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Review

# The Microbiome as a Modifier of Neurodegenerative Disease Risk

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The gut microbiome is increasingly implicated in modifying susceptibility to and progression of neurodegenerative diseases (NDs). In this review, we discuss roles for the microbiome in aging and in NDs. In particular, we summarize findings from human studies on microbiome alterations in Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, and Huntington's disease. We assess animal studies of genetic and environmental models for NDs that investigate how manipulations of the microbiome causally impact the development of behavioral and neuropathological endophenotypes of disease. We additionally evaluate the likely immunological, neuronal, and metabolic mechanisms for how the gut microbiota may modulate risk for NDs. Finally, we speculate on cross-cutting features for microbial influences across multiple NDs and consider the potential for microbiome-targeted interventions for NDs.

## Introduction

Neurodegenerative diseases (NDs) are characterized by progressive functional loss of neurons in the central nervous system (CNS) or peripheral nervous system (PNS), which leads to long-term motor and/or cognitive impairments. While genetic susceptibilities are major risk factors for developing NDs, environmental factors experienced throughout life also impact the onset, progression, and severity of NDs. For example, Parkinson's disease (PD) has been positively associated with pesticide exposure (Menegon et al., 1998) and gastrointestinal (GI) infection (Fasano et al., 2015), and Alzheimer's disease (AD) has been increasingly associated with herpes simplex virus (HSV) (Itzhaki, 2017). Exactly how genetic and environmental risk factors are conveyed to together predispose to NDs remains poorly understood. However, emerging evidence suggests that genetically and environmentally induced alterations in the gut microbiome may contribute in part to ND risk.

The GI tract harbors trillions of microorganisms, collectively called the gut microbiota, that is shaped by both host genetics and environmental exposures (Lozupone et al., 2012), and that profoundly modulates the development and function of the CNS and PNS across model organisms (Fung et al., 2017). Relevant to aging-related disorders, like some NDs, the composition and activity of the gut microbiota changes over the lifespan, and manipulating the gut microbiota in animal models alters aging-associated immune function, metabolic activity, and ultimately longevity (Kundu et al., 2017). Additional environmental risk factors for NDs, such as diet, chemical exposures, and infection also shape the gut microbiota in humans and animal models (Sherwin et al., 2018). Consistent with this, an increasing number of studies report changes in the microbiota in individuals with PD, AD, amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD) (Table 1) as well as in a subset of animal models for these disorders (Table 2). Across animal models for NDs, disruptions in the gut microbiome can positively or negatively impact the manifestation of neuropathological and behavioral endophenotypes of disease. Although the precise mechanisms underlying these phenomena remain unclear, increasing evidence supports the notion of a "microbiota-gut-brain axis," whereby intestinal microorganisms influence the CNS by modulation of neuroimmune function, sensory neuronal signaling, and metabolic activity. Altogether, these findings raise timely interest in how the microbiome is shaped by risk factors for NDs, how functional activities of the microbiome impact the nervous system, and how microbiome-gut-nervous system interactions may predispose to NDs.

## The Gut Microbiome on Aging and Longevity

Preceding interest in the microbiome and aging-related NDs were several pioneering studies on the gut microbiome and healthy aging. Aging is commonly accompanied by chronic inflammation, increased intestinal permeability, impaired digestion, and disrupted nutrient absorption, each of which exhibits bidirectional interactions with the gut microbiome (An et al., 2018). Consistent with these interactions, a preponderance of sequencing studies reports shifts in the composition of the gut microbiota from adulthood to old age (Langille et al., 2014; O'Toole and Jeffery, 2015). However, there is little consensus on the particular aging-associated taxa found across these studies. For example, increases in *Bacteroidetes* were correlated with aging in some studies (Claesson et al., 2011; Odamaki et al., 2016), whereas decreases in *Bacteroidaceae*, *Lachnospiraceae*, and *Ruminococcaceae* and increases in *Akkermansia*, *Bifidobacterium*, and *Christensenellaceae* were reported in another (Biagi et al., 2016). Aside from differences in study design and technical approaches, several confounding factors are likely to contribute to the lack of study consensus. In a study of hospitalized elderly and healthy controls, for example, medication intake correlated negatively with *Massilia* and *Lachnospiraceae* and positively with *Bradyrhizobium*, *Coprobacter*, *Helicobacter*, and *Prevotella* (Ticinesi et al., 2017). In a 60-year longitudinal study, married individuals exhibited greater microbial diversity and richness compared with individuals living alone (Dill-McFarland et al., 2019), which

**Table 1. Microbiome Alterations in Clinical Cohorts for NDs**

| Subject | Microbiota Alterations | Sample | Other Variables | Method | Reference |
|---|---|---|---|---|---|
| Parkinson's Disease: Microbiota Alterations in Humans | | | | | |
| Human, PD patients | increased: *Bifidobacterium*; decreased: *Prevotellaceae*, *Roseburia* | n = 64, feces | sex matched; age matched; diet: no difference; medication: variable | 16S rRNA gene sequencing | Aho et al., 2019 |
| Human, L-dopa-naive PD patients | family/genus increased: *Verrumicrobiaceae* (genus *Akkermansia*), unclassified bacteria, Firmicutes; family/ genus decreased: *Prevotellaceae* (*Prevotella*), *Erysipelotrichaceae (Eubacterium)*; species increased: *Akkermansia muciniphila*, *Alistipes shahii*; species decreased: *Prevotella copri*, *Eubacterium bioforme*, *Clostridium saccharolyticum* | n = 31, feces | males, age matched; diet: no difference; medication: no L-DOPA therapy | shotgun metagenomic sequencing | Bedarf et al., 2017 |
| Human, PD patients | increased: *Lactobacillus*; decreased: *Clostridium coccoides*, *Bacteroides fragilis* | n = 52, feces | sex unmatched age matched diet: no difference medication: unknown | 16S rRNA gene qPCR | Hasegawa et al., 2015 |
| Human, PD patients | increased *Akkermansia* | n = 76, feces | sex unmatched; age matched; diet: unknown; medication: variable | 16S/18S rRNA gene sequencing | Heintz-Buschart et al., 2018 |
| Human, PD patients | family increased: *Bifidobacteriaceae*, *Lactobacillaceae, Tissierellaceae, Christensenellaceae, Verrucomicrobiaceae*; family decreased: *Lachnospiraceae*, *Pasteurellaceae*; genus increased: *Akkermansia, Christensenellaceae, Lactobacillus, Bifidobacterium*; genus Decreased: *Blautia*, *Roseburia*, *Lachnospiraceae*, *Faecalibacterium* | n = 197, feces | sex adjusted; age adjusted; diet adjusted; medication adjusted | 16S rRNA gene sequencing | Hill-Burns et al., 2017 |
| Human, PD patients | increased: *Lactobacillaceae, Barnesiellaceae, Enterococcaceae* | n = 29, feces | sex unmatched; age matched; diet: no difference; medication: unknown | 16S rRNA gene sequencing | Hopfner et al., 2017 |
| Human, PD patients | mucosal genus increased: *Faecalibacterium*, *Ralstonia*; fecal genus increased: *Blautia*, *Coprococcus*, *Roseburia* | n = 38, sigmoid mucosal biopsies, feces | sex matched; age: PD patients are older than HC; diet: no difference; medication: variable | 16S rRNA gene sequencing | Keshavarzian et al., 2015 |
| Human, PD patients | increased: *Escherichia-Shigella*, *Streptococcus*, *Proteus, Enterococcus*; decreased: *Blautia*, *Faecalibacterium*, *Ruminococcus* | n = 24, feces | sex matched; age matched; diet: unknown; medication: unknown | 16S rRNA gene sequencing | Li et al., 2017 |

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**Table 1.** *Continued*

| Subject | Microbiota Alterations | Sample | Other Variables | Method | Reference |
|---|---|---|---|---|---|
| Human, PD patients | increased: *Ruminococcaceae, Verrucomicrobiaceae, Porphyromondaceae, Hydrogenoanaerobacterium, Lachnospiraceae*; decreased: *Bacteroides, Prevotellaceae* | n = 10, feces | sex unmatched; age matched; diet: unknown; medication: unknown | 16S rRNA gene sequencing | Li et al., 2019 |
| Human, PD patients | increased: *Eubacteriaceae, Bifidobacteriaceae, Aerococcaceae, Desulfovibrionaceae*; decreased: *Streptococcaceae, Methylobacteriaceae, Comamonadaceae, Halmonadaceae, Brucellaceae, Xanthomonadaceae, Lachnospiraceae, Actinomycetaceae, Aphingomonadaceae, Pasturellaceae, Micrococcaceae, Intrasporangiaceae, Methanobacteriacea, Idomarinaceae, Brevibacteriaceae, Gemellaceae* | n = 75, feces | sex matched; age matched; diet: no influence on results; medication: variable | 16S rRNA gene sequencing | Lin et al., 2018 |
| Human, PD patients | Genus increased: *Christensenella, Catabacter, Lactobacillus, Oscillospira, Bifidobacterium*; genus decreased: *Dorea*, *Bacteroides*, *Prevotella*, *Faecalibacterium*; species increased: *Christensenella minuta, Catabacter hongkongensis, Lactobacillus mucosae, Ruminococcus bromii Palpillibacter cinnamivorans*; species decreased: *Bacteroides massiliensis*, *Stoquefichus massiliensis*, *Bacteroides coprocola*, *Blautia glucerasea*, *Dorea longicatena*, *Bacteroides dorei*, *Bacteroides plebeus*, *Prevotella copri*, *Coprococcus eutactus*, *Ruminococcus callidus* | n = 89, feces | sex unknown; age matched; diet unknown; medication unknown | 16S rRNA gene sequencing | Petrov et al., 2017 |
| Human, PD patients | increased: *Clostridium IV, Aquabacterium, Holdemania, Sphingomonas, Clostridium XVII, Butyricicoccus, Anaetroncus* | n = 45, feces | sex matched; age matched; diet matched; medication: antiparkinsonian medications | 16S rRNA gene sequencing | Qian et al., 2018 |
| Human, PD patients | family increased: *Enterobacteriaceae*; phylum decreased: *Bacteroidetes*; family decreased: *Prevotellaceae* | n = 43, feces | sex unmatched; age matched; diet: no difference; medication: no dopaminergic drugs | 16S RT-qPCR | Unger et al., 2016 |

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**Table 1.** ***Continued***

| Subject | Microbiota Alterations | Sample | Other Variables | Method | Reference |
|---|---|---|---|---|---|
| Human, PD patients | increased: *Akkermansia*, *Bifidobacterium* (trending); decreased: *Prevotella* (trending) | n = 9, feces | sex unknown; age: 35–95; diet: unknown; medication: unknown | 16S rRNA gene sequencing | Vidal-Martinez et al., 2020 |
| Human, PD patients with normal cognition (NC) or mild cognitive impairment (MCI) | PD-NC versus PD-MCI and HC: increased: *Blautia*, *Ruminococcus*; PD- MCI versus PD-NC and HC: increased: *Alistipes*, *Barnesiella, Butyricmonas, Odoribacter, Anaerotruncus* | n = 13 (MCI); n = 14 (NC), feces | sex adjusted; age adjusted; diet: no restriction; medication: unknown | 16S rRNA gene sequencing | Ren et al., 2020 |
| AD: Microbiota Alterations in Humans | | | | | |
| Human, AD patients | phylum level: mild decrease in *Bacteriodetes*decrease in *Verrucomicrobia*; class level: increase in *Actinobacteria* and *Bacilli*decrease in *Negativicutes* and *Bacteroidia*; order level: increase in *Lactobacillales*decrease in *Bacteroidales and Selenomonadales*; family level: increase in *Ruminococcaceae*, *Enterococcaceae*, and *Lactobacillaceae*decrease in *Lanchnospiracea*, *Bacteroidaceae* and *Veillonellaceae* | n = 43, feces | sex matched; age matched; diet: unknown; medication: unknown | 16S rRNA gene sequencing | Zhuang et al., 2018 |
| Human, AD patients | phylum level: increase in *Bacteriodetes*decrease in *Firmicutes* and *Actinobacteria*; family level: increase in *Gemellaceae, Bacteroidaceae and Rikenellaceae* decrease in *Bifidobacteriaceae, Ruminococcaceae, Turicibacteraceae, Peptostreptococcaceae, Clostridiaceae*, and *Mogibacteriaceae*; genus level: increase in *Bacteroides*, *Alistipes*, *Bilophila*, *Blautia*, *Phascolarctobacterium*, and *Gemella* decrease in *SMB53, Dialister, Clostridium, Turicibacter, cc115*, *Bifidobacterium* and *Adlercreutzia* | n = 25, feces | sex matched; age matched; diet: no difference; medication: variable | 16S rRNA gene sequencing | Vogt et al., 2017 |
| Amyotrophic Lateral Sclerosis: Microbiome Alterations in Humans | | | | | |
| Human, ALS patients | increased *Methanobrevibacter*; decreased *Faecalibacterium* and *Bacteroides* | n = 8, feces | sex matched; age matched; diet: no difference; medication: unknown | 16S rRNA gene sequencing | Zhai et al., 2019 |
| Human, ALS patients | no significant alterations | n = 25–32, feces | sex matched; age matched; diet unknown; medication unknown | 16S rRNA gene sequencing | Brenner et al., 2018 |

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**Table 1.** *Continued*

| Subject | Microbiota Alterations | Sample | Other Variables | Method | Reference |
|---|---|---|---|---|---|
| Human, ALS patients | increased *Dorea*; decreased *Oscillibacter*, *Anaerostipes*, *Lachnospiraceae* | n = 5–6, feces | sex unknown; age unknown; diet unknown; medication unknown | 16S rRNA gene sequencing | Fang et al., 2016 |
| Human, ALS patients | marginally increased *Anaerostipes hadrus* and *Bacteroidales bacterium*; marginally decreased *Bifidobacterium pseudocatenulatum*, *Clostridium leptum* and *Escherichia coli* | n = 29–37, feces | sex mixed; age matched; diet unknown; medication: no constipation medication | shotgun metagenomic sequencing | Blacher et al., 2019 |

may be related to reported influences of social interaction on the gut microbiota.

Given the technical challenges of conducting mechanistic aging-related research in humans, animal models have been particularly useful for demonstrating proof of principle for microbial effects on aging. Manipulation of the gut microbiota can modify lifespan across rodents, fish, flies, and worms (Gordon et al., 1966; Lee and Hase, 2014; Obata et al., 2018; Smith et al., 2017; Thevaranjan et al., 2017). In *Drosophila melanogaster*, age-related alterations in the gut microbiota preceded and predicted age-associated intestinal barrier dysfunction and subsequent decline (Clark et al., 2015). Specific depletion of *Acetobacter* from the gut in response to early life oxidant exposure extended longevity (Obata et al., 2018), whereas overgrowth of *Lactobacillus plantarum* shortened lifespan via lactic acid-mediated overproduction of reactive oxygen species (Iatsenko et al., 2018). In *C. elegans*, gut colonization of *Bacillus subtilis* extended lifespan via biofilm formation and the production of nitric oxide and quorum-sensing pentapeptide (Donato et al., 2017). In the African turquoise killifish, transplanting microbiota from young donors into middle-aged recipients extended lifespan, which correlated with engraftment of *Exiguobacterium*, *Planococcus*, *Propionigenium*, and *Psychrobacter* (Smith et al., 2017). Consistent with reported age-associated reductions in *Akkermansia muciniphila* in mice and humans (Fransen et al., 2017; Langille et al., 2014; Sovran et al., 2019), supplementation of *A. muciniphila* to the $Lmna^{G609G/G609G}$ mouse model of progeria significantly extended lifespan (Bárcena et al., 2019). While little is known regarding the exact molecular and cellular mechanisms underlying these distinct phenomena, several studies suggest that microbial modulation of immune homeostasis may be involved. Germ-free mice colonized with microbiota from old, but not young, conventional mice displayed elevated inflammatory cytokines in the serum and higher intestinal permeability, suggesting that aging-associated changes in the gut microbiota enhance systemic inflammation (Thevaranjan et al., 2017). Consistent with this, impaired proliferation of B cells in the Peyer's patches of aged mice was corrected by fecal microbiota transplantation from young mice (Stebegg et al., 2019), indicating protective effects of the microbiota from young mice against aging-associated immune dysregulation. These foundational studies on roles for the microbiome during normal aging highlight temporal changes in the microbiome that impact health span and lifespan, likely through immune and stress responses. Given that aging is a major risk factor for many NDs, these studies further pave the way for active interest in microbial influences on aging-related neurodegenerative disorders, such as PD and AD.

## The Gut Microbiome and PD

Of all NDs, PD has the richest history of interest in peripheral contributions to central neural dysfunction. PD is a multifactorial neurodegenerative disease, believed to be caused by both environmental and genetic risk factors. It is characterized by the presence of neurotoxic alpha-synuclein inclusions that result in striatal dopaminergic cell death and motor deficits. A role for the GI tract in the pathogenesis of PD has been hypothesized since the 1980s when alpha-synuclein inclusions were first observed in enteric tissues from PD patients (Wakabayashi et al., 1988). In the years to follow, Braak and colleagues went on to characterize the topographical spread of alpha-synuclein inclusions, noting that regions receiving input from peripheral nerve fibers, such as the dorsal vagal brainstem motor nuclei and olfactory bulb are affected early on (Braak and Braak, 2000; Braak et al., 2002, 2000). These observations lead to the development of the "dual-hit" staging hypothesis, suggesting that PD pathology originates from insults in peripheral organs where alpha-synuclein is seeded, such as the GI tract and nasal cavity, before translocating to the brain (Hawkes et al., 2007). In line with this hypothesis, many PD patients experience hyposmia and GI issues prior to the onset of motor symptoms, and patients with inflammatory bowel disease (IBD) are at a greater risk of developing PD.

In the past decade, this interest in GI associations with PD has extended to include alterations in the gut microbiota as a potential disease biomarker and/or disease modifier. Clinical studies profiling the microbiota of PD patients reported many alterations relative to healthy controls, including increases in the relative abundance of *Bifidobacterium* (Hasegawa et al., 2015; Peng et al., 2018), *Lactobacillus* (Hasegawa et al., 2015; Petrov et al., 2017), and *Verrucomicrobiaceae* (Hasegawa et al., 2015; Petrov et al., 2017; Unger et al., 2016). Consistent with the latter, increases in the abundance of *Akkermansia* have been widely reported in individuals with PD (Hill-Burns et al., 2017; Keshavarzian et al., 2015; Scheperjans et al., 2015; Unger et al., 2016). Decreases in *Blautia* and *Coprococcus* (Bedarf et al., 2017; Hasegawa et al., 2015; Unger et al., 2016) as well as *Prevotellaceae* (Hasegawa et al., 2015; Heintz-Buschart et al., 2018; Hill-

**Table 2. Microbiome Alterations in Animal Models for NDs**

| Subject | Microbiota Alterations | Sample | Method | Reference |
|---|---|---|---|---|
| PD: Microbiota Alterations in Animal Models | | | | |
| Mice, Thy1-A30P:SNCA | decreased: *Verrumicrobiae* | n = 26, feces | 16S rRNA gene sequencing | Gorecki et al., 2019 |
| Mice, rotenone | no observed differences with FDR-*P*; With less stringent analysis, decreased: *Actinobacteria* | n = 14, feces | 16S rRNA gene sequencing | Dodiya et al., 2020 |
| Rats, rotenone | (RT-qPCR) increased: *Bifidobacterium*; (sequencing, SI) phylum increased: *Actinobacteria*, *Proteobacteria*; (sequencing, SI) phylum decreased: *Bacteroidetes*, *Cyanobacteria*, *Firmicutes*; (sequencing, colon) phylum decreased: *Cyanobacteria*, *Firmicutes*; (sequencing, SI/colon) family increased: *Bifidobacteriaceae*, *Alcaligenaceae, Clostridiaceae*; (sequencing, SI/colon) family decreased: *S24-7, Prevotellaceae, Paraprevotellaceae, Lachnospiraceae*; (sequencing, SI/colon) genus increased: *Bifidobacterium*, *Lactobacillus*, *Turicibacter*, *Sutterella*; (sequencing, SI/colon) genus decreased: *Prevotella, S24-7, Oscillospira* | n = 12, distal small intestine and colon contents | qPCR, shotgun metagenomic sequencing | Johnson et al., 2018 |
| Mice, MPTP | phylum decreased: Proteobacteria (2 days and 3 weeks following treatment); order increased: *Erysipelotrichales* (3 weeks following treatment); order decreased: *Clostridiales* (3 weeks following treatment); family increased: *Prevotellaceae* (2 days following treatment), *Erysipelotrichaceae* (3 weeks following treatment); family decreased: *Lachnospiraceae* (2 days and 3 weeks following treatment) | n = 10, feces | 16S rRNA gene sequencing | Lai et al., 2018 |
| Mice, MPTP | Mucosa phylum increased: Bacteroidetes, Firmicutes Mucosa; phylum decreased: Actinobacteria Lumenal content; phylum increased: Proteobacteria Lumenal content; phylum decreased: Actinobacteria Mucosa and Lumenal content; family increased: *Rikenellaceae, S24-7, Clostridiales* (unclassified), *Ruminococcaceae* mucosa and lumenal content; family decreased: *Bifidobacterium* mucosa; genus increased: *Allobaculum* | n = 9–10, cecum mucosal-associated and lumenal contents | 16S rRNA gene sequencing | Perez-Pardo et al., 2018 |
| Mice, MPTP | phylum increased: *Proteobacteria*; phylum decreased: *Firmicutes*; order increased: *Turicibacterales, Enterobacteriales*; order decreased: *Clostridiales* | n = 7, feces | 16S rRNA gene sequencing | Sun et al., 2018 |
| Mice, rotenone, within comparison over 4 weeks | phylum increased: *Firmicutes* (3, 4 weeks of treatment); phylum decreased: *Bacteroidetes* (3, 4 weeks of treatment); genus increased: *Lactobacillus* (3 and 4 weeks of treatment); genus decreased: *Clostridium* (1–4 weeks of treatment), *Sutterella* (1–4 weeks of treatment), *Lactococcus*(1–3 weeks of treatment), *Desulfovibrio* (2–4 weeks of treatment), *Aldercreutzia* (2 weeks of treatment), *Paraprevotella* (1 and 3 weeks of treatment) | n = 5–8, feces | 16S rRNA gene sequencing | Yang et al., 2017 |

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**Table 2.** *Continued*

| Subject | Microbiota Alterations | Sample | Method | Reference |
|---|---|---|---|---|
| AD: Microbiota Alterations in Animal Models | | | | |
| Mice, tau P301L | phylum level: increase in *Bacteroidetes*decrease in *Firmicutes*, *Actinobacteria* and *Tenericutes*; genus level: increase in *Ruminococcaceae_NK4A214_group*, *Bacteroides*, *Anaeroplasma*, *norank_f_Clostridiales_vadinBB60_group*, *norank_f_Bacteroidales_24_7_group*, *Phascolarctobacterium*, *Parabacteroides*, and *unclassified_o_Bacteroidales*, *Anaerovorax*, *Peptococcus*, *Caproiciproducens*, *Ruminoclostridium*, and *Oscillibacter*; decrease in *Lactobacillus, Enterorhabdus*, *Staphylococcus*, *Bifidobacterium*, *Gemella*, *Roseburia*, *norank_f_Mycoplasmataceae*, *Lachnoclostridium*, *Klebsiella*, and *Streptococcus* | n = 32, feces | 16S rRNA gene sequencing | Sun et al., 2019a |
| Mice, APP/PS1 | phylum level: increase in *Proteobacteria* and *Verrucomicrobia*; genus level: decrease in *Ruminococcus* and *Butricicocus* | n = 24, feces | 16S rRNA gene sequencing | Zhang et al., 2017a |
| Mice, 5 × FAD | phylum level: increase in *Firmicutes*; decrease in *Bacteriodetes* | n = 18, feces | 16S rRNA gene qPCR | Brandscheid et al., 2017 |
| ALS: Microbiome Alterations in Animal Models | | | | |
| Mice, SOD1$^{G93A}$ | decreased *Butyrivibrio Fibrisolvens* | n = 3, feces | 16S rRNA gene sequencing | Zhang et al., 2017b |
| Mice, SOD1$^{G93A}$ | decreased *Akkermansia muciniphila*, *Parabaceroides distasonis*, *Rikenellaceae*, *Prevotella*, *Lactobacillus murinus*, *Alistipes unclassified*, *Eggertella unclassified*; increased *Sutterella*, *Allobaculum*, *Desulfovibrionaceae*, *Coprococcus*, *Oscillospira*, *Bifidobacterium*, *Helicobacter hepaticus*, *Lactobacillus johnsonii*, and *Lactobacillus reuteri* | n = 6, feces | 16S rRNA gene sequencing, shotgun metagenomic sequencing | Blacher et al., 2019 |
| HD: Microbiota Alterations in Animal Models | | | | |
| Mice, R6/1 | sex difference in the bacterial signature of R6/1 mice; male: an increase in *Bacteroidales*, *Lactobacillales* and a decrease in *Clostridiales*; female: an increase in *Coriobacteriales*, *Erysipelotrichales*, *Bacteroidales*, *Burkholderiale* and a decrease in *Clostridiales* | n = 7–8, feces | 16S rRNA gene sequencing | Kong et al., 2020 |

Burns et al., 2017) have also appeared across studies. One metagenomic study reported that increased abundance of *A. municiphila*, *Prevotella copri*, and *Eubacterium* in L-DOPA-naive patients correlated with alterations in microbial metabolism of tryptophan and beta-glucuronide (Bedarf et al., 2017). Interestingly, longitudinal studies assessing the temporal dynamics of microbiota composition in PD patients indicate that microbial changes remain relatively stable over years following the onset of PD symptoms (Aho et al., 2019; Minato et al., 2017). However, as the samples were collected 5–9 years after diagnosis, it remains unclear whether there are PD-associated changes in the microbiota on a shorter temporal scale. Despite these advances, there remain many contradictory findings likely due to inter-study variability in patient cohort design, sample preparation and sequencing methodology, and data analysis relative to confounding variables. Rigorous, standardized methodology and study design are thus required in order to draw stronger conclusions regarding whether there exist common microbiome signatures for PD.

Changes in the gut microbiota are also seen in some genetic and pharmacological animal models with face and construct validity for PD. In one study, mice overexpressing human alpha-synuclein (ASO) exhibited reduced *Verrucomicrobiaceae*, which contrasts the increased levels of this taxon reported in some

aforementioned human studies (Gorecki et al., 2019). When raised as germ-free or treated with antibiotics in order to deplete the microbiota during adulthood, ASO mice displayed improved motor function compared with transgenic littermates harboring a complex microbiota (Sampson et al., 2016), which suggested that ASO-associated alterations in the microbiota contribute to motor symptoms of PD. Furthermore, transplantation of ASO mice with human microbiota from PD patients induced more severe motor dysfunction and reactive microglia relative to mice transplanted with human microbiota from healthy controls, suggesting that select human-derived microbes from PD patients can exacerbate motor deficits and neuroinflammation in mice (Sampson et al., 2016). In a rat model for PD, chronic treatment with the pesticide, rotenone, altered the small intestinal and colonic microbiota, with significant increases in *Actinobacteria* and *Proteobacteria*, but decreases in *Bacteroidetes* and *Cyanobacteria*. Alongside these observed shifts, their model was able to reproduce symptoms of gastroparesis before the development of nigrostriatal pathology (Johnson et al., 2018). Mice receiving similar exposures to rotenone show decreases in the relative abundance of *Bifidobacterium* and alterations in associated metabolic pathways, such as glycosaminoglycan degradation in the cecum (Perez-Pardo et al., 2018). Another neurotoxin model for PD involving low-dose, chronic administration of methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) also alters the microbiota, increasing *Prevotellaceae* and *Erysipelotrichaceae* whereas decreasing *Lachnospiraceae* (Lai et al., 2018). Another group reported similar changes, where MPTP-treated mice exhibited decreased *Firmicutes* and *Clostridiales* and increased *Proteobacteria, Turicibacterales, and Enterobacteriales* (Sun et al., 2018). Notably, transplantation of the MPTP-associated microbiota into conventional mice impaired motor function and reduced striatal dopamine levels relative to controls. Additionally, administration of a single bacterium, *Proteus mirabilis*, to mice was able to exacerbate MPTP-induced motor deficits, dopaminergic neuronal damage, and inflammation in brain structures relevant to PD, such as the substantia nigra (Choi et al., 2018). Altogether, these studies indicate that alterations in the gut microbiome can contribute to the presentation of PD-associated endophenotypes in animals.

### The Microbiome and Intestinal Inflammation in PD

Exactly how the microbiome may impact PD-related symptoms remains unclear. However, several lines of inquiry are now converging on the notion that microbial regulation of intestinal inflammation may be involved. Some PD patients experience GI disturbances, such as chronic gastritis and constipation, several years prior to the onset of motor symptoms, leading scientists to consider GI issues as early prodromal features of PD in subsets of patients (Cersosimo and Benarroch, 2012; Pfeiffer, 2011). In a large Danish cohort where individuals with IBD were tracked over 30 years, IBD patients exhibited a 22% increase in PD incidence compared with the unaffected controls (Villumsen et al., 2019). In this clinical investigation, patients with ulcerative colitis exhibited a significantly higher risk of PD compared with patients with Crohn's disease (Villumsen et al., 2019). In a nationwide Swedish cohort, the incidence of PD was 30% higher in the individuals with IBD compared with those without IBD (Weimers et al., 2019). Another study of an American cohort also observed elevated risk of PD in patients with IBD compared with non-IBD individuals (Peter et al., 2018). Accordingly, alpha-synuclein accumulation was observed in the submucosal layer of the intestine in PD patients with colitis (Grathwohl et al., 2019), providing a potential link between IBD and PD pathology. Notably, the IBD patients who received anti-TNF therapy had a 78% reduction in the incidence of PD, suggesting that suppressing peripheral inflammation may protect against PD (Peter et al., 2018). While the biological links between intestinal autoimmune disease and PD remain elusive, genetic studies of IBD and PD have converged on some shared genetic risk factors. For example, mutations in the kinase domain of leucine-rich repeat kinase 2 (LRRK2) has been identified in both the IBD patients and PD patients, with the N2081D mutation found in IBD and the G2019S mutation representing the most common monogenic cause of PD (Hui et al., 2018). Notably, both N2081D and G2019S mutations result in increased kinase activity of LRRK2, which is associated with impaired function of autophagy and lysosomal formation (Hui et al., 2018). Previous studies had found decreased *Firmicutes* and increased *Enterobacteriaceae* in the microbiome of IBD patients (Frank et al., 2007; Schirmer et al., 2019). These microbial features in IBD patients align with some microbial alterations reported across several studies of PD patients (Unger et al., 2016; Vogt et al., 2017). Other specific bacteria implicated in immune tolerance and IBD, such as *Bacteroides fragilis* and *B. vulgatus* have not been specifically investigated in PD. Overall, these studies reveal associations between PD and IBD, a condition for which the gut microbiome is strongly implicated (Johnson et al., 2019).

An increasing number of animal studies provide causal evidence that intestinal disruptions can trigger PD-related pathology. For example, intestinal injury by dextran sodium sulfate (DSS) triggered the aggregation of alpha-synuclein in enteric neurons and dopaminergic neurons of the substantia nigra, ultimately leading to neuronal degeneration in aged alpha-synuclein transgenic mice (Grathwohl et al., 2019). In ASO mice, lipopolysaccharide injection reduced intestinal barrier integrity and expedited the onset of motor symptoms (Gorecki et al., 2019). Intestinal infection of Pink1$^{-/-}$ mice with *Citrobacter rodentium* induced severe motor deficits and loss of striatal dopaminergic axons as compared with uninfected Pink1$^{-/-}$ mice and infected wild-type littermates (Matheoud et al., 2019), suggesting interactions between intestinal inflammation and genetic risk for PD. Moreover, in rotenone-treated mice, depletion of Toll-like receptor (TLR) 4 ameliorates rotenone-induced intestinal infiltration of CD3$^{+}$ T cells and intestinal dysfunction as well as rotenone-induced motor deficits and loss of dopaminergic neurons (Perez-Pardo et al., 2019). These studies provide proof of principle that intestinal inflammation can induce or exacerbate behavioral and pathological symptoms in mouse models of PD. Additional research is needed to examine potential relationships between the microbiome, intestinal disease, and PD and to further determine whether microbial regulation of peripheral immunity may underlie any of the existing links between the microbiome and PD in humans and animal models.

### The Microbiome and Amyloidogenesis in PD

Beyond potential immune-mediated pathways linking the microbiome to PD-related neurophysiological and behavioral symptoms, several studies suggest that select microbes and microbial products could stimulate alpha-synuclein aggregation, leading

to the presence of neuronal inclusions comprised misfolded alpha-synuclein protein. Early clinical characterization of amyloid pathology in post-mortem brains of sporadic PD patients by Braak and colleagues uncovered particular peripheral organs susceptible to aggregates at different stages in the development of PD (Braak and Braak, 2000; Braak et al., 2002). Histological examination of post-mortem intestinal tissue from non-symptomatic elderly patients uncovered the early presence of alpha-synuclein inclusions in the intestine, in the absence of Parkinsonian motor symptoms (Bloch et al., 2006; Braak et al., 2006; Shannon et al., 2012). Subsequent studies in animal models of PD have demonstrated the presence of intestinal alpha-synuclein during disease (Drolet et al., 2009; Kelly et al., 2014; Wang et al., 2012) that coincides with alterations in gastric motility and permeability (Kelly et al., 2014). Further, intestinal injection of alpha-synuclein pre-formed fibrils (PFFs) (Pan-Montojo et al., 2010) or alpha-synuclein from human brain isolates (Holmqvist et al., 2014) results in the appearance of alpha-synuclein inclusions in the CNS. Duodenal inoculation of alpha-synuclein PFFs to aged ASO mice leads to loss of striatal dopamine in the mid brain (Challis et al., 2020). Together, these data implicate the ability for peripheral alpha-synuclein aggregation to propagate to the CNS and induce PD-related pathology. Alpha-synuclein overexpression is associated with alterations in vagal neuronal gene expression (Noorian et al., 2012), the dorsal motor vagal nucleus is one of the first brain regions in which alpha-synuclein inclusions appear, and there is a decreased risk of developing PD in vagotomized patients (Svensson et al., 2015). The vagus nerve possesses dense varicose projections to the intestinal villi of the small intestine, situating its terminals immediately adjacent to the intestinal lumen whereby the members of the host microbiota reside. One proposed mode of transport is via trans-neuronal propagation of alpha-synuclein along the vagus nerve. Indeed, alpha-synuclein released from enteric neurons or alpha-synuclein PFFs injected into the GI tract are unable to reach the brain following vagotomy (Kim et al., 2019; Pan-Montojo et al., 2012; Uemura et al., 2018), suggesting a role for the vagus nerve in propagating alpha-synuclein spread from the periphery to the brain in a prion-like manner.

CNS alpha-synuclein burden is decreased in germ-free animals lacking a microbiome compared with specific pathogen-free mice harboring a complex microbiome (Sampson et al., 2016). One possible mechanism by which the microbiota may contribute to the development of PD alpha-synuclein pathology is through pathogenic cross-seeding, whereby one amyloidogenic protein causes another to adopt a beta-sheet structure, between alpha-synuclein and bacterial amyloids, such as curli in *Escherichia coli* in the gut. Curli are highly conserved surface organelles that mediate binding of bacteria to soluble matrix proteins (Olsén et al., 1989). Additionally, the key element, CsgA, contains amyloidogenic peptide repeat motifs shared by human prions (Miraglia et al., 2018). Two genes in the curli operon, *CsgE* and *CsgC,* have been shown to modulate alpha-synuclein aggregation *in vitro* (Chorell et al., 2015), and oral administration of curli-producing bacteria, but not curli-deficient mutant strains, to wild-type rats results in intestinal accumulation of alpha-synuclein (Chen et al., 2016). A recent study reproduced these findings in mice overexpressing alpha-synuclein and additionally demonstrated that introduction of curli-producing bacteria to a healthy, complex human microbiota exacerbated alpha-synuclein pathology and behavioral abnormalities in a CsgA-dependent manner (Sampson et al., 2020). These findings correlated with morphological alterations in midbrain microglia, suggesting an inflammatory state. However, curli are also considered microbial-associated molecular patterns and are recognized by TLRs to induce activation of the innate immune system (Rapsinski et al., 2015). Due to the role of inflammation in facilitating alpha-synuclein aggregation, more research is needed to determine whether bacterial amyloids from the gut microbiome may act to either directly or indirectly to increase peripheral alpha-synuclein aggregation. This amyloid hypothesis has also recently been extended to non-proteinaceous metabolites that are able to form fibrillar amyloid-like assemblies that seed alpha-synuclein aggregation *in vitro* (Tavassoly et al., 2018). Due to the vast array of metabolic biotransformations carried out by the microbiota, additional efforts are warranted to evaluate effects of microbially modulated metabolites on amyloidogenesis.

Most recently, alpha-synuclein was found to be cleaved and enriched particularly in the appendices of healthy individuals (Gray et al., 2014; Killinger et al., 2018), suggesting potential biological relevance to PD pathology. This may be relevant as the appendix has been proposed to serve as a reservoir for the microbiota (Lautenschlager et al., 2017). However, whether appendectomy associates with decreased risk of PD remains controversial. Several epidemiological studies show no or limited correlation between appendectomy and the incidence of PD (Palacios et al., 2018; Svensson et al., 2016; Yilmaz et al., 2017), whereas one very large study indicates that removal of the appendix is associated with lower risk of PD, particularly for people living in rural areas (Killinger et al., 2018).

Notably, subsets of PD patients do not exhibit PNS-CNS spread of alpha-synuclein, with some completely lacking alpha-synuclein pathology in the dorsal motor nucleus of the vagus. This has led to the development of a hypothesis for a subtype of PD that is "CNS-first" (Borghammer and Van Den Berge, 2019). In these cases, it is possible that efferent signaling from the CNS during pathology may drive alterations in the microbiota, as both stress and immune signaling have been demonstrated to alter its composition (Bonaz et al., 2018). However, more evidence is needed to clearly determine the effects of efferent-induced alterations in the microbiota on PD outcomes. Similarly, additional understanding is needed for how comorbidities during the prodrome may alter the microbiota to impact the development of PD. Rapid eye movement (REM) sleep behavioral disorder, anxiety, and depression are of particular interest given their prevalence during the PD prodrome (Borghammer and Van Den Berge, 2019) and strong evidence linking both circadian issues and stress to alterations in the microbiome (Gubert et al., 2020; Heintz-Buschart et al., 2018; Voigt et al., 2014). Additionally, a recent study demonstrated alterations in the microbiota compositions of multiple system atrophy (MSA) patients (Engen et al., 2017), highlighting the importance of considering the role of the microbiome in additional synuclein-based NDs, such as MSA and Lewy body dementia (LBD).

## The Gut Microbiome and AD

In addition to PD, the gut microbiome is increasingly implicated in the manifestation of AD-related symptoms. AD is a complex

neurodegenerative disorder that is characterized by the progressive loss of cognition, memory, and motor ability. A hallmark of AD is the accumulation of extracellular Aβ plaques and intracellular hyperphosphorylated tau tangles in the brain while other neuropathological signs include neuronal loss and brain atrophy. The lack of effective single-target therapeutic treatments and failures of clinical trials, particularly those targeting the amyloid pathways, are evidence that the current understanding of the pathogenesis of AD must be extended (Bullain and Doody, 2020). A few lines of evidence motivate interest in the microbiome and AD. From fundamental behavioral observations in laboratory animals, several studies reveal that manipulations of the microbiome, like germ-free rearing, antibiotic treatment, and severe dietary alterations, alter learning and memory-related behavior (Abbott et al., 2019; Fröhlich et al., 2016; Luczynski et al., 2016). In addition, the microbiome is emerging as an important regulator of the development and function of the neuroimmune system (Fung et al., 2017), which is implicated in AD among several other neurological disorders (Giau et al., 2018; Kowalski and Mulak, 2019). As yet, however, there is only some evidence of microbiome alterations in human AD. In one study, decreases in the *Negativicutes* and *Lachnospirceae* and an increase in *Ruminococcaceae* were seen in AD participants compared with healthy controls (Zhuang et al., 2018). In another study, analysis of fecal samples obtained from AD participants reported alterations in the gut microbiota with decreased relative abundance of *Ruminococcaceae*, *Turicibacteraceae*, *Peptostreptococcaceae*, *Clostridiaceae*, and *Mogibacteriaceae* and increased relative abundance of *Bacteroidaceae* and *Rikenellaceae* compared with the healthy control group (Vogt et al., 2017). Notably, increases in bacterial abundance were correlated with alterations in cerebrospinal fluid (CSF) biomarkers suggestive of increased amyloid burden in the brain. In particular, elevated abundance of *Bacteroides* and *Blautia* corresponded to a decrease in CSF $A\beta_{42}/A\beta_{40}$ and increase in CSF p-tau and p-tau/$A\beta_{42}$ (Vogt et al., 2017). Microbiome profiling of mouse models for AD has also suggested alterations in the gut microbiota (Brandscheid et al., 2017; Zhang et al., 2017a). For the 5xFAD mouse model, which expresses human transgenes for amyloid precursor protein (APP) and presenilin-1 (PS1) containing three familiar AD mutations in APP and two mutation in PS1, *Firmicutes* increased from 6 weeks of age to 18 weeks while *Bacteroidetes* decreased (Brandscheid et al., 2017). In the APP/PS1 mouse model, which expresses the human transgenes containing the Swedish mutation in APP and lacking exon 9 in PS1, *Proteobacteria* doubled in relative abundance by 6 months of age, and *Verrucomicrobia* increased by 6-fold by 12 months of age (Zhang et al., 2017a). Increases in *Firmicutes* and decreases in *Bacteroidetes* were also reported in the APP/PS1 line, consistent with findings from 5xFAD mice (Zhang et al., 2017a). However, a longitudinal study of the microbiota in the P301L tau mouse model of AD reported the opposite relationship, wherein the abundance in *Bacteriodetes* increased starting at 3 months of age while *Firmicutes* decreased (Sun et al., 2019a). By 10 months of age, *Candidatus_saccharimonas*, *Alistipes*, *Rikenella*, *Odoribacter*, *Blautia*, *Ruminococcaceae*, *Eubacterium_xylanophilum*, *Paraprevotella*, *Butyricicoccus*, and *Parvibacter* were among the bacteria that were negatively associated with tau pathology in the brain. In contrast, *Bacteroides*, *Parabacteroides*, *Escherichia-Shigella, and Clostridium_innocuum* were positively correlated with tau pathology. Across these studies examining the microbiota in human AD and AD mouse models, there is as yet no clear microbial signature for AD.

### The Microbiome and Neuroinflammation in AD

Despite limited evidence for a defined shift in the composition of the microbiota in human AD and AD mouse models, a growing number of studies report that manipulating the gut microbiota impacts the severity of Aβ pathology and cognitive impairment in genetic models for AD. Several of these studies suggest that microbial regulation of neuroimmunity may contribute to AD symptoms. For example, rearing APP/PS1 mice as germ free resulted not only in reduction of Aβ aggregates in the brain but also a 40% decrease in Iba-1-positive microglia leading to an overall decrease in neuroinflammation compared with conventionalized mice (Harach et al., 2017). The pro-inflammatory cytokine IL-1β was also significantly decreased by 36% in germ-free APP/PS1 mice compared with conventionally colonized APP/PS1 controls. Another study using antibiotics to acutely deplete the microbiota of APP/PS1 mice saw similar results. One week of antibiotic treatment led to a decrease in Aβ load and down regulation of pro-inflammatory cytokine IL-6 compared with the vehicle-treated group (Minter et al., 2017). These alterations corresponded with reductions in numbers of plaque-associated microglia and astrocytes, suggesting that depletion of the microbiome reduced neuroinflammation and gliosis in the brain. In a separate study, treatment of APP/PS1 mice with an antibiotic cocktail led to a reduction in Aβ plaques and astrogliosis in male mice only (Dodiya et al., 2019). Tg2576 mice, which express a variant of human APP, exhibited an aging-related increase in intestinal *Bacteroides fragilis* relative to wild-type controls. Notably, administering *B. fragilis* to APP/PS1 female mice promoted Aβ plaque deposition in the cortex, which was rescued by calorie restriction (Cox et al., 2019). While exact mechanisms are unknown, the findings appear to counter existing literature reporting that *B. fragilis* elicits immunosuppressive effects that may ameliorate symptoms of other neurological diseases. Together these two studies suggest that microbiome alterations may modulate AD pathology in a sex-dependent manner. Overall, these studies provide some evidence that large alterations in the gut microbiota can influence the progression and manifestation of AD-related features in genetically susceptible animals.

### Infection and AD

While many studies on the microbiota and AD focus on symbiotic microorganisms indigenous to the gut, a growing body of literature links AD to bacterial and viral pathogens. RNA sequencing of brain tissue from deceased patients with a variety of NDs revealed the presence of infectious agents in the CNS (Bennett et al., 2019). For AD in particular, reads aligning to *Toxoplasmosis gondii*, *Trichinella sp. T6*, *Babesia microti*, *Borrella burgdorferi*, *Porphyromonas gingivalis*, and *Treponema denticola* were observed in samples of the frontal cortex from postmortem AD brains. Similarly, another transcriptomics study mapped HSV6 and 7 to multiple brain regions in AD patients (Readhead et al., 2018), which aligns with multiple case and epidemiological studies on HSV and AD. This could be due to direct interactions between neurotropic viruses and amyloids,

as HSV1 was capable of catalyzing Aβ plaque formation by binding Aβ-peptides using the protein corona (Ezzat et al., 2019). In addition, data from the National Health and Nutrition Examination Surveys database revealed a positive association between AD mortality and *H. pylori* (Beydoun et al., 2018). One study showed that *H. pylori* is able to induce tau hyperphosphorylation by activating the tau kinase glycogen synthase kinase-3β (Wang et al., 2015). Additional studies reported that Aβ precursor was upregulated in tissues from AD patients with periodontitis relative to unaffected AD controls (Nezu et al., 2017). Consistent with this, across several mouse models for AD, infection with *Porphyromonas gingivalis* exacerbated AD pathology resulting in an increase in neuroinflammation, neurodegeneration, and amyloidosis (Dominy et al., 2019; Ilievski et al., 2018; Ishida et al., 2017). This evidence linking bacterial and viral infection to AD may be related to some studies proposing that native function of Aβ is an antimicrobial peptide involved innate immune defense (Eimer et al., 2018; Kumar et al., 2016; Moir et al., 2018). Whether the gut microbiome may play a role in regulating host responses to AD-associated infections remains poorly understood.

### Emerging Studies of the Gut Microbiome in Other NDs

ALS is a fatal neurological disease accompanied with the progressive degeneration of motor neurons in the brain and spinal cord. A few studies have compared the gut microbial composition in patients with ALS and healthy controls and highlighted certain species. In an investigation of 37 patients with ALS and 29 healthy familial controls, metagenomic sequencing of gut microbiome revealed a significantly distinct microbial composition compared with healthy controls, among which the abundance of *Anaerostipes hadrus*, *Bacteroidales bacterium*, *Bifidobacterium pseudocatenulatum* are marginally increased whereas *Clostridium leptum* and *Escherichia coli* are marginally decreased in ALS patients compared with non-affected controls (Blacher et al., 2019). In addition, reduced levels of nicotinamide (NAM), correlated with decreased abundance of *Bifidobacterium pseudocatenulatum*, was observed in the serum and CSF of patients with ALS by metabolomic analysis (Blacher et al., 2019). Another study of 6 ALS patients highlights the reduced ratio of *Firmicutes*/*Bacteroidetes* in fecal microbiota of ALS patients, accompanied with increased abundance of *Dorea* and decreased abundance of *Oscillibacter*, *Anaerostipes*, and *Lachnospiraceae* (Fang et al., 2016). By contrast, a study involving 25 ALS patients did not find significant differences in the gut microbial composition between ALS patients and healthy controls, even though an increase of the overall number of microbial species was observed in ALS patients compared with healthy control (Brenner et al., 2018). The contradictory results from different studies may be due at least in part to the limited power of the studies, which highlights the need for systematic investigation of the microbiota in large cohorts of ALS patients and controls.

In addition to independent studies reporting correlations between microbiota composition and ALS, one study reported a role for the microbiota in protecting against ALS symptoms in the Sod1 transgenic mouse model. Depletion of gut microbiota by broad-spectrum antibiotics exacerbated the motor deficits in Sod1 mice, as evaluated in the rotarod test and wire-hanging test (Blacher et al., 2019). In line with this, Sod1 mice reared as germ free exhibited higher mortality rates than those raised as conventionally colonized. This contrasts findings from mouse models of PD and AD, where absence of the microbiota abrogates pathology and behavioral abnormalities. 16S rRNA sequencing and shotgun metagenomic sequencing revealed that Sod1 mice exhibited reduced abundance of *A. muciniphila* compared with wild-type littermates. Colonizing antibiotic-treated Sod1 mice with *A. muciniphila* not only extended lifespan but also ameliorated the brain atrophy and motor deficits of the mice. Consistent with the association of reduced nicotinamide with ALS incidence in a companion clinical study, increased levels of nicotinamide were observed in the sera of Sod1 mice administered *A. muciniphila*, and administering nicotinamide to Sod1 mice recapitulated the protective effect of *A. muciniphila* (Blacher et al., 2019). Overall, this study suggests that gut microbes can regulate motor and neurophysiological endophenotypes of ALS by altering the systemic bioavailability of select metabolites. In another study of the C9orf72 mouse model of ALS and FTD, researchers similarly identified a role for microbiome alterations in regulating the severity of motor impairments and lifespan (Burberry et al., 2020). The microbiome alterations correlated with increases in pro-inflammatory cytokines and activated microglia in the spinal cord, suggesting a neuroimmune-mediated path. Altogether, the findings contribute to growing interest in uncovering functions for previously uncharacterized metabolites that are regulated by the gut microbiome.

Similar links between the microbiota and HD are just beginning to be explored. HD is a progressive neurological disorder caused by a trinucleotide expansion in the huntingtin gene, which causes death of neurons in various brain regions, leading to chorea and abnormal alterations in behavior, emotion, and cognition. Severe gliosis was observed in the post-mortem brains of individuals with HD (Singhrao et al., 1999). Given that the microbiota can affect the neuronal activity and glial function in the brain, studying the involvement of microbiota in HD can improve our understanding of whether alterations in environment factors can modulate risk for HD. Although no studies to date have sequenced the human gut microbiome from HD patients, one study reported the presence of various fungal and bacterial species in the striatum of HD patients, including *Candida*, *Pseudomonas*, *Acinetobacter*, and *Burkholderia* (Alonso et al., 2019). While the study suggests that microbial infection may be associated with HD, the technical contamination of brain tissue needs to be excluded. Another study compared the gut microbiota in the R6/1 mouse model of HD with wild-type littermates and reported sexual dimorphism in the bacterial signature of R6/1 mice. An increase in *Bacteroidales* and *Lactobacillales* and a decrease in *Clostridiales* were observed in male R6/1 mice, whereas an increase in *Coriobacteriales*, *Erysipelotrichales*, *Bacteroidales*, *Burkholderiale*, and a decrease in *Clostridiales* were observed in females. In addition, male R6/1 mice show higher microbial diversity compared with both female R6/1 mice and wild-type littermates (Kong et al., 2020). Whether these microbiota alterations contribute to symptoms of the R6/1 model remain unclear. However, in the bacterial artificial chromosome (BACHD) model, mice reared as germ free exhibited reduced levels of myelin-related proteins and decreased numbers of mature oligodendrocytes in the prefrontal cortex compared with mice reared conventionally colonized (Radulescu et al., 2019). This generally aligns with findings from the Sod1 model, where

absence of the microbiota exacerbates endophenotypes of disease.

There are as yet only a few studies of the microbiome in other NDs such as MSA. A study analyzing 15 fecal samples from Chinese MSA patients found increased *Roseburia hominis*, *Akkermansia muciniphila*, *Alistipes onderdonkii*, *Streptococcus parasanguinis*, and *Staphylococcus xylosus* and decreased *Bacteroides coprocola*, *Megamonas funiformis*, *Bifidobacterium pseudocatenulatum*, *Clostridium nexile*, *B. plebeius*, and *Granulicatella adiacens* relative to healthy controls (Wan et al., 2019). Another study sequencing 17 fecal samples of Malaysian MSA patients identified increased abundance of *Bacteroides* and reduced abundance of *Paraprevotella* (Tan et al., 2018). For LBD, there is as yet no study examining the microbiome. Additional research is warranted to replicate and extend interrogations into the microbiota in ALS, HD MSA, and LBD, among other NDs.

## Comparing Microbiomes Across NDs

Some NDs share common risk factors and symptomatology, raising the question of whether there are any shared features of the microbiome across different NDs. Decreases in *Lachnospiraceae* have been commonly reported in aging, PD, and ALS as well as Crohn's disease (Gevers et al., 2014). *Lachnospiraceae* suppresses colonic inflammation in experimental colitis models and there are negative correlations between *Lachnospiraceae* and pro-inflammatory intestinal microbes. Moreover, *Lachnospiraceae* are a major producer of short-chain fatty acids (SCFAs), which elicit widespread effects on host metabolism, immunity, and neurophysiology. SCFAs have been shown to activate GPR109 on myeloid cells, which in turn induces regulatory T cell polarization to attenuate intestinal inflammation (Singh et al., 2014). Such interactions between select microbiota alterations, microbiome-dependent metabolites, and immunity could be relevant to links between infection, inflammation, and NDs, such as AD and PD. Additionally, the SCFA propionate was reported to reduce oxidative stress via GPR41 in cells of the blood brain barrier (Hoyles et al., 2018), further warranting future investigation into the impact of SCFAs on NDs.

Another notable bacterial species associated with NDs is *Akkermansia*, which exhibits increased abundance in both PD and AD but decreased abundance in aging and the ALS mouse model. Administration of *Akkermansia* ameliorates motor impairment in the Sod1 ALS mouse model via select microbial metabolites like nicotinamide (Blacher et al., 2019). A previous study has found that administration *Akkermansia* with *Parabacteroides* to epilepsy mouse models elevated levels of GABA relative to glutamate levels in the hippocampus (Olson et al., 2018). While the signaling pathways and the downstream effects of metabolites deriving from *Akkermansia* are still elusive, these observations suggest that there may be distinctive effects of *Akkermansia* in different NDs. Nevertheless, the reported increased abundance of *Akkermansia* in PD/AD could well be a result or confounding effect, rather than a modifier of PD/AD pathogenesis. Therefore, further studies are needed to discern any causal links between microbial alterations and the onset or development of NDs.

## Microbiome-Related Interventions for NDs

Despite our limited mechanistic understanding of how the microbiota may predispose to symptoms of NDs, efforts to manipulate the microbiota through transplantation and probiotic treatment highlight the potential for microbial amelioration of ND-related pathology and behavior in laboratory animals. Transplantation of conventional microbiota into MPTP mice ameliorated motor deficits, increased dopaminergic neurons in the substantial nigra, and reduced numbers of activated microglia and astrocytes (Sun et al., 2018). Similarly, AD mouse models exhibited improved learning and memory behavior, reductions in brain Aβ and tau aggregation, decreased neuroinflammation, and attenuated synaptic dysfunction after microbiota transplantation (Kim et al., 2020; Sun et al., 2019b). In the ADLP$^{APT}$ mouse line, which expresses the five mutations in APP and PS1 found in the 5xFAD model in addition to the P301L mutation in tau, daily fecal microbiota transplant (FMT) from wild-type mice into ADLP$^{APT}$ mice over 4 months resulted in improved spatial short-term memory and long-term memory (Kim et al., 2020). In addition, measuring the total plaque area of the frontal cortex and hippocampus revealed a decrease in Aβ plaque burden in treated mice compared with control ADLP mice. Furthermore, FMT decreased tau aggregates, reduced the number of activated microglia and attenuated gliosis. In the APP/PS1 mouse model, daily FMTs over 4 weeks also resulted in improved cognition as indicated by better performance in the Morris water maze and object recognition test (Sun et al., 2019b). Aβ deposition and hyperphosphorylated tau in the hippocampus were reduced in the treated mice compared with controls. Targeting select bacteria for treatment has also revealed beneficial effects in ND animal models. Administration of probiotic bacterium *Bacillus subtilis* to transgenic *C. elegans* expressing human alpha-synuclein increased clearance of alpha-synuclein, ultimately leading to a reversal of aggregation (Goya et al., 2020). Another series of experiments examining the effects probiotic treatment used the 3xTg mouse model, which express single mutation in each of the human transgenes for APP, PS1, and tau. Chronic treatment of 3xTg mice with a cocktail of *Lactobacillus* and *Bifidobacteria* (SLAB51) promoted working memory, increased cortical thickness, and decreased amyloid burden in the brain (Bonfili et al., 2017). Furthermore, bacterial treatment activated sirtuin-1-dependent pathways to promote antioxidative responses (Bonfili et al., 2018), improved glucose reuptake in the brain, and reduced levels of hyperphosphorylated tau (Bonfili et al., 2020). Similarly, APP/PS1 mice treated with *Clostridium butyricum* had reduced microglial activation and decreased signs of neurodegeneration (Sun et al., 2020). These studies provide proof of concept for microbiome interventions in animal models. However, there is as yet no evidence that such approaches would be safe or effective for clinical NDs.

Other microbiome-based interventions that aim to enhance the efficacy of existing treatments for NDs may be more tractable for clinical translation. With the finding that select bacteria metabolize L-dopa, a common treatment for PD (Maini Rekdal et al., 2019; van de Steeg et al., 2018; van Kessel et al., 2019), there is increased interest in whether inhibiting or reducing microbial metabolism of L-dopa could promote drug efficacy in PD. Similar approaches of modulating microbial metabolism of existing medications may be relevant more broadly to neurology, as select microbes from the gut microbiome have been reported to interact with a variety of common medications for neurological diseases (Cryan et al., 2020; Maini Rekdal et al., 2019). In

**Table 3. Effects of Microbiome Manipulations in Animal Models for NDs**

| Subject | Perturbation | Sample | Test | Result | Reference |
|---|---|---|---|---|---|
| Parkinson's Disease: Microbial Effects on Pathology and Behavior in Animal Models | | | | | |
| Pink1$^{-/-}$ mice | oral administration of *Citrobacter rodentium* | n = 5–72 males and females | grip strength test pole test; open field test; flow cytometry for brain infiltrated immune subsets; histology for dopaminergic neurons in the striatum | impaired motor ability shown in *Citrobacter rodentium* treated Pink1$^{-/-}$ mice; decreased density of dopaminergic axonal varicosities and enhanced CD8$^{+}$ T cells in the brain of *Citrobacter rodentium* treated Pink1$^{-/-}$ mice | Matheoud et al., 2019 |
| Thy1-αSyn mice | administration of microbiota from PD patients | n = 3–6 males | beam traversal test; pole descent test; nasal adhesive removal; test hindlimb clasping reflexes | PD-derived gut microbiota promotes motor impairments | Sampson et al., 2016 |
| Thy1-αSyn mice | GF versus SPF | n = 4–6 males | beam traversal test; pole descent test; nasal adhesive removal test; hindlimb clasping reflex; western blot and immunofluorescence staining for α-Syn aggregation; representative 3D reconstructions of Iba1-stained microglia in the caudoputamen | reduced locomotor deficits, α-syn accumulation, and decreased activation of microglia in GF Thy1-αSyn mice | Sampson et al., 2016 |
| Thy1-αSyn mice | monocolonization of curli-sufficient versus curli-deficient *E. coli* | n =7–9 males | ELISA and immunofluorescence staining for α-Syn aggregation in the brain and gut; immunofluorescence staining for TH neurons; microglia activation in the brain; beam traversal; pole descent test; nasal adhesive removal test; hindlimb clasping reflex; Wire hang test | increased locomotor deficits, α-syn accumulation, and enhanced activation of microglia in Thy1-αSyn mice colonized with curli-sufficient Ecoli | Sampson et al., 2020 |
| Aged Fischer 344 rats | oral administration of curli-sufficient *E. coli* versus curli-deficient *E. coli* | n = 9–13 males | histology for astrocytes; α-syn aggregation; serum cytokine profiling | increased a-syn accumulation and enhanced activation of astrocytes in rats with curli-sufficient E. coli | Chen et al., 2016 |
| *C. elegans* expressing human α-syn | curli-sufficient versus curli-deficient *E. coli* feeding | n = 15; sex unknown | immunofluorescence staining for α-syn aggregation | increased α-syn accumulation in *C. elegans* feed with curli-sufficient *E. coli* | Chen et al., 2016 |
| Thy1-αSyn mice | drinking water containing 10 μg/ml LPS; 12 consecutive days treatment | n is unknown; males and females | hindlimb clasping reflex | mildly exacerbated motor impairments in LPS-treated Thy1-αSyn mice | Gorecki et al., 2019 |

(*Continued on next page*)

**Table 3. *Continued***

| Subject | Perturbation | Sample | Test | Result | Reference |
|---|---|---|---|---|---|
| MPTP mouse model<br>Rotenone mouse model | probiotic cocktail containing *Lactobacillus rhamnosus GG*, *Bifidobacterium animalis lactis* and *Lactobacillus acidophilus*; 4 weeks treatment before animal model establishment | n = 3–4 males | cylinder test; beam traversal test; challenge beam test; stride length test; histology for dopaminergic neurons and glial activation in the striatum and substantial nigra | mitigated behavioral impairments, ameliorated dopaminergic neuronal loss, and gliosis in probiotics-treated MPTP and rotenone mouse model | Srivastav et al., 2019 |
| 6-OHDA rat model | antibiotic (neomycin, 2 mg/mL; vancomycin 0.2 mg/mL; bacitracin, 0.5 mg/mL; pimaricin 1.2 μg/ mL); treatment for 14 days before animal model establishment | n = 4–12 males | cylinder test; forepaw adjusted steps test; amphetamine-induced rotation; histology for TH neuron loss in striatum and substantial nigra Q-PCR for expression of pro-inflammatory cytokines in striatum | attenuated motor deficits, decreased dopaminergic neuron loss, and lower expression of pro-inflammatory cytokines in antibiotic-treated 6-OHDA rat model | Koutzoumis et al., 2020 |
| MPTP mouse model | fecal microbiota of transplantation after animal model establishment | n = 15 males | pole test; traction test; histology for dopaminergic neuron loss and glial activation in the substantial nigra Western blot for striatal TH expression | attenuated motor deficits, decreased dopaminergic neuron loss, and glial activation in the MPTP mouse model transplanted fecal microbiota from wild-type littermates | Sun et al., 2018 |
| *C. elegans* model of Synucleinopathy | *Bacillus subtilis* probiotic strain PXN21 feeding | n = 25; sex unknown | histology for αSyn aggregation | reduced αSyn aggregation in the *C. elegans* feed with *Bacillus subtilis* | Goya et al., 2020 |
| MPTP mouse model | oral administration of *Proteus mirabilis* | n = 9–12 males | rotarod test; open field test; histology for dopaminergic neuron loss and glial activation in the substantial nigra histology for TH-positive axons loss in the striatum | exacerbated motor deficits, loss of dopaminergic neurons, and glial activation in MPTP mouse model received *Proteus mirabilis* | Choi et al., 2018 |
| MPTP mouse model | antibiotic (ampicillin 1 g/L, neomycin sulfate 1 g/L, metronidazole 1 g/L); treatment before animal model establishment | n = 10 males | histology for dopaminergic neuron loss in the substantial nigra | attenuated dopaminergic neuron loss in the antibiotic-treated MPTP mouse model | Pu et al., 2019 |
| AD: Microbial Effects on Pathology and Behavior in Mice | | | | | |
| APP/PS1 mice | GF versus CONV | n = 5–8; males and females | histopathology for Aβ in brain; immunostaining for microglia; western blot for Aβ; ELISA for Aβ38, Aβ40, Aβ42, and cytokines | for GF-APP/PS1 mice: lower Aβ deposition in cortex and hippocampus, Aβ levels in western blot and Aβ42 levels in ELISA reduction in Iba-1-positive microglial leading to reduction in cortical neuroinflammation; for CONV-APP/PS1 mice: increase in IL-1β, INF-χ, IL-2, IL-5 | Harach et al., 2017 |

(*Continued on next page*)

**Table 3.** ***Continued***

| Subject | Perturbation | Sample | Test | Result | Reference |
|---|---|---|---|---|---|
| APP/PS1 mice | antibiotics (gentamycin 1 mg/mL, vancomycin 0.5 mg/mL, metronidazole 2 mg/mL, neomycin 0.5 mg/mL, ampicillin 1 mg/mL, kanamycin 3 mg/mL, colistin 6,000 U/mL, cefaperazone 1mg/mL) for 1 week | n = 5–14 males | flow cytometry for immune cells; immunohistochemistry for Aβ, Iba-1, GFAP; ELISA for Aβ cytokine/chemokine array | for ABX-treated mice: reduced Aβ deposition in cortex and hippocampus; reduction in Aβ plaque localized microglial and astrocytes leading to reduction in neuroinflammation; elevation in $FOXP3^+$ $T_{regs}$ upregulation of CCL11, IL-1β, IL-2, IL-3, SCF and downregulation of IL-6 | Minter et al., 2017 |
| ADLP mice | fecal microbiota transplant daily for 4 months | n = 14–16; sex unknown | behavioral tests: Y-maze, contextual fear conditioning, open field; ELISA for Aβ; Immunohistochemistry for Aβ, tau, GFAP | for FMT-treated mice: better performance on behavioral tests, reduction in Aβ deposition in frontal cortex and hippocampus, reduction in cortex Aβ40 levels, reduction in tau aggregates in hippocampus, reduction in activated microglia and astrocytes in frontal cortex | Kim et al., 2020 |
| APP/PS1 mice | fecal microbiota transplant daily for 4 weeks | n = 4–6 males | behavioral tests: Morris Water Maze, object recognition test, ELISA for Aβ40 and Aβ42, Western blot for tau, immunostaining for PSD-95, CD11b and COX-2, NMR for SCFAs | for FMT-treated mice: better performance on behavioral tests compared with controls, reduction in Aβ deposition in cortex and hippocampus and in Aβ40/Aβ42 levels, reduction in tau phosphorylation, increase in PSD-95 staining, reduction in COX-2 and CD11b levels increase in butyrate levels | Sun et al., 2019b |
| APP/PS1 mice | oral administration of *Clostridium butyricum* for 4 weeks | n = 20; sex unknown | behavioral tests: Morris Water Maze, object recognition test, histology for Aβ and CD11b, ELISA for Aβ42 butyrate assay | treated mice performed better on behavioral tests compared with controls decrease of Aβ levels in the brain for treated mice, reduction of activated microglial in treated mice; reduction of IL-1β and TNF-α in treated mice; higher levels of butyrate in treated mice | Sun et al., 2020 |
| 3×Tg mice | SLAB51 probiotic treatment | n = 48–64 males | behavioral test: open field, novel object recognition, and elevated maze; GC for SCFAs; ELISA for cytokines; Histology and immunostaining for Aβ; western blot for tau | for SLAB51 treated mice: better performance on behavioral tests; reduction in Aβ deposition in hippocampus; reduction in tau phosphorylation; reduction in oxidative stress | Bonfili et al., 2017, 2018, 2020 |

(*Continued on next page*)

**Table 3.** *Continued*

| Subject | Perturbation | Sample | Test | Result | Reference |
|---|---|---|---|---|---|
| APPPS1-21 mice | antibiotics (kanamycin 4 mg/mL, gentamicin 0.35 mg/mL, colistin 8,500 U/mL, metronidazole 2.15 mg/mL, vancomycin 0.45 mg/mL) from P14-P21 | n = 6–10; males and females | immunofluorescence for Ab and microglia activation | antibiotics treatment reduces Aβ pathology and activation of microglia only in the male brain but not female | Dodiya et al., 2019 |
| ALS: Microbial Effects on Pathology and Behavior in Mice | | | | | |
| $SOD1^{G93A}$ mice | 2% sodium butyrate in water | n = 5–10; sex unknown | ALS progression (body weight loss, lifespan); intestinal tight junction (western blot and immunofluorescence staining for ZO-1) | delayed ALS progression; increased the intestinal tight junction | Zhang et al., 2017b |
| $SOD1^{G93A}$ mice | repeated oral administration of *Akkermansia muciniphila* into antibiotic; pre-treated Sod1 mice at 6-day intervals for a total of 15 treatments | n = 5–61; males and females | lifespan analysis; rotarod locomotor test; hanging-wire grip test; histological staining for spinal cord T2 relaxation time | extended lifespan; ameliorated locomotor deficits; increased number of motor neurons in spinal cord; reduced brain atrophy | Blacher et al., 2019 |
| HD: Microbial Effects on Pathology and Behavior in Mice | | | | | |
| BACHD mice | GF versus SPF | n = 4–12; males and females | transmission electron microscopy for myelination; qPCR and western blot for myelin-based protein | reduced levels of myelin-related proteins and decreased numbers of mature oligodendrocytes in the prefrontal cortex of BACHD mice reared in GF condition | Radulescu et al., 2019 |

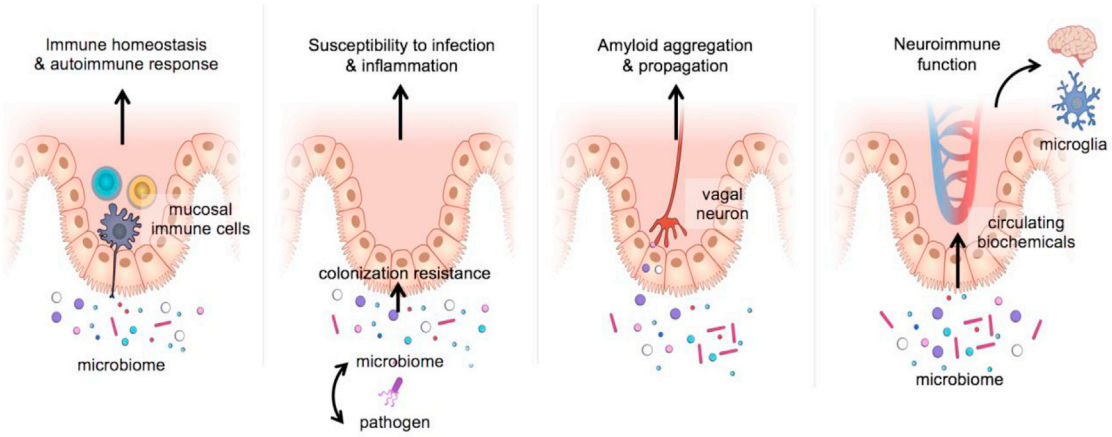


**Figure 1. Proposed Pathways for Microbial Modulation of Neurodegenerative Diseases**
The gut microbiome is increasingly implicated in modifying risk for NDs. While precise mechanisms are unknown, a few pathways have been proposed. The gut microbiome is critical for conditioning peripheral immune homeostasis and autoimmune responses, which could influence the manifestation of NDs (far left). In addition, the gut microbiome is important for promoting intestinal colonization resistance and protecting against pathogens, which could influence the likelihood of host exposure to infection and inflammation, as risk factors for subsets of NDs (center left). The gut microbiome regulates a vast repertoire of biochemicals, some of which may interact directly with amyloids to promote aggregation in neurons and propagation from the periphery to the brain (center right). The gut microbiome modulates biochemicals that modulate neuroimmune development and function, including the activity of brain microglia, which could impact the manifestation of subsets of NDs (far right).

addition, a wealth of evidence suggests that adherence to the Mediterranean diet reduces susceptibility to AD (Berti et al., 2018; Rainey-Smith et al., 2018), but whether the microbiome may mediate the effects of the diet on host physiology remains unclear. Indeed, the microbiome was altered in patients with mild cognitive impairment after treatment with the modified Mediterranean-ketogenic diet (Nagpal et al., 2019), and the microbiome was reported to mediate the neuromodulatory effects of the ketogenic diet in mouse models of refractory epilepsy (Olson et al., 2018). While diet-related interventions are being pursued for protecting against cognitive impairment in AD, further epidemiological research on potential relationships between diet, microbiome, and risk for NDs is warranted.

## Future Outlooks

Overall, studies in animal models provide intriguing evidence that perturbing the microbiota can modify risk for developing pathological and behavioral endophenotypes of PD, AD, ALS, and HD (Table 3). In various genetic models of PD and AD, complete lack of or depletion of the microbiota yields animals with reduced amyloid pathology and improved motor or cognitive behavior. While only a few such studies have been conducted in mouse models of ALS and HD, they commonly report that the absence or depletion of the microbiota exacerbates neuropathological and behavioral symptoms of disease. While these studies provide intriguing proof of concept that the microbiome as a whole is important for host physiological responses related to NDs, whether more subtle alterations in complex microbial communities could predispose to disease remains largely unexplored. Intriguingly, microbiota transplant and bacterial treatments are capable of reducing symptoms, suggesting that modifying the gut microbiota in animal models of NDs can ameliorate neuroinflammatory, neuropathological and/or behavioral abnormalities triggered by genetic risk. These findings raise the question of whether there are cross-cutting features and shared influences of the gut microbiota across subsets of ND models (Figure 1). Microbial regulation of peripheral immune and neuroimmune function may be a point of convergence across the disorders that warrants future study. Direct effects of microbial products or microbially regulated biochemicals on protein misfolding is another area for continued investigation.

While an increasing number of studies continue to link the microbiome to the modulation of ND symptoms in animal models, the most formidable challenge will be in assessing the relevance of these preclinical studies to the complex and varied manifestations of human NDs. Expanded epidemiological and translational research on the microbiome and NDs is needed to identify associations between the microbiome and various NDs. These studies will need to account for the many influences of diet, medical co-morbidity, and environmental exposures, among other factors, to variation in the human microbiome. The findings from these clinical studies will be paramount to guiding hypothesis generation and experimental design toward ensuring that basic studies of the microbiome in NDs will ultimately be relevant to the human conditions. The future holds tremendous opportunity to uncover the influences of the gut microbiota on risk for symptoms of NDs, with the potential to reveal fundamental interactions across the microbiota-gut-brain axis and inform new approaches for identifying and treating NDs.

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