 3.1.2. Autism spectrum disorder (ASD)

ASD is a neurodevelopmental disorder characterized by repetitive behaviors and impairments in social communication and cognitive functions [116,117]. A combination of genetic and nongenetic factors contributes to ASD. Candidate genes involved in ASD etiology include genes coding for postsynaptic scaffolding proteins, contactins, neurexin family, and chromatin remodeling proteins [118]. Nongenetic factors include parental age, preterm birth, and other environmental factors [118]. Gastrointestinal symptoms and high levels of intestinal immune inflammation linked with gut dysbiosis are common in children with ASD [114,119]. The gut microbiome of ASD patients differs from that of healthy individuals. The increased levels of *Lactobacillus* and *Clostridium* have been consistently observed in ASD patients in a few reports [95,96,98–101]. However, controversial results were also observed for *Bifidobacterium*, *Alistipes*, *Bacteroidetes*, and *Bacteroidetes* in ASD patients [98–100].

NGS of 16S rRNA gene amplicons was used to compare fecal samples from autism patients and healthy controls. The relative abundance of the *Lactobacillaceae*, *Bifidobacteraceae*, and *Veillonellaceae* families was higher in ASD patients than that in healthy controls [94]. For comparison, fecal samples from two groups (Indian and American patients with ASD) having different diets were also analyzed. Despite differences in the diets of Indian and American patients with ASD, *Lactobacillus* species were the most dominant in both groups. *Lactobacillus* is one of the common lactic acid producers present predominantly in the gut of infants and its level reduces with age because of different diets. However, the association between the increased populations of *Lactobacillus* with autism pathology is unclear.

Bacterial tag-encoded FLX-titanium amplicon pyrosequencing (bTEFAP) of the 16S rDNA and 16S rRNA analyses of fecal samples from autism patients and healthy controls were conducted [95]. The microbial alpha-diversity was higher in autism patients. Additionally, autism patients displayed higher levels of the genera *Caloramator*, *Sarcina,* and *Clostridium* and of the *Alistipes* and *Akkermansia* species.

The microbiota composition of fecal samples from autism patients and healthy controls was also analyzed by 16S rRNA gene sequencing [97]. There was no difference in the microbiome diversity, namely species richness and evenness, between autism patients and healthy controls. However, a higher abundance of the genus *Burkholderia* and lower amounts of the genus *Neisseria* were found in autism patients. Moreover, two *Bacteroides* species and *Escherichia coli* were decreased in autism patients.

Compared with control mice, GF mice transplanted with ASD donor feces had increased repetitive behavior, decreased sociability, and decreased mobility [120]. The offspring of GF mice implanted with an ASD fecal sample also displayed ASD-like behaviors. Mice lacking ephrin type-B receptor 6 (EphB6) exhibited ASD-like behaviors as well as microbial dysbiosis and their fecal microbiota contained less *Deferribacteres* [121]. Specific pathogen-free (SPF) C57BL/6J mice transplanted by the fecal microbiota from EphB6-defective mice displayed ASD-like behaviors measure with the open field test, elevated plus maze test, and social behavior tests. On the other hand, ASD-like behaviors were improved in EphB6-defective mice transplanted with fecal microbiota from wild-type mice.

It has been reported that the administration of bacterial metabolites, such as taurine and 5-aminovaleric acid, into an autism mouse model, enhanced ASD-like behavior [120]. Arentsen *et al*. investigated the effect of bacterial peptidoglycan cognitive protein (PGN) in brain development [122]. PGN2-deficient mice showed alteration in the autism risk gene c-Met and social behavior change. Lipopolysaccharides produced by Gram-negative bacteria and lipoproteins and peptidoglycan produced by Gram-positive bacteria are recognized by Toll-like receptors and then stimulate the production of cytokines. Additionally, they are transported to the brain across the blood–brain barrier and are associated with the expression of the ASD risk gene c-Met.

### 3.1.3. Attention deficit hyperactivity disorder (ADHD)

ADHD is a neurodevelopmental disorder characterized by impulsivity, hyperactivity, and inattention. ADHD is usually present from birth and its onset is always before the age of seven. Risk factors of developing ADHD are genetic and environmental [123,124]. The causes of ADHD are unknown. However, the dopamine (DA) transporter gene (DAT1) and the dopamine D4 receptor gene were associated with ADHD [124]. ADHD patients showed a density of DA receptors lower than normal. Recently, the mutations A559V and R615C of DAT-1 were identified in ADHD patients, suggesting a hyperactive DA response [125].

Arts *et al*. examined the gut microbiome of adolescents and adults diagnosed with ADHD. They observed increased levels of bacteria belonging to the *Bifidobacterium* and *Eggerthella* genera in patients with ADHD [126]. They postulated that this increase was associated with enhanced dopamine precursor synthesis, which was associated with changes in reward expectation responses, a hallmark of ADHD [126]. In another study, *Faecalibac-*

*terium* was reduced in children with ADHD [127]. In patients with ADHD, the cytokine level is increased, and there is a positive correlation between the symptoms of ADHD and cytokines [128]. *Faecalibacterium* has an anti-inflammatory effect on the host [129,130], which suggests that a low level of *Faecalibacterium* increases inflammation [131].

GF mice transplanted with fecal microbiota of ADHD participants showed anxiety in the open field test and a decreased *Lachnoclostridium* content, which was also found in ADHD patients [132]. Pärtty *et al.* conducted a double-blind randomized placebo-controlled trial in which 75 infants received *Lactobacillus rhamnosus* GG (ATCC 53103) or placebo during the first 6 months of life. The diagnostic of ADHD or Asperger syndrome (AS) was posed when the children were 13 years old based on the ICD-10 diagnostic criteria. Interestingly, 17.1% of the children in the placebo group were diagnosed with ADHD or AS, whereas none of the children in the probiotics group were diagnosed [133]. Kumperscak *et al.* investigated the effects of supplementation with the probiotic strain *Lactobacillus rhamnosus* GG (ATCC53103). The scores obtained in the PedsQLTM Child-Self Report were significantly improved in children and adolescents with ADHD who received probiotics supplementation (n = 18) for 3 months compared with those of placebo controls (n = 14). In addition, the proinflammatory factors IL-12 p70, IL-10, and tumor necrosis factor-α (TNF-α) were significantly decreased in the probiotics group. The proinflammatory IL-6 was significantly reduced in both groups compared with the levels 3 months before. These controversial results were suggested to be the result of the small sample size and the short observation period [134].

### 3.1.4. Depression

Depression is a common psychiatric disorder characterized by depressed mood, loss of interest, and loss of pleasure for 2 weeks or longer. It also comes with symptoms such as sleep disorder, loss or gain of weight, and diminished ability to think or concentrate [135]. Depression was associated to abnormalities in neurotransmitters like a decrease in serotonin (5-HT) transporter binding and 5-HT1A receptor binding [136].

The association between depression and gut microbiota has been studied in depression patients. Obvious differences in gut microbiota composition between depressed patients and healthy controls were evidenced using 16S rRNA gene sequencing and shotgun metagenomic analysis. Briefly, the lower abundance of *Allobaculum* has been observed in patients with depression compared with healthy subjects [90,91]. However, a controversial result was also observed for *Bacteroidetes* and *Actinobacteria* [91–93].

Kelly *et al.* analyzed the composition of human fecal samples from depressed patients and healthy controls by using 16S rRNA sequencing [91]. In depressed patients, the relative proportion of *Prevotellaceae* was decreased, whereas that of *Thermoanaerobacteriaceae* was increased. At the genus level, the relative proportions of *Eggerthella, Holdemania, Gelria, Turicibacter, Paraprevotella,* and *Anaerofilum* were increased in the depressed patients, whereas those of *Prevotella* and *Dialister* were decreased. Moreover, microbiota-depleted rats transplanted with the fecal microbiota from depressed patients developed depression-related behaviors, which suggested that depression was related to the decreased gut microbiota richness and diversity.

An increased abundance of *Actinobacteria* and decreased *Bacteroidetes* amounts were observed in depressed patients compared with the levels in healthy controls [92]. The mice that received fecal samples from depressed patients displayed depression-like behaviors. These results suggest that dysbiosis of the gut microbiome is associated with the development of depression. Naseribafrouei *et al.* showed an association between depression and gut microbiota: specifically, *Oscillibacter* and *Alistipes* were abundant in depressed patients [40]. Jiang *et al.* found that *Enterobacteriaceae* and *Alistipes* levels were increased in patients with major depressive disorder (MDD), whereas *Faecalibacterium* was less abundant [93]. Moreover, the alpha-diversity was higher in the active-MDD patients. They suggested that *Alistipes* species was indole-positive and that it affects tryptophan availability, a precursor of serotonin closely related to depression [93,137,138]. In another study, the analysis of the fecal microbiota of patients with depression revealed the level of *Bifidobacterium* and *Lactobacillus* [139] to have decreased. Bravo *et al.* indicated that *L. rhamnosus* had a beneficial effect in treating depression and anxiety in stress-induced mice [140]. Long-term treatment with *L. rhamnosus* decreased corticosterone level and depressive-like behaviors in stress-induced mice. GABA is one of the inhibitory neurotransmitters of the CNS and is associated with the pathogenesis of anxiety and depression. The administration of *L. rhamnosus* decreased $GABA_{B1b}$ mRNA levels in the hippocampus, which was consistent with the antidepressant-like effect of $GABA_B$ receptor antagonists [140].

Akkasheh *et al.* conducted a randomized, double-blind, and placebo-controlled clinical trial that included 40 patients with a diagnosis of MDD based on the criteria from the diagnostic and statistical manual of mental disorders, fourth edition (DSM-IV). The patients were given probiotics (*Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium bifidum*) or placebo for 8 weeks. Probiotic supplements had beneficial effects on Beck Depression Inventory total scores, serum insulin levels, homeostasis model assessment of insulin resistance, and serum high sensitivity C-reactive protein (hs-CRP) concentrations [141]. Ghorbani *et al* also investigated the effects of symbiotic supplement on depression [142]. They provided synbiotic supplements to 40 patients (moderate depression and placebo-controlled) who met the DSM-V criteria for moderate depression. All patients received fluoxetine (20 mg/day) for 4 weeks. Either a synbiotic (*Lactobacillus casaei*, *Lactobacillus acidofilus*, *Lactobacillus bulgarigus*, *Lactobacillus rhamnosus*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Streptococus thermophilus,* fructooligosaccharide as prebiotic) or the placebo was added to the administration for 6 weeks. The synbiotic group obtained a significantly reduced HAM-D score compared with that of the placebo group [142].

However, another study showed no beneficial effect of probiotics supplement on depression. Romijn *et al.* followed seventy-nine participants who received probiotics (*Lactobacillus helveticus* and *Bifidobacterium longum*) or placebo for 8 weeks [143]. They found no significant changes in depression severity and inflammatory markers in probiotics-supplemented depression patients.

## 3.2. Gut microbiome and neurodegenerative diseases

Researchers have identified a role of the gut microbiome in the development of neurodegenerative diseases such as Alzheimer's, Parkinson's, and amyotrophic lateral sclerosis. The gut microbiome also alters vulnerability to and progress of neurodegenerative diseases. In this section, we discuss the involvement of the microbiome in the pathogenesis of the neurodegenerative diseases.

### 3.2.1. Alzheimer's disease (AD)

AD, one of the major neurodegenerative disorders, is characterized by amyloid plaques and tau tangles. Patients with AD usually exhibit memory impairments and, less frequently, non-amnestic cognitive impairments [144]. Genetic factors, such as rare mutations, are critical risk factors in the development of AD [145].

The gut microbiome of patients with AD differs from that of other individuals. In reports, the decreased abundance of *Bifidobacterium* has been observed in AD patients, and supplementing *Bifidobacterium* alleviated cognitive symptoms of AD patients

[63,64]. However, controversial results have been reported for the abundances of *Firmicutes* and *Bacteroidetes* in AD patients [64,66,68].

A study showed differences in bacterial abundance, including reduced *Firmicutes* and *Bifidobacterium* levels and increased abundance of *Bacteroidetes*, in fecal samples obtained from patients with AD using 16S rRNA gene sequencing [64]. Phylum *Bacteroidetes* includes different Gram-negative commensal bacteria in the gut such as *Bacteroides*. Gram-negative bacteria have lipopolysaccharide (LPS) that induces inflammation. It also revealed that patients with AD tended to have the lower microbial alpha-, beta-diversity compared with healthy controls. The intestinal absorption of significant LPS and amyloids from bacteria promotes the production of proinflammatory cytokines and is implicated in the pathogenesis of AD [146]. LPS and Gram-negative *E. coli* fragments were colocalized with an amyloid plaque in a postmortem brain tissue of patients with AD. The abundance of Gram-negative bacteria in the gut may increase LPS translocation, induce inflammation, and then aggravate pathology [64]. In another study, the analysis of fecal sample microbiota using 16S rRNA sequencing exhibited a decreased abundance of *Bacteroidaceae* and *Lachnospiraceae*. It increased the abundance of *Actinobacteria* and *Ruminococcaceae* in patients with AD compared with controls [65]. Although bacterial metabolites may be associated with AD, additional in-depth microbiome studies are required because the results of microbiome analysis are often controversial [64,65].

The fecal microbiomes and fecal SCFA composition of healthy and AD mice were also compared. The microbiota composition and diversity were perturbed and the levels of SCFAs were decreased in AD mice. At the phylum level, *Proteobacteria* and *Verrucomicrobia* levels were increased in AD mice as revealed by 16S rRNA gene sequencing of fecal samples [67]. At the genus level, *Ruminococcus* and *Butricicocus* were less abundant in AD mice. The findings showed that AD might exacerbate cognitive deficits through the reduction of SCFAs by altering the gut microbiota.

Mice transplanted with feces from AD patients had lower levels of nervous system-related metabolites such as GABA, taurine, and valine compared with those in mice transplanted with healthy human feces. They also displayed significant cognitive impairment [147]. Chen *et al.* demonstrated that mice with AD-linked 5 mutations (5xFAD), a rodent model for AD, experienced gut dysbiosis in an age-dependent manner, and that antibiotic treatment improved cognitive performances and reduced amyloidogenic processes [148]. Moreover, probiotic R13 treatment reduced amyloid aggregates in the gut of 5xFAD mice and boosted the beneficial effects of bacterium *L. salivarius*.

### 3.2.2. Parkinson's disease (PD)

PD is a neurodegenerative disorder characterized by movement dysfunctions. It also has a non-motor dimension that involves cognitive impairments and depression [149]. Misfolding and aggregation of α-synuclein, mitochondrial dysfunction, malfunctioning protein clearance systems, including the ubiquitin–proteasome and autophagy-lysosome systems, and neuroinflammation, play vital roles in the progression and onset of PD [100]. The dark pigmented areas in the pars compacta of substantia nigra and locus coeruleus are lost in the brains of patients with PD due to the death of dopaminergic neuromelanin-containing neurons and noradrenergic neurons [100].

Several studies have analyzed the link between gut microbiota and PD from fecal samples of PD patients. In a few reports, the increased abundance of *Bifidobacteriaceae*, *Lactobacillaceae* and *Verrucomicrobiaceae* families, and the decreased abundance of *Prevotellaceae* family have been consistently observed in PD patients [72,73,76,79,82]. The higher level of *Lactobacillus* and lower level *Ruminococcus* have been also observed in PD patients compared with that of healthy controls [71,74,77,80]. However, there were controversial results about the abundance of *Faecalibacterium* in PD patients [74,78,80].

Vidal-Martinez *et al.* observed the increased amounts of several microorganisms (*Verrucomicrobiaceae* [*Akkermansia muciniphila*] and unclassified *Firmicutes*) and decreased levels of other microorganisms (*Prevotellaceae* [*Prevotella copri*] and *Erysipelotrichaceae* [*Eubacterium biforme*]) in PD patients [72]. *Akkermansia muciniphila* is a mucin degrader that improves the gut barrier function by restoring the intestinal mucus layer and the underlying epithelium and is considered beneficial for human health [150]. However, Ring, C. *et al.* showed that *A. muciniphila* had the opposite outcome: degradation of mucin and increase in intestinal inflammation and intestinal permeability [151]. *A. muciniphila* is also abundant in constipated individuals, which is one of the major non-motor symptoms in PD [152]. In another study, 16S rRNA gene sequencing of fecal samples of 197 PD patients and 130 healthy controls was conducted. It revealed that the abundances of *Bifidobacteriaceae*, *Lactobacillaceae*, *Tissierellaceae*, *Christensenellaceae,* and *Verrucomicrobiaceae* were increased, and the abundances of *Lachnospiraceae* and *Pasteurellaceae* were decreased in PD patients [83]. Collectively, *Verrucomicrobiaceae, Bifidobacteriaceae, Christensenellaceae*, as well as *Lactobacillaceae* increased and *Bacteroidaceae* and *Prevotellaceae* decreased in patients with PD. In contrast, changes in *Ruminococcaceae* and *Lachnospiraceae* families were not consistent [65,69–76,78–80,82].

Higher levels of *Proteus* sp., *Bilophila* sp., and *Roseburia* sp. as well as loss of members of the families *Lachnospiraceae*, *Rikenellaceae*, and *Peptostreptococcaceae* were found in GF mice transplanted with PD patient fecal microbiota compared with the levels in GF mice transplanted with healthy control microbiota. GF mice transplanted with PD microbiota showed impairment in behavioral tests for motor dysfunction, such as the beam traversal, pole descent, and nasal adhesive removal tests [153]. The levels of beneficial bacteria such as *Bifidobacterium* increased in M83 transgenic mice treated with adoptive cellular therapy. It was also shown that immunotherapy outcomes had an impact on the gut microbiome of PD patients [154].

### 3.2.3. Amyotrophic lateral sclerosis (ALS)

ALS is a neurodegenerative disease inducing the loss of muscle control. Several studies have been conducted to investigate the association between gut microbiota and ALS. The found a strong link between several bacterial species and ALS.

The analysis of fecal samples from ALS patients and healthy controls with 16S rRNA gene sequencing revealed that *Escherichia coli* and *Enterobacteriaceae* abundance were higher in ALS patients than that in healthy controls. Additionally, *Clostridium* amounts were lower in ALS patients [85]. Blacher *et al.* employed shotgun metagenomic sequencing to analyze gut microbiota and found that *Anaerostipes hadrus* and *Bacteroidales bacterium* were marginally more abundant in ALS patients, whereas the levels of *Bifidobacterium pseudocatenulatum*, *Clostridium leptum*, and *Escherichia coli* were decreased [86]. Zhai *et al.* analyzed the fecal samples of ALS patients and healthy controls by 16S rRNA gene sequencing and found that *Methanobrevibacter* levels tended to be higher in ALS patients compared with those of healthy controls, whereas *Faecalibacterium* and *Bacteroides*, which are known beneficial microorganisms, were less abundant in ALS patients [89].

ALS presents with swallowing disturbance, muscle atrophy, alterations in metabolic activity, and weight loss due to eating problems [155]. Compared with healthy controls, the levels of SCFAs, $NO_2$-N/$NO_3$-N, and GABA, which are metabolites and substrates of the gut microbiome, were higher in patients with ALS [89]. Wu S *et al.* obtained fecal samples from ALS transgenic mice (G93A) and analyzed bacterial profiles using 16r RNA sequencing.

They showed that G93A mice exhibited increased gut permeability and higher levels of the inflammatory cytokine IL-17. G93A mice also showed a decrease level of in butyrate-producing bacteria (*Butyrivibrio fibrisolvens*) in young mice before the onset of ALS [88]. The changes in *E. coli* abundance and bacterial metabolites are still unclear in ALS. The abundance of identified microorganisms was often inconsistent. Thus, further in-depth research is required to determine the clear association between microbiota and ALS.

## 4. Summary

The advancement in microbiome analysis methods allowed us to understand which microorganism populations and bacterial metabolites were altered in a disease state compared with the normal state. In this review, the advantages and limitations of microbiome analysis methods and online tools for GBA discovery were summarized, such as metabolomic and metagenomic analyses. In addition, the potential relationship between alteration of the gut microbiome and brain disorders reported to date was summarized. These findings have provided researchers with a new insight into the pathophysiology of brain diseases and a new path to explore successful disease treatments based on probiotics or fecal transplantation [156].

## CRediT authorship contribution statement

**Jae Gwang Song:** Writing – original draft. **Myeong-Sang Yu:** Writing – original draft. **Bomi Lee:** Writing – original draft. **Jingyu Lee:** Writing – original draft. **Su-Hee Hwang:** Writing – original draft. **Dokyun Na:** Writing – review & editing. **Hyung Wook Kim:** Writing – review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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