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

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.

Keywords:
Animal model
Brain
Genomic analysis
Gut microbiome
Metabolomics

Contents

1. Introduction ................................................................. 1098
2. Analysis methods and bioinformatics tools for analyzing the microbiome ........................................ 1098
   2.1. Marker gene analysis ........................................ 1098
   2.2. Whole genome analysis .................................... 1098
   2.3. Meta-transcriptome analysis ............................ 1099
   2.4. Metabolome and meta-proteome analyses .......... 1099
3. Gut-brain axis (GBA) ...................................................... 1100
   3.1. Gut microbiome and neuropsychiatric disorders .......................................................... 1100
      3.1.1. Schizophrenia (SCZ) .................................. 1100
      3.1.2. Autism spectrum disorder (ASD) ............... 1104
      3.1.3. Attention deficit hyperactivity disorder (ADHD) .................................................. 1104
      3.1.4. Depression .................................................. 1105
   3.2. Gut microbiome and neurodegenerative diseases .......................................................... 1105
      3.2.1. Alzheimer’s disease (AD) .......................... 1105
      3.2.2. Parkinson’s disease (PD) .......................... 1106
      3.2.3. Amyotrophic lateral sclerosis (ALS) ........... 1106
4. Summary ................................................................. 1107
CRediT authorship contribution statement ................................................................. 1107
Declaration of Competing Interest ................................................................. 1107
Acknowledgments ................................................................. 1107
References ................................................................. 1107

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

https://doi.org/10.1016/j.csbj.2022.02.024
2001-0370/C211 2022 Published by Elsevier B.V. on behalf of Research Network of Computational and Structural Biotechnology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
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-pituitary-adrenal (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 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...
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 about 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

| Name                          | Description                                                                                                                                                                                                 | Analysis                                                                 | Link                                                                 |
|-------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------|----------------------------------------------------------------------|
| Mothur [15]                   | Includes useful tools such as SONS, DOTOUR, TreeClimber, LIBSHUFF, j/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]                  | Plug-in 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://benjneb.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.bioc conductor.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://bioc conductor.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 analysis                                                      | 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.nowiki/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, FaniPhlAn 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-raft.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/                       |
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 affect 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, Megaphtera, 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, Bifidobacteraceae, 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
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 (*Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum*, and *Lactobacillus fermentum*) 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 PD patients. |
| 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 *Selenomonadaceae*. |
| 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. |
| AD [67] | n = 24 | Mice 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 PD patients. |
| AD [68] | n = 18 | Mice fecal samples | 16S rRNA gene sequencing (qPCR) | At the phylum level, *Proteobacteria* and *Verrucomicrobia* levels were increased in AD mice. |
| PD [65] | PD patients = 75, healthy controls = 45 | Human fecal samples | 16S rRNA gene sequencing | At the phylum level, *Firmicutes* levels were increased in AD mice. *Bacteroidetes* were less abundant in AD mice. |
| 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 cocoides* 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 biforum*) 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, Oscillibacter, Bifidobacterium, Christensenella minuta,* *Catabacter hongkongensis,* *Lactobacillus mucosae,* *Ruminococcus bromii,* and *Papillibacter cinnamivorans,* and decreased levels of *Dorea, Bacteroides, Prevotella, Faecalibacterium, Bacteroides massiliensis,* *Streptococcus massiliensis,* *Bacteroides coprocola,* *Blautia lenta,* *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 and mucosal 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, Porphyromonadaceae, 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. The levels of 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 Raistonia 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, Anaerobacterium, Holdemania, Sphingomonas, Clostridium XVIII, Butyricoccus, and Anaerotrubus 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. Proteobacteria levels were significantly increased in SCZ patients. |
| Schizophrenia [83] | SCZ patients = 64, healthy controls = 53 | Human fecal samples            | 16S rRNA gene sequencing (metagenomes, PICRUSt analysis) | SCZ patients 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. 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 [85]         | ALS patients = 50, healthy controls = 50 | Human fecal samples            | 16S rRNA gene sequencing with qPCR | Decreased levels of Akkermansia muciniphila, Parabacteroides distasonis, Rikenellaceae, Prevotella, Lactobacillus murinus, Alstipes unclassified, and Eggertella uncultured as well as increased Sutterella, Allobaculum, Desulfovibrioaceae, Coprococcus, Oscillibacter, Bifidobacterium, Helicobacter hepaticus, Lactobacillus johnsonii, and Lactobacillus reuteri in ALS mice. |
| ALS [86]         | ALS patients = 6, healthy controls = 5 | Mice fecal samples             | 16S rRNA gene sequencing, Shotgun metagenomic sequencing | The amounts of Anaerostipes hadas and Bacteroides bacterium were increased marginally in ALS patients and Bifidobacterium pseudocatenulatum, Clostridium leptum, and Escherichia coli levels were decreased. Dorea levels were increased and the amounts of Oscillibacter, Anaerostipes, and Lachnospiraceae were decreased in ALS patients compared with those of healthy controls. A gut dysbiosis was evidenced in ALS mice. particularly in terms of reduced levels of butyrate-producing bacteria, including Butyrivibriofibrisolvens and E. coli. Increased amounts of Methanobrevibacter and decreased levels of Faecalibacterium and Bacteroides in ALS patients. |
| ALS [87]         | ALS patients = 6, healthy controls = 5 | Human fecal samples            | 16S rRNA gene sequencing         | The amounts of Anaerostipes hadas and Bacteroides bacterium were increased marginally in ALS patients and Bifidobacterium pseudocatenulatum, Clostridium leptum, and Escherichia coli levels were decreased. Dorea levels were increased and the amounts of Oscillibacter, Anaerostipes, and Lachnospiraceae were decreased in ALS patients compared with those of healthy controls. A gut dysbiosis was evidenced in ALS mice. particularly in terms of reduced levels of butyrate-producing bacteria, including Butyrivibriofibrisolvens and E. coli. Increased amounts of Methanobrevibacter and decreased levels of Faecalibacterium and Bacteroides in ALS patients. |
| ALS [88]         | not mentioned (454 16S rRNA 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. Increased amounts of Methanobrevibacter and decreased levels of Faecalibacterium and Bacteroides in ALS patients. |
| ALS [89]         | ALS patients = 8, healthy controls = 8 | Human fecal samples            | 16S rRNA gene sequencing         | The amounts of Anaerostipes hadas and Bacteroides bacterium were increased marginally in ALS patients and Bifidobacterium pseudocatenulatum, Clostridium leptum, and Escherichia coli levels were decreased. Dorea levels were increased and the amounts of Oscillibacter, Anaerostipes, and Lachnospiraceae were decreased in ALS patients compared with those of healthy controls. A gut dysbiosis was evidenced in ALS mice. particularly in terms of reduced levels of butyrate-producing bacteria, including Butyrivibriofibrisolvens and E. coli. 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). At the family level, the relative proportions of Bifidobacteriaceae, Coriobacteriaceae, Porphyromonadaceae, Candidate Division TM7 uncultured bacterium, Caldicoprobacteraceae, Akaligenaceae were decreased in FMT rats. |
| Depression [92]                              | Depressed patients = 58, healthy controls = 33 | Human fecal samples                         | 16 s rRNA sequencing                      | At the family level, the relative proportions of Prevellaceae were decreased, whereas those of Thermoanaerobacteriaceae were increased in depressed patients. At the genus level, there was an increase of the relative proportions of Eggertellhae, Holdemania, Gébria, Turiccibacter, Paraprevotella and Anaeroplasm, and decreased amounts of Prevotella and Dialister in depressed patients. |
| 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             | Actinobacteria levels were increased, whereas Bacteroidetes amounts were decreased in depressed patients compared with the levels in healthy controls. 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. 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. Depressed patients had increased Enterobacteriaceae and Alstipes levels and decreased amounts of Faecibacterium. |
| 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 Prevellaceae family. 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 Alstipes and Akkermansia. 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 [95]                              | Autism patients = 20, healthy controls = 10  | Human fecal samples                         | 16S rRNA sequencing (by using Bacterial tag-encoded FLX-titanium amplicon pyrosequencing [V3EAP]) | 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. 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 Alstipes, Bilophila, Dialister, Parabacteroides, and Veillonella, and increased amounts of Collinsella, Corynebacterium, Dorea, and Lactobacillus in autistic patients. |
| Autism/ASD [96]                              | Autism patients = 13, healthy controls = 8   | Human fecal samples                         | 16S rRNA gene sequencing                 | Much lower levels of Bifidobacterium, slightly lower levels of Enterococcus, and the much higher levels of Lactobacillus in autism patients. Autism patients were likely to have Bacillus spp. and less likely to have Klebsiella oxytoca. |
| 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. 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 Alstipes, Bilophila, Dialister, Parabacteroides, and Veillonella, and increased amounts of Collinsella, Corynebacterium, Dorea, and Lactobacillus in autistic patients. |
| Autism/ASD [98]                              | Autism patients = 40, healthy controls = 40  | Human fecal samples                         | 16S rRNA gene pyrosequencing             | Much lower levels of Bifidobacterium, slightly lower levels of Enterococcus, and the much higher levels of Lactobacillus in autism patients. Autism patients were likely to have Bacillus spp. and less likely to have Klebsiella oxytoca. |
| 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. Autism patients were likely to have Bacillus spp. and less likely to have Klebsiella oxytoca. |
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 PANS anxiety/depression score were improved and their interleukin 22 (II-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, Bacteroides, 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 Clostridium, Sarcina, and Clostridioides 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 implanted 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 Deferrribacteres [121]. Specific pathogen-free (SPF) C57BL/6 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 lipopolysaccharides 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 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 with 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 Thermomonas and Prevotellaceae was increased. At the genus level, the relative proportions of Eggerthella, Holdemania, Gelria, Turicibacter, Paraprevotella, and Anaeroflibacterium 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. Nasibi-afrouei et al. showed an association between depression and gut microbiota: specifically, Oscillibacter and Alistipes were abundant in depressed patients [40]. Jang 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 GABAβ1 mRNA levels in the hippocampus, which was consistent with the anti-depressant-like effect of GABAβ1 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 symbiotic 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 symbiotic (Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus rhamnosus, Bifidobacterium breve, Bifidobacterium longum, Streptococcus thermophilus, fructooligosaccharide as prebiotic) or the placebo was added to the administration for 6 weeks. The symbiotic 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 seventeen 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-aminergic 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 Butyricoccus 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 [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 [5xFAD]. 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, NO2-N/NO3-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 (*Butyrovibrio 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. Jinyu 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.

Acknowledgments

This work was supported by the Chung-Ang University Research Grants in 2021 and the National Research Foundation (NRF) of Korea (NRF-2018R1A5A1025077 to D Na and NRF-2021R1F1A1059574 To HW Kim).

References

[1] Appleton J. The gut-brain axis: influence of microbiota on mood and mental health. Int Med (Encinitas, Calif) 2018;17(4):28–32.
[2] Rhee SH, Pothoulakis C, Mayer EA. Principles and clinical implications of the brain-gut-enteric microbiota axis. Nat Rev Gastroenterol Hepatol 2009;6(5):306–14.
[3] Ghash SH, Whitley CS, Farah B, Yula VR. Regulation of intestinal barrier function by microbial metabolites. Cell Mol Gastroenterol Hepatol 2021;11(5):1463–82.
[4] Lyte M, Varcoe J, Bailey MT. Anxiogenic effect of subclinical bacterial infection in mice in the absence of overt immune activation. Physiol Behav 1998;65(1):63–8.
[5] Goehler LE, Gaykema RP, Opitz N, Reddaway R, Badr N, Lyte M. Activation in vagal afferents and central autonomic pathways: early responses to intestinal infection with Campylobacter jejuni. Brain Behav Immun 2005;19(4):334–44.
[6] Sudo N, Chida Y, Aiba Y, Sonoda J, Oyama N, YU XN, et al. Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. J Physiol 2004;558(Pt 1):263–74.
[7] Azzam I, Gid S, Limor L, Nisen N, Greenman Y. Cholin stimulation by hypothalamic-pituitary-adrenal axis activation depends on increasing cortisol levels. Endoc Connect 2017;6(8):847–55.
[8] Picciotto MR. Galanin and addiction. Cell Mol Life Sci 2008;65(12):1872–9.
[9] Cheng HY, Ning MX, Chen DK, Ma WT. Interactions between the gut microbiota and the host innate immune response against pathogens. Front Immunol 2019;10:607.
[10] Danter K. Neuroimmune interactions: from the brain to the immune system and vice versa. Physiol Rev 2018;98(1):477–504.
[11] Braniste V, Al-Ashmakh M, Kowal C, Anuar F, Abbaspour A, Toth M, et al. The gut microbiota influences the blood-brain permeability in mice. Sci Transl Med 2014;6(245):245ra158.
[12] Mayer EA, Tillisch K, Gupta A. Gut-brain axis: the microbiota. J Clin Invest 2015;125(3):926–38.
[13] Brolt S, Kupferberg A, Rolger H, Gasler G. Vagus nerve as modulator of the brain-gut axis in psychiatric and inflammatory disorders. Front Psychiatry 2018;9(44):44.
[14] Moraes LH, Schreiber HLT, Mazmanian SK. The gut microbiota-brain axis in behaviour and brain disorders. Nat Rev Microbiol 2021;19(4):241–55.
[15] Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EA, et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 2009;75(23):7537–41.
[16] Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 2019;37(8):852–7.
[17] Callahan BJ, McMurdie PJ, Rosen JI, Han AW, Johnson AJA, Holmes SP. DADA2: high-resolution sample inference from illumina amplicon data. Nat Methods 2016;13(7):581–3.
[18] McMurdie PJ, Holmes S. Phylseq: an R package for reproducible interactive visualization and graphical exploration of phylogenetic data. PLoS ONE 2013;8(4):e60117.
[19] Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O’Hara R, et al. Package ‘vegan’. Community ecology package, version 2013;2(9):1–295.
[20] Love M, Huber W, Vah Re MM, Genomeics I, Biobase B, et al. Package ‘DESeq2’. 2013.
[21] Peng Y, Leung HCM, Yiu SM, Chin FYL. IDBA-UD: a de novo assembler for de novo assembly of single-cell and metagenomic sequencing data with highly uneven depth. Bioinformatics 2012;28(11):1420–8.
[22] Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 2012;19(5):455–77.
[23] Li DH, Luo RB, Liu CM, Leung CM, Ting HF, Sadakane K, et al. MEGAHIT v1.0: a fast and scalable metagenome assembler driven by advanced methodologies and community practices. Methods 2016;102:3–11.
[24] Bagnini F, Melci LV, Blanco-Miguez A, Dubois L, Asnicar F, Maharanj S, et al. Integrative, taxonomic, functional, and strain-level profiling of diverse microbial communities with biobakery. 3. Elite 2021;10:65088.
[25] Meyer F, Paarmann D, D’Souza M, Olson R, Glass EM, Kuba M, et al. The metagenomics RAST server—a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinform 2009;8:936.
[26] Douglas CM, Mafee VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, et al. PICRUS2 for prediction of metagenome functions. Nat Biotechnol 2020;38(6):685–8.
[27] Luo RB, Liu BH, Xie YL, Li ZY, Huang WH, Yuan JY, et al. SPAdenovo2: an empirically improved memory-efficient short-read de novo assembler. GigaScience 2012;1(1):18.
[28] Schlunzen F, Toci M, Zarifch V, Harms J, Gluehmann M, Janell D, et al. Structure of functionally activated small ribosomal subunit at 3.3 angstrom resolution. Cell 2000;102(5):615–23.
[29] Wu CD, Lewis JD. Analysis of the human gut microbiome and association with clinical Gastroenterol Hepatol 2013;11(7):774–7.
[30] Duong S, Higgins PD, Metodios KP. The human gut microbiome: current knowledge, challenges, and future directions. Transl Res 2012;160(4):52–67.
[31] Czaprowski JG, Kuczynski J, Stombaugh J, Bittertinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods 2010;7(5):335–6.
[32] Cole JR, Wang Q, Fish JA, Chai BL, McGarrell DM, Sun YN, et al. Ribosomal database project: data and tools for high-throughput rRNA analysis. Nucleic Acids Res 2014;42(9):D631–42.
[33] Suzuki MT, Giovannoni SJ. Bias caused by template annealing in the based profiling of the human infant gut microbiota is strongly influenced by 3.3 angstrom resolution. Cell 2000;102(5):615–23.
[34] Wu CD, Lewis JD. Analysis of the human gut microbiome and association with clinical Gastroenterol Hepatol 2013;11(7):774–7.
[35] Duong S, Higgins PD, Metodios KP. The human gut microbiome: current knowledge, challenges, and future directions. Transl Res 2012;160(4):246–57.
[36] Caporaso JG, Kuczynski J, Stombaugh J, Bittertinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods 2010;7(5):335–6.
[37] Cole JR, Wang Q, Fish JA, Chai BL, McGarrell DM, Sun YN, et al. Ribosomal database project: data and tools for high-throughput rRNA analysis. Nucleic Acids Res 2014;42(9):D631–42.
[38] Suzuki MT, Giovannoni SJ. Bias caused by template annealing in the based profiling of the human infant gut microbiota is strongly influenced by 3.3 angstrom resolution. Cell 2000;102(5):615–23.
Lin H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics 2010;26(5):589–95.

Li Y, Chen YX, Wu DS, Yuan JY, Shi YJ, Zhang H, et al. Comparison of the two major classes of assembled reads: overlap-layout-consensus and de-bruijn graph. Brief Funct Genomics 2012;11(1):25–37.

Ranjan R, Rani A, Mettawaly A, McGee HS, Perkins DL. Analysis of the microbial profile of whole genome shotgun versus 16S amplicon sequencing. Biochem Biophys Res Commun 2016;469(4):967–77.

Bashiardeh S, Zilberman-Schapira G, Eliran E. Use of metatranscriptomics in microbiome research. Bioinfon Biol Insights 2016;10:19–25.

Shalaya M, Lo CC, Sin-PG. Advances and challenges in metatranscriptomics analysis. Front Genet 2019;10:904.

Chen MX, Wang SY, Kuo CH, Tsai IL. Metabolome analysis for investigating host-gut microbiota interactions. J Formos Med Assoc 2019;118:510–22.

Petrov V, Saltykova I, Zhukova I, Alifirova V, Zhukova N, Dorofeeva YB, et al. Potential functional implications of microbial and viral gut metagenome changes in early stage L-DOPA-naive Parkinson’s disease patients. Genome Med 2017;9:115–13.

Hill-Burns EM, Debeles JW, Morton JT, Wissemann WT, Lewis MR, Wallen ZD, et al. Parkinson’s disease and Parkinson’s disease medications have distinct signatures of the gut microbiome. Mov Disord 2017;32(5):739–49.

Kushak RI, Winter HS, Buie TM, Cox SB, Phillips CD, Ward NL. Analysis of the duodenal microbiome in autistic individuals: association with carbohydrate metabolism. J Pediatr Gastroenterol Nutr 2017;64(5):e110–6.
Romano S, Savva GM, Bedarf JR, Charles IG, Hildebrand F, Narbad A. Meta-
alysis of the Parkinson's disease gut microbiome suggests alterations
linked to intestinal inflammation. NPJ Parkinsons Dis 2021;7(1):27.

Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GC, Ilhan ZE, et al. Gut
microbiota regulate motor deficits and neuroinflammation in a model of
Parkinson’s disease. Cell 2016;167(6):1469-80.e12.

Klann E, Tagliamonte MS, Ukhanova M, Mai V, Vedam-Mai V. Gut microbiota
dynamics in parkinsonian mice. ACS Chem Neurosci 2020;11(20):3267–76.

Ioannides ZA, Ngo ST, Henderson RD, McCombe PA, Steyn FJ. Altered
metabolic homeostasis in amyotrophic lateral sclerosis: mechanisms of
energy imbalance and contribution to disease progression. Neurodegener Dis
2016;16(5–6):382–97.

Evrensel A, Ceylan ME. Fecal microbiota transplantation and its usage in
neuropsychiatric disorders. Clin Psychopharm Neurosci 2016;14(3):231–7.