Movement Disorders
CLINICAL PRACTICE

REVIEW

Gut Vibes in Parkinson's Disease: The Microbiota-Gut-Brain Axis

Clara Bullich, MSc,[1] Ali Keshavarzian, MD,[2] Johan Garssen, PhD,[1,3] Aletta Kraneveld, PhD,[1,4] and Paula Perez-Pardo, PhD[1,]*

ABSTRACT: Background: The complexity of the pathogenic mechanisms underlying neurodegenerative disorders such as Parkinson's disease (PD) is attributable to multifactorial changes occurring at a molecular level, influenced by genetics and environmental interactions. However, what causes the main hallmarks of PD is not well understood. Recent data increasingly suggest that imbalances in the gut microbiome composition might trigger and/or exacerbate the progression of PD.

Objective: The present review aims to (1) report emerging literature showing changes in microbiota composition of PD patients compared to healthy individuals and (2) discuss how these changes may initiate and/or perpetuate PD pathology.

Methods: We analyzed 13 studies published from 2015 and included in this review. Altered microbial taxa were compiled in a detailed table summarizing bacterial changes in fecal/mucosal samples. The methodology was systematically reviewed across the articles and was also included in a table to facilitate comparisons between studies.

Results: Multiple studies found a reduction in short-chain fatty-acid–producing bacteria that can rescue neuronal damage through epigenetic mechanisms. Overall, the studies showed that changes in the gut microbiota composition might influence colonic inflammation, gut permeability, and α-synuclein aggregation, contributing to the neurogenerative process.

Conclusion: Further studies with larger cohorts and high-resolution sequencing methods are required to better define gut microbiota changes in PD. Furthermore, additional longitudinal studies are required to determine the causal link between these changes and PD pathogenesis as well as to study the potential of the intestinal microbiota as a biomarker.

Microbiota-Gut-Brain Axis

Recent neurobiological research suggests a strong impact of the microbiota-gut-brain axis on the pathogenesis of neurodegenerative disorders.[1–3] The brain-gut axis is a long-recognized bidirectional communication system with great implications in health and disease.[4] There is growing evidence that the gut microbiota (GM) modulates the crosstalk between the brain and gastrointestinal tract to maintain homeostasis, giving rise to a more inclusive concept, the microbiota-gut-brain axis.[4–6]

During early postnatal stages, the mammalian intestine is colonized by bacteria, reaching an individually distinct microbiota during the first year of extrauterine life. The diversity and composition of the GM reaches the adult microbial profile at 3 to 5 years of age,[7,8] and it comprises around 3.8×10^{13} microorganisms in the colon.[9] This bacterial colonization has a vital role in the development, maturation, and modulation of the nervous and immune systems. On one hand, studies with germ-free (GF) mice demonstrate that a successfully established enteric microbiota is essential to establish the neurochemical signaling profile in the central nervous

[1]*Division of Pharmacology Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, The Netherlands;* [2]*Department of Medicine, Division of Allergy-Immunology, Rush University Medical Center, Chicago, Illinois, USA;* [3]*Nutricia Reasearch, Utrecht, The Netherlands;* [4]*Institute for Risk Assessment Sciences, Faculty of Veterinary Medicine Utrecht University, Utrecht, The Netherlands*

***Correspondence to:** Dr. Paula Perez-Pardo, Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, visiting address Room 2.68, Universiteitsweg 99, 3584 CG Utrecht, The Netherlands; E-mail: p.perezpardo@uu.nl
Keywords: microbiota, short chain fatty acids, inflammation, gut permeability.
Dr. Kraneveld and Dr. Perez-Pardo shared senior authorship.
Relevant disclosures and conflicts of interest are listed at the end of this article.
Received 11 June 2019; revised 8 August 2019; accepted 20 August 2019.
Published online 23 October 2019 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mdc3.12840

system (CNS).[10,11] In addition, GF mice show reduced expression of proteins involved in synaptogenesis[12,13] and deficient microglia function and maturation.[14] On the other hand, bacterial colonization and composition shape the host's adaptive and innate immunity by promoting differentiation of specific T-cell lines that has a protective effect against pathogens and maintains a balanced immune activity[15,16] and therefore prevents the onset of autoimmune responses and other immune-related disorders.[17]

Modulation Mechanisms of CNS Function by the Gut Microbiota

The bottom-up regulation of GM on brain function occurs by neural, hormonal, and immune pathways. Based on previous evidence, Galland presented multiple routes and mechanisms, presented hereafter.[2] First, the GM communicates directly with the CNS through the vagal-mediated pathway, which stimulates intrinsic primary afferent neurons of the enteric nervous system (ENS), which transmit the signals to the brain through the vagal nerve that innervates the intestine and proximal colon.[2,18,19] Second, based on its composition, the GM induces the release of diverse cytokines, resulting in different cytokine profiles, with more pro- or more anti-inflammatory characteristics.[2,20,21] Cytokines released from the enteric mucosal immune cells or bacterial components, such as lipopolysaccharide (LPS), can reach the brain either by the ENS-vagal–mediated pathway route or through the bloodstream.[16] Third, the GM produces molecules with neuroactive properties that are able to induce changes in the CNS, like short-chain fatty acids (SCFAs) and neurotransmitters such as gamma-aminobutyric acid, noradrenaline, acetylcholine, dopamine, and serotonin.[2,18,22]

GM in Parkinson's Disease

The high complexity of the pathogenic mechanisms underlying neurodegenerative disorders such as Parkinson's disease (PD) is attributable to multifactorial changes occurring at a molecular level, influenced by genetics and environmental interactions. However, what causes the main hallmarks of PD remains to be elucidated. Still, emerging data increasingly confirm the contribution of imbalances in the GM community structure as an etiological factor triggering or exacerbating the progression of PD.[23,24]

PD is a neurodegenerative movement disorder occurring late in life.[25] The main hallmark of PD is α-synuclein (αSyn) misfolding and aggregation in neurons, forming the so-called Lewy bodies.[26] αSyn aggregates particularly affect the dopaminergic neurons in the SNc by inducing neurodegeneration, ultimately leading to motor impairment.[27] According to the dual-hit hypothesis that Braak and colleagues postulated, PD pathology originates in the nasal and/or the enteric neurons and ultimately reaches the brain by the olfactory tract and the vagal nerve, respectively.[28] Based on this hypothesis, it has been suggested that αSyn aggregates may potentially spread from the gut (ENS) to the brain (CNS) as a prion-like protein by cell-to-cell transfer.[26,29] These assumptions seem to be consistent with the long-reported nonmotor symptoms of PD patients, often occurring before the onset of motor symptoms, including olfactory impairment and gastrointestinal (GI) dysfunctions, such as constipation and colonic inflammation.[1–33]

The mechanisms by which changes in the GM composition may cause or perpetuate PD pathology are not yet fully established. However, based on experimental evidences, hypothetical mechanisms have been proposed. Imbalances in the gut microbiota composition may overstimulate the innate immune system of the intestinal mucosa, possibly through Toll-like receptor 4 activation,[33] increasing the levels of oxidative stress in the gut. Oxidative stress may activate enteric neurons and enteric glial cells, contributing to misfolding and accumulation of αSyn in the ENS.[23,34–38] In fact, enteric neurons in colon biopsies of early untreated PD patients show αSyn accumulation before the onset of PD's characteristic motor symptoms,[39–41] although other studies have shown that αSyn immunoreactivity was also observed in some of the healthy individuals.[42–45]

αSyn aggregation in the ENS may reach the brain through systemic and vagal routes. Regarding the systemic route, sustained gut inflammation and increased intestinal permeability might trigger systemic inflammation, which may induce microglia activation in the brain through enhancement of blood–brain barrier (BBB) permeability.[36,38,46,47] Regarding the vagal route, αSyn accumulation in the intestine may be transferred from cell to cell from the enteric neurons to the brain through the vagal nerve in a prion-like propagation fashion.[28,48] The resulting αSyn aggregation in the brain activates microglia, which causes an increase in oxidative stress that exacerbates neuroinflammation. αSyn-induced microglia activation would, in turn, potentiate further αSyn aggregation and propagation specifically in nigrostriatal dopaminergic neurodegeneration, thus contributing to PD progression.[27,49] Another possible mechanism for the GM to induce oxidative stress and neuroinflammation is through gut-derived microbial products that could impact the brain though systemic mechanisms, including BBB disruption described in PD patients.[50]

Methods

To determine whether there are objective evidences supporting the hypothesis that changes in the intestinal microbiota composition are involved in the pathogenesis of PD, we identified 13 human studies published from 2015 and included in this review. Twelve studies were case-control studies and one was a longitudinal study. All studies were based on fecal samples collected from PD patients and control participants (age matched in most cases), except one study that also included mucosal samples. Altered microbial taxa were compiled in a table summarizing bacterial changes in fecal/mucosal samples (Supporting Information Table S2). The methodology was reviewed across the articles and was also included in a table to facilitate the comparison between studies (Supporting Information Table S1). Using available data in literature, we then attempted to propose a potential mechanism by which disrupted microbiota composition/function might initiate and/or perpetuate PD pathology.

Results

Bacterial Taxonomic Differences

To date, 13 studies have described gut bacterial changes in fecal samples of PD patients,[51–62] including one longitudinal study[61] (Supporting Information Tables S1 and S2).

To address the hypothesis that the GM composition is altered in PD, the case-control studies examined the differences in fecal microbiome composition of PD patients compared to control participants.[51–60,62] To analyse the GM during PD progression, the follow-up study examined the changes in fecal microbiome of PD patients at two time points 2 years apart.[61] The fecal microbiome was analyzed at multiple taxonomic levels, as described below in more detail.

At the phylum level, a reduction in the abundance of Bacteroidetes[54,59] and an increase in Firmicutes abundance[57] were reported in fecal samples of PD patients compared to control participants. Only Keshavarzian and colleagues found a trend toward a lower Firmicutes/Bacteroidetes (F/B) ratio in fecal samples, but not in mucosal biopsies, of PD patients.[52] Furthermore, Keshavarzian and colleagues and Li and colleagues reported an increased abundance of Verrucomicrobia[52] and Actinobacteria,[59] respectively, in fecal samples of PD patients compared to controls. Both studies also confirmed increased Proteobacteria[52,59] (Supporting Information Table S2).

At the family level, Scheperjans and colleagues found a significant reduction of 78% in the abundance of Prevotellaceae in feces of PD patients compared to healthy controls,[51] confirmed by the also significant decrease observed in the Heintz-Buschart and colleagues[60] and reinforced by the strong downward trend reported in three other studies here reviewed, which failed to reach statistical significant differences.[52,54,59] In contrast to Prevotellaceae, Verrucomicrobiaceae was found to be enriched in four studies.[51,52,55,57] Other reproducible findings were increased abundance of Lactobacillaceae,[51,55,58,63] only refuted by the Unger and colleagues study,[54] and reduced abundance of Lachnospiraceae.[52,55,59,63]

In addition, fewer studies detected significant reduced levels of Pasteurellaceae,[55,59] as well as increased levels of Enterobacteriaceae[54,59] and Enterococcaceae[58,59] in fecal samples of PD patients, although the abundance of the latter was reduced in the Unger and colleagues study.[54] Only Heintz-Buschart and colleagues showed a significantly lower abundance of Ruminococcaceae,[60] together with a remarkable, but not significant, lower abundance in the Li and colleagues study.[59] In contrast, two studies observed a significant enrichment of Ruminococcaceae.[51,55] This increase was suggested to be compensating the decreased abundance of Prevotellaceae in PD patients rather than being associated with the disease itself.[51] Recently, Johnson and colleagues published a study about GM alteration in a rotenone-induced rat model of PD.[64] Consistently with the studies aforementioned, lower levels of Prevotellaceae and Lachnospiraceae were reported in these rotenone rats, as well as lower levels of Ruminococcaceae levels,[64] which still does not enlighten the ambiguous findings regarding the abundance of Ruminococcaceae across the studies concerned[51,55,59,60] (Supporting Information Table S2).

At the genus level, several works reported overlapping findings. On one hand, the levels of *Akkermansia* were significantly increased in feces of PD patients compared to control groups in four studies,[52,55,57,60] with particular mention to the increase in *Akkermansia muciniphila*.[54,57] Multiple studies also revealed an increase in *Bifidobacterium*[50–52] and *Lactobacillus*,[53,55,56] aligning with the increase in the abundance of these bacteria in the colon and small intestine of rotenone-treated rats[64] and in stool samples of rotenone-treated mice.[65] On the other hand, the abundances of genera such as *Faecalibacterium*[52,55,56,59] (including the species *Faecalibacterium prausnitzii*[54,55]), *Blautia*[52,55,59] (including the species *Blautia glucerase*[56]), and *Prevotella*[56,57,60] (including *Prevotella* levels with a remarkable but nonsignificant reduction[52,59]) were significantly impoverished in fecal samples of PD patients. In addition, Keshavarzian and colleagues observed a nonsignificant decrease in *Prevotella* in mucosal biopsies of PD patients.[52] Other differences in bacterial abundances less characterized across the studies reviewed included an elevation of *Oscillospira*,[52,56] contrary to the Johnson and colleagues findings,[64] and *Anaerotruncus*[60,65]; whereas *Dorea*[52,56] and *Roseburia*[52,55,63] showed lowered levels in PD groups.

Besides the reproducible findings previously mentioned, the changes in the abundances of specific bacterial taxa differed among studies. For instance, Clostridiaceae, as well as several clusters and genera from this family, were increased.[52,60,62] However, specific *Clostridium* species were reduced.[53,57] Moreover, *Bacteroides* showed lower abundance at the genus[56,60] and species[53,56] levels, except in the Keshavarzian and colleagues study.[52] Finally, bacteria from the family Ruminococcaceae were more elevated in feces of PD patients compared to controls, including most Ruminococcaceae genera,[51,52,55,56] except for *Ruminococcus*, which showed to be reduced.[59] Two studies reported reduced levels of Ruminococcaceae, although they missed the level of significance[59,60] (Supporting Information Table S2).

In general, the inconsistencies among studies, absence of changes in some phyla,[54,59] and lack of analysis at the phylum level indicate that the examination of bacterial changes at lower taxonomic ranks (family, genus, and species) may better define modifications in the microbiota composition. In fact, relative abundances of bacterial families and genera can change within a phylum without altering the phylum's overall relative abundance.

It should be noted that these studies were conducted in different parts of the United States,[52,55] Europe (including Western[51,54,57,58,60,63] and Eastern Europe[56]), and Asia.[53,59,61,62] All studies found abnormal microbiota composition in PD compared to controls. The observed differences in microbiota compositions in PD patients among studies could be attributable to differences in environmental factors known to impact microbiota structures like the dietary habits in study subjects.

Overall, the case-control studies here reviewed suggest changes in the gut environment in PD caused by an imbalance in the GM. As highlighted by Mertsalmi and colleagues, patients suffering from PD and inflammatory bowel disease share some bacterial taxa,[66] and inflammatory bowel disease increases the risk of developing PD.[67] Even if these common bacteria may not have a PD-specific predictive value, they confirm the intestinal inflammatory component in PD pathology.

Bacterial-Derived Products and Potential Effects in PD Pathology

To address the potential effects of the aforereviewed GM composition changes in PD pathology, the consequences of the presumably altered microbial products secretion and metabolic activity are examined henceforth.

SCFAs

The studies here examined reported a decrease in the abundance of putative SCFA-producing bacteria[68] from bacterial families, such as Prevotellaceae, Lachnospiraceae, and Ruminoccocaceae, in feces and/or intestinal mucosa of PD patients. A reduction in the abundances of SCFA-producing bacteria seems to fit well with the observed significant reduction in fecal concentrations of health-promoting neuroactive SCFAs, such as butyrate, propionate, and acetate, in PD patients.[54] SCFAs have potent anti-inflammatory and -oxidant properties,[69,70] and they enhance intestinal epithelial barrier function.[47,71,72] Thus, as suggested by several of the reviewed studies, a reduction of SCFA-producing bacteria leading to SCFA deficiency may result in detrimental effects in PD patients, including increased colonic inflammation, gut leakiness, increased risk of αSyn deposition in the GI tract, and microglial activation in the brain.[52,54,55,59]

Butyrate

The studies here analyzed reported a decrease in putative-butyrate–producing (pBP) bacteria,[68,70] such as *Faecalibacterium prausnitzii*, *Blautia*, *Coprococcus*, Roseburia, and Eubacterium. Only the abundances of *Clostridium* clusters IV and XIV, also pBPs, were increased (Supporting Information Table S2).

Butyrate is a well-known SCFA with anti-inflammatory properties, which is able to regulate the expression of cell-survival–, regeneration-, and plasticity-related genes.[68,73] Butyrate exerts neuroprotection against cell death by its histone deacetylase (HDAC) inhibitory activity.[68,73,74] For instance, a treatment with sodium butyrate prevents MPTP-induced dopaminergic neurodegeneration in mice[75]and improves motor impairment in a rotenone-induced Drosophila model of PD.[76] Interestingly, under the assumption that αSyn in PD can modulate gene transcription by altering the acetylation status of histones, a study with a dopaminergic neuronal cell model demonstrated the ability of sodium butyrate to rescue the DNA damage induced by αSyn.[77] Furthermore, by inhibiting HDAC activity, butyrate strengthens the integrity of the BBB, known to be disrupted in PD,[50,78] and reinforces the intestinal barrier integrity by modulating tight junction protein expression between epithelial cells.[79] Besides this, the anti-inflammatory effects of butyrate are achieved by promoting apoptosis in colonic T cells and inducing differentiation of T-regulatory cells,[80,81] which could possibly reduce the colonic inflammation characterizing PD.

Propionate

As observed in multiple studies, feces from PD patients were shown to have a decreased abundance of *Prevotella*, which is known to produce propionate.[82] Same as butyrate, propionate is an HDAC inhibitor[68,74] with anti-inflammatory properties and protective properties in BBB integrity.[83]

Acetate

By a cross-feeding mechanism, the SCFA acetate is used by pBP- and propionate-producing bacteria as a substrate to synthetize butyrate and propionate, respectively.[68,70] The studies here reviewed detected an increased abundance of several putative-acetate–producing bacteria,[68,70] such as *Bifidobacterium*, *Lactobacillus*, *Clostridium* clusters, and *Akkermansia muciniphila*. Similarly, Wang and colleagues found enriched levels of *Bifidobacterium* and *Lactobacillus* accompanied by a reduction of pBP bacteria in fecal samples of patients with inflammatory bowel disease (IBD).[84] Gargari and colleagues recently showed that intake of *Bifidobacterium bifidum* decreases butyrate concentrations in stools of healthy individuals.[85] In contrast, the examined studies also reported a reduction in other acetate producers,[68,70] such as *Prevotella*, *Bacteroides*, *Blautia*, *Clostridium* spp, and *Ruminococcus*. Intriguingly, Gargari and colleagues showed in the aforementioned study that administration of *Bifidobacterium bifidum* also decreased the relative abundance of Prevotellaceae (including *Prevotella*) and led to an enrichment in Ruminococcaceae,[85] in line with the results compiled in the present review. In addition, acetate concentrations detected in feces of PD patients were lower compared to controls.[54]

In contrast to the neuroprotective function attributed to bacterial-derived SCFAs above described, Sampson and colleagues suggested a negative impact of these compounds on inflammation, potentially leading to αSyn aggregation and subsequently promoting αSyn-dependent microglia activation. This would exacerbate αSyn pathology, ultimately enhancing impairment of motor function.[27] First, Sampson and colleagues' findings are not fully consistent with the depletion of SCFAs detected in human PD feces and seem to contradict the beneficial role attributed to SCFAs, especially considering the findings from the human PD studies here analyzed. Second, the adverse effects attributed to SCFAs in the Sampson and colleagues study should be interpreted carefully, given that the GF mice were orally administered with a mixture of acetate, propionate, and butyrate[27] in a ratio that may not reflect the proportion of bacterial-derived SCFAs in the gut[86] (either in a "healthy gut" or in a "PD gut"). Third, the use of GF mice in Sampson and colleagues' experiments does not recapitulate or model PD patients' gut microenvironment, including GM-ecosystem interactions on the overall SCFA production and consumption, and their subsequent effects in PD pathology. Finally, GF mice have a different mucosal and neuronal immune system that might have a different response to SCFAs compared to a host with intestinal microbiota.

Mucin-Degrading Activity

The under-representation of Prevotellaceae (including *Prevotella*) and the genus *Ruminococcus* in PD fecal samples observed across the studies reviewed seems to be consistent with the increased intestinal permeability reported in PD.[36] *Prevotella* and *Ruminococcus* are important mucin degraders,[87] and therefore a reduced abundance

of these bacteria may reveal a lower mucin synthesis in the colonic mucosal layers, which is related to higher mucosal permeability.[88] Subsequently, translocation of bacteria to the inner mucus layers is facilitated, stimulating epithelial immune cells and ultimately triggering colonic inflammation.[89,90]

Similarly to *Prevotella* and *Ruminococcus*, the genus *Akkermansia* comprises mucin-degrading species.[87] *Akkermansia muciniphila*, for instance, is closely associated with the mucosal layer by adhesion to enterocytes. *Akkermansia muciniphila* has been proposed to strengthen the integrity of the intestinal epithelium[91,92] by stimulating mucus synthesis. However, other studies reported an association between increased abundance of *Akkermansia muciniphila* and leaky gut, presumably through mucus degradation.[93] Based on these studies, it can be hypothesized that mucus degradation by *Akkermansia muciniphila* might lead to a compensatory increased synthesis of mucus and anti-inflammatory effects in the host[94,95]; then, *Akkermansia muciniphila* would strengthen barrier integrity. However, if the host cannot create thiscompensatory response, Akkermansia muciniphila will lead to an increase in gut leakiness and inflammatory state. Intriguingly, five of the studies here reviewed reported an increased abundance of *Akkermansia* and/or *Akkermansia muciniphila* in PD feces, suggesting that *Akkermansia muciniphila* is associated with a proinflammatory state in PD. In general, the studies analyzed revealed a decrease of *Prevotella* and *Ruminococcus*, together with an increase of *Akkermansia* and/or *Akkermansia muciniphila*. Interestingly, a negative correlation between *Akkermansia* and *Prevotella* has been found in dietary-fiber–deprived mice[96] and in human enterotypes,[87] suggesting a compensatory effect of *Akkermansia* in response to the reduction of *Prevotella* abundance as an attempt to maintain the mucin degradation status.

Hydrogen Production

The reduction in abundance of *Prevotella* across the studies reviewed may be associated with the impairment in endogenous hydrogen production observed in PD, as noted by Cakmak.[97,98] Hydrogen sulfide (H_2S) is an intestinal gaseous neurotransmitter produced by *Prevotella*[99] that exerts a protective effect in dopaminergic neurons of the SN in mouse and rat models of PD.[100–102] Therefore, a decrease in *Prevotella* is likely to drive to H_2S intestinal underproduction, limiting its availability in nigrostriatal dopaminergic neurons.[98] As suggested by Cakmak, the increased gut permeability in PD might be a compensatory mechanism of the body to enhance a better intestinal H_2S intake in response to reduced *Prevotella* levels.[97] Similarly to *Prevotella*, some reviewed studies reported a reduction in *Roseburia* population (Supporting Information Table S1), another putative hydrogen-producing genus.[103] H_2S might have beneficial properties[104]; its depletion could cause an exacerbation of LPS-induced microglial inflammation leading to neurodegeneration.[105,106] Moreover, a recent study demonstrates a reduction of nitrated αSyn after H_2S treatment in MPTP-treated mice,[102] reinforcing the negative impact of a reduction in hydrogen-producing bacteria in PD.

In contrast, the abundance of the putative hydrogen-consumer *Blautia*[107] was reduced, possibly caused by the afore-mentioned depletion of H_2. Intriguingly, the levels of Enterobacteriaceae, also putative hydrogen-producing bacteria,[108] were increased, although this enrichment seems to be more relevant for motor symptoms development in PD pathology (see Motor Symptoms).

Vitamin Biosynthesis

The GM enterotype dominated by *Prevotella* is associated with a higher capacity of thiamine biosynthesis.[87] Therefore, the reduction of Prevotellaceae and *Prevotella* detected in PD feces in some of the studies here examined is consistent with the already reported deficiencies in thiamine in PD patients[109,110] and with the olfactory dysfunction associated with low levels of thiamine in early stages of the disease.[111] Similarly, *Bacteroides* are associated with an enriched riboflavin biosynthesis,[87] which has shown potential neuroprotective effects in PD[112] given that riboflavin deficiency may cause an upregulation of PD pathways.[113]

Intestinal Ghrelin Secretion

The findings of the reviewed studies showed a decrease in *Prevotella* levels, as well as an increase in *Bifidobacterium* and *Lactobacillus* levels in PD feces. These bacterial changes have been associated with a reduction of ghrelin secretion in the intestine.[114] Accordingly, the concentration of ghrelin is low in plasma of PD patients, regardless of the disease stage,[115] and its acylated isoform has neuroprotective effects in dopaminergic neurons of the SN in a MPTP-induced mouse model of PD.[116,117]

Correlation Between GM Changes and PD Clinical Features

To explore whether changes in GM are associated with PD clinical features, most of the reviewed studies found relevant correlations between these two aspects.

Disease Severity

The severity of the disease (total or specific UPDRS scores) was positively correlated with the abundances of Enterococcus and *Escherichia-Shigella*[59,62] and negatively correlated with the abundance of *Bacteroides fragilis*, *Bifidobacterium*, *Blautia*, *Ruminococcus*, and *Faecalibacterium*.[59,61,62] In line with this, Li and colleagues found a remarkable decrease of *Faecalibacterium* in the severe PD group compared to a lower decrease detected in the mild PD group.[59] In addition, Prevotellaceae abundance was related to UPDRS-III.[51] These findings suggest that GM may shift as the disease progresses, apparently toward a more proinflammatory bacterial profile.[52,118] Furthermore, Minato and colleagues could build a model based on *Bifidobacterium* and *Atopobium* abundances at the beginning of the study to predict worsening of total UPDRS scores in 2 years.[61] However, only *Bifidobacterium* was correlated with worsening of UPDRS-I.[61]

PD Duration

PD duration was positively correlated with Bacteroidetes, Enterococcus, *Lactobacillus* spp, Proteobacteria, and Ruminococcaceae[52,53,55,59,62] and negatively correlated with *Blautia*, *Clostridium* spp, *Faecalibacterium*, *Ruminococcus*, Lachnospiraceae, and Firmicutes.[52,53,59,62] Interestingly, most bacteria showing negative correlations with disease duration are SCFA producers.[68] Therefore, a gradual decrease of these bacterial taxa may lead to a depletion of SCFA production, which would entail a progressive reduction in the antioxidant, anti-inflammatory, and neuroprotective effects of SCFAs, together with an increase in inflammation and intestinal permeability along time. However, two studies found opposite results regarding the correlation of *Escherichia-Shigella* with PD duration,[59,65] which is not sufficient evidence to speculate about their role in disease duration.

Medication

Regarding medication, *Dorea* and *Phascolarctobacterium* were found to be negatively correlated with levodopa equivalent doses,[62] and Enterobacteriaceae had a negative correlation with catechol-O-methyl transferase inhibitor intake.[51]

Overall, these studies suggest that changes in the GM composition may occur even in early diagnosed PD patients. These changes worsen with longer duration of the disease, possibly as a consequence of: PD medication,[55] constipation,[119] disrupted sleep,[120] and more severe inflammatory state of the intestine.[121] Moreover, a recent study shows that L-dopa availability is compromised by high abundance of intestinal bacterial tyrosine decarboxylase in patients with PD,[122] suggesting that not only medication can affect GM composition, but GM can affect drug effectivity.

Motor Symptoms

Motor symptoms were related to *Anaerotruncus* (Clostridiaceae, Firmicutes), *Aquabacterium* (Proteobacteria), *Peptococcus* (Firmicutes), *Clostridium* XIVa, and Lachnospiraceae[60,62] abundances. In addition, Scheperjans and colleagues found that the severity of motor deficits in PD patients with postural instability and gait difficulty (PIGD) was positively correlated with the abundance of Enterobacteriaceae.[51] As suggested in that study, PIGD phenotype may imply a more severe αSyn pathology in the colon.[51] Another study showed *Escherichia coli* staining to be positively correlated with αSyn staining in PD patients.[36] Therefore, the association between the levels of Enterobacteriaceae and the mentioned motor phenotype may be explained by an increased translocation of *E. coli* (Enterobacteriaceae) into the colonic mucosa, causing a more severe αSyn pathology potentially leading to the PIGD phenotype. In contrast to Scheperjans and colleagues, Unger and colleagues did not find any differences in the abundance of Enterobacteriaceae between PD motor phenotypes, probably because of the small sample size of PD patients.[54]

Nonmotor Symptoms

Nonmotor symptoms were in general associated with *Akkermansia* and *Anaerotruncus* (Clostridiaceae).[60] Bradyrhizobiaceae and Verrucomicrobiaceae were related to constipation in PD patients.[51] For instance, an increase in *Akkermansia*, a mucin-degrading bacterium from the family Verrucomicrobiaceae, has been detected in IBD patients,[123] suggesting a role of these bacteria in the development of GI problems characterized in PD.[124]

Gut Microbiota Changes as Predictors for PD

Based on the abundance of bacterial taxa in PD fecal samples, four of the reviewed studies generated predictive models to identify PD using the receiver operating characteristic (ROC) curve for diagnostic tests[51,57,58,62] (Supporting Information Table S1). They included taxa with significant different abundances in PD compared to control participants. At the family level, Scheperjans and colleagues and Hopfner and colleagues found four and three different taxa, respectively, predicting PD.[51,58] At the genus level, Bedarf and colleagues and Qian and colleagues found 5 and 18 different taxa, respectively, predicting PD.[57,62] The areas under curve were similar among the four studies and indicated that the taxa included may be a valuable tool to identify PD patients before motor symptoms development. In contrast with the Scheperjans and colleagues study,[51] Bedarf and colleagues did not find any added predictive value of the constipation degree added to the bacterial taxa predicting PD.[57]

Potential Confounding Factors in the Observed PD-Associated GM Changes

Age and Sex

Five of the case-control studies matched study participants by age and sex to rule out any associations between these two parameters and GM composition in PD[125,126] (Supporting Information Table S1). Regarding sex, the Hill-Burns and colleagues study had an imbalanced sex distribution,[55] which could have influenced the bacterial relative abundances measured.[126] Based on this potential confounder, Bedarf and colleagues included only males in their study.[57] Regarding age, case and control groups significantly differed in the Keshavarzian and colleagues study,[52] which might have biased the results. Advanced age is the greatest risk factor for developing PD.[125] Interestingly, some GM changes detected in PD have opposite trends when compared to GM changes in the elderly. For instance, *Bacteroides* and Eubacterium are elderly-associated bacteria[127] that showed decreased abundances in PD among the studies here examined. Moreover, the elderly present a lower *Akkermansia* abundance compared to young individuals[128]; however, a remarkable enrichment of *Akkermansia* was widely reported among the studies analyzed. Besides, some studies did not find significant associations between bacterial composition in feces

of PD patients and age.[51,52] Altogether, these evidences support the association between the abundance of certain bacteria and PD pathology rather than the aging process. Nevertheless, age and sex seem to have low impact on GM composition changes in PD patients compared to the influence of environment in PD-associated GM changes, as discussed below.

Environmental Factors

Keshavarzian and colleagues found that GM composition in feces or mucosa of control participants did not differ between whites and blacks,[52] suggesting that the environment, rather than ethnicity, is a key factor influencing GM, including diet and geographical region.

GM is strongly influenced by diet, which can lead to shifts in bacterial taxa abundances depending on the dietary habits.[129] For instance, evidences show that fiber-rich diets increase the production of SCFAs through gut bacterial fiber fermentation,[70,73,82] thus determining the GM's capacity to maintain a functional mucosal barrier to prevent pathogen susceptibility.[96] Among the studies reviewed, only Keshavarzian and colleagues compiled information about dietary habits of the participants. Li and colleagues did not strictly control for diet as a confounder, only dietary habits considered to have great potential confounding effects on the GM were ruled out from the analysis.[59] In the follow-up study by Minato and colleagues, only consumption of *Lactobacillus*-fermented milk was considered, but not other dietary habits.[61] To minimize the strong effects that environmental factors may have on GM changes, three studies included individuals sharing daily living environment and diet, being most of them the spouses of PD patients.[53,55,58]

Medication can have profound effects on GM composition.[130] Hill-Burns and colleagues found a remarkable decrease in the association between *Bifidobacterium* and *Blautia* and PD after excluding patients taking COMT inhibitors and/or anticholinergics.[55] In addition, the negative correlations of *Dorea*, *Phascolarctobacterium*, and Enterobacteriaceae with parkinsonian drugs found in two studies[51,62] supports the idea that medication can have a strong impact on GM composition. In contrast, Heintz-Buschart and colleagues did not find any effects of medication in differentially abundant bacteria between PD patients and controls.[60]

Limitations of the Studies Reviewed and Future Directions

Study Design

From the 13 studies reviewed, 12 were case-control studies,[51–60,62] whereas Minato and colleagues' was a longitudinal study.[61] An advantage of the latter is that GM changes during disease progression can be examined, which is not possible in a case-control study. Further large-sample and longitudinal studies using additional controls like household control and first-degree non-PD controls with careful information regarding factors that could impact microbiota composition/function (e.g., diet, sleep, exercise, socioeconomic state, stress, and crowding)[120,121,131,132] and using validated questionnaires are required to further interrogate the contribution of the microbiota in pathogenesis, disease phenotype, and disease course of PD.

Demographics and Clinical Characteristics

Including the 2-year follow up study from Minato and colleagues, the studies reviewed had a limited sample size, except for five studies,[51,55,56,60,63] and therefore the absence/presence of a certain abundance difference in these studies might be caused by insufficient sample size. Moreover, the cohorts from each study included participants from different geographic areas, disease duration, and disease stages (Supporting Information Table S1). The clearest example is the Bedarf and colleagues study, which included early-stage PD patients,[57] thus restricting the implications of the findings in PD pathology in more advanced stages. While most of the PD patients examined were taking PD medication, the Bedarf and colleagues study included L-dopa-naïve patients,[57] which may have influenced the results compared to the other studies. Still, most of the studies included PD patients under diverse antiparkinsonian medications, which may have differential effects on GM in a drug-specific manner.

Methodology

First, Keshavarzian and colleagues analyzed the microbiota from both feces and intestinal mucosa,[52] whereas the rest of the studies only examined fecal samples (Supporting Information Table S1). Analyzing microbiota and bacterial-derived products, such as SCFAs, also from mucosal biopsies would provide higher accuracy in determining the relative abundance of mucosal-associated bacterial taxa and SCFA concentrations in the intestine of PD patients, given that fecal samples may not completely reflect the intestinal environment.[65,86] The sequencing approaches used to analyze GM differed among studies. Most of the studies sequenced the bacterial 16S rRNA gene. And only three studies used nonsequencing techniques to identify target bacteria: Unger and colleagues used quantitative polymerase chain reaction,[54] whereas Hasegawa and colleagues and Minato and colleagues used the Yakult Intestinal Flora-SCAN (YIF-SCAN)[53,61] (Supporting Information Table S1). The YIF-SCAN allows species resolution, determines the exact microbial cell quantification, and counts only life bacteria by analyzing ribosomal RNA (rRNA) instead of rRNA gene sequences.[133] Still, by using this technique, Hasegawa and colleagues and Minato and colleagues could analyze a very limited number of target microbial taxa, which could also have been biased given that the amount of rRNA quantified depends on bacterial metabolism.[53,61,133] Additionally, Bedarf and colleagues used metagenomic shotgun sequencing of single-copy marker genes, allowing a high resolution of GM analysis at a

species level[57] (Supporting Information Table S1). Moreover, in a recent study, Barichella and colleagues showed that the significant changes in GM composition were reduced after adjusting for confounders, including disease duration and clinical profile.[63]

In general, the relative abundances of bacterial taxa in PD may not be directly comparable across the studies because of environmental, clinical, and methodological differences. However, there are some clearly overlapping findings across studies, as reported along the Discussion section, that may add valuable information about the GM in PD.

Together, the studies here examined suggest the need for better designed human studies to assess GM changes in all stages of PD. First, studies with larger cohorts and from wider geographical regions are needed to draw more definitive conclusions about GM changes in PD. Second, the use of age- and sex-matched controls should be maintained in future studies to avoid any possible confounding effects on GM. Based on the study from Unger and colleagues,[54] future studies could also include young controls to better assess potential effects of aging in GM of PD patients. Third, the use of spouses/household controls seems to be an appropriate strategy to minimize the environmental impact on GM composition changes in PD. However, evidence suggests that PD can also occur in couples exposed to shared environmental risk factors,[134] thus questioning their validity as controls. For this reason, using two complete sets of controls, spouses and nonspouses, could be an optimal approach. Fourth, although the influence of medication appears to be difficult to control, studies with PD patients under different medications could be grouped and assessed both independently and as a single group. Fifth, given that GM changes during PD progression may be key for a better understanding of the mechanisms of GM-host interactions in PD pathology, follow-up evaluations should be carried out. To further elucidate the causal role of GM in the onset, development, and/or exacerbation of the pathology, studies should focus on all PD stages.[135] Sixth, in contrast to Minato and colleagues' longitudinal study,[61] the inclusion of control participants in follow-up studies is necessary to take into account the potential effects of time in GM changes. Additionally, a wide case-control design involving PD patients in all disease phases, including prodromal stages, could be a valid alternative to longitudinal designs. Finally, It should be noted that published studies only interrogated bacteria composition. It is now well established that fungi, archaea, and virus are an important part of intestinal microenvironment that could influence the host biology.[136] Future studies are required to use available techniques, such as shot gun metagenomics, transcriptomics, and metabolomics, to not only interrogate bacteria composition, but also bacterial function, archaea, virome, and mycobiota in PD.

Gut Bacteria as Biomarkers for PD Diagnosis: Is There a Microbial Signature for PD?

Based on the 13 studies reviewed, no specific bacterium seems to be linked to PD. For instance, the decreased abundance of *Prevotella* has also been reported in multiple sclerosis,[137,138] type 1 diabetes,[139] and autism spectrum disorders.[140,141] High *Prevotella* levels in feces would unlikely be a PD-excluding biomarker, given that the diet has a marked and immediate impact on *Prevotella* abundance with consumption of a plant-based diet significantly increasing its abundance in stools.[51] In addition, enrichment in *Lactobacillus* was also reported in type 2 diabetes and constipation,[142–144] suggesting that its predictive value is not disease specific. In fact, multiple bacterial taxa were reported to be changed in PD, and the potential interactions between them indicate that the effects of the GM in PD may be the result of complex community relationships within the entire GM, as well as with the host.[51,87] The studies here examined consistently reported abundances of some bacteria discriminating PD patients from control participants. Therefore, in the future, it might be possible to link a microbial profile to PD.

ROC analyses conducted in four of the studies examined showed potential GM predictive values for PD diagnosis, always including several bacterial taxa simultaneously[51,57,58,62] (Supporting Information Table S1). For example, Lactobacillaceae was identified as a predictor of PD together with other bacterial taxa, but not alone, in two of the studies.[51,58] The bacterial taxa involved in ROC analyses were used to predict PD after the onset of motor symptoms, given that three of four studies included PD patients with a disease duration (years from onset of motor symptoms) higher than 5 years.[51,57,58,62] However, predictive analyses of the GM are also required in the prodromal phases of PD. In this respect, Heintz-Buschart and colleagues did not perform predictive analysis in either PD patients or idiopathic rapid-eye-movement sleep behavior disorder (iRBD) patients.[60] Interestingly, *Prevotella* and *Bacteroides* were reduced in both PD and iRBD patients[60] (Supporting Information Table S2), suggesting the potential of these two bacterial taxa as useful biomarkers for PD diagnosis in premotor phases.

Overall, the use of the GM as a diagnostic biomarker for PD pathophysiology may be a valuable noninvasive tool in the future.[145] To this end, future studies should focus on the development of predictive models with putative PD-associated bacteria as potential biomarkers for PD.

GM Changes in PD: Cause or Consequence of the Disease?

Although PD patients seem to have different bacterial abundances compared to controls, the causal relationship between GM composition and PD is still under discussion. On one hand, Heintz-Buschart reported changes in the GM in the prodromal stages of PD,[60] suggesting that it may precede motor symptoms. On the other hand, Hill-Burns suggested that reduction of SCFA-producing bacteria may be a consequence of several diseased states, rather than a PD-specific cause, given that other disorders also show a depletion in SCFA-producing bacteria.[55] It might also be a common causal factor to different disease.

Intriguingly, epidemiological studies have associated the exposure to pesticides and herbicides with PD,[146] and studies with

mutated αSyn transgenic animal models show that these toxic compounds cause dopaminergic neurodegeneration and motor dysfunction by exacerbating αSyn pathology.[147,148] Thus, a role for xenobiotics in the disturbance of the gut bacterial community has been hypothesized[149] that could, in turn, initiate PD pathology in genetically susceptible individuals.

The GM may have different effects in the host depending on its genetic predisposition to develop PD. Sampson and colleagues demonstrated that human PD-derived fecal transplants in GF mice induce motor impairment in αSyn transgenic mice.[27] In the same animal model (αSyn-overexpressing mice), the investigators showed that bacterial depletion with antibiotics ameliorates PD-like pathophysiology in adult mice.[27] These results together suggest that the GM can trigger PD symptoms in genetically susceptible individuals. Furthermore, several risk loci have been associated to the disease, including inflammatory-associated loci, such as HLA-DRB5.[150] As mentioned above, epigenetic changes induced by bacterial-derived products, such as SCFAs, strongly influence the expression and differentiation of proinflammatory molecules and immune cells.[74] Thus, the GM composition, including changes in both pathobionts and nonpathogenic commensal bacteria, may exacerbate gut and brain inflammation in a PD-genetically susceptible host carrying risk loci associated with inflammation. Therefore, the GM may ultimately enhance neuronal degeneration by aggravating and perpetuating inflammatory responses in the brain.

Further microbiota-directed interventional studies and long-term longitudinal studies in a high-risk population are required to determine the causal link between imbalances in the GM and PD. One possible scenario is that the GM imbalances might simply be a marker of the environmental factor(s) that triggers PD pathological processes in a susceptible host. This concept is supported by the fact that microbiota are exquisitely susceptible to environmental factors and changes in their composition in PD might simply be "the canary in the coal mine" representing noxious environmental factor(s) that trigger and promote PD. This scenario is compatible with the recent rise of PD incidence in "modern" societies. Interrogating microbiota and identifying environmental factors leading to a similar shift in the microbiota community could lead to the identification of the pathogenic culprit that might be the novel therapeutic target to prevent PD.

Conclusion

Based on the aforementioned[37,52,151] findings, a hypothesis seems to emerge to explain the potential consequences of GM changes in PD. A proinflammatory and mucus degrading microbiota community might increase intestinal barrier permeability by disrupting the apical junctional complex and mucus layer, leading to enteric and systemic exposure to LPS and other bacterial products resulting in intestinal and neuroinflammation in PD. Decreased levels of bacterial derived anti-inflammatory products might also impact the proinflammatory state. This state would lead to an increased intestinal oxidative stress, which may increase immunoreactivity to αSyn and perpetuate its overexpression, misfolding, and accumulation in the ENS. Subsequently, αSyn pathology could reach the brain though the vagus nerve in a prion-like fashion. Alternatively, bacterial products and peripheral immune cells could reach the brain through blood circulation, facilitated by an LPS-induced increased BBB permeability, and activate microglia, ultimately leading to dopaminergic cell loss.

Although there are clear differences in methodology, the studies here reviewed reveal similar patterns in structural gut bacterial changes in PD patients. Examination of GM changes has been mainly performed at the genus level, probably because of technical limitations, but specific strains and species as well as their functional properties are the ultimate point of interest. Still, the causal role of GM in PD pathology needs to be understood. In the future, GM changes may not only be a therapeutic target, but might also become effective biomarkers with highly predictive value on prodromal diagnosis for PD.

Author Roles

(1) Research Project: A. Conception, B. Organization, C. Execution; (2) Statistical Analysis: A. Design, B. Execution, C. Review and Critique; (3) Manuscript Preparation: A. Writing of the First Draft, B. Review and Critique.

CB: 1B, 1C, 2A, 2B, 3A
AK: 1C, 2C, 3B
ADK: 1A, 1C, 2C, 3B
PPP: 1A, 1C, 2A, 2C, 3B

Disclosures

Ethical Compliance Statement: The authors confirm that the approval of an institutional review board and patient consent were not required for this work. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this work is consistent with those guidelines.

Funding Sources and Conflicts of Interest: This work is part of the Utrecht University "Focus en Massa program" and financially supported by Nutricia Research, Utrecht, The Netherlands. Prof. Dr. Johan Garssen is an employee of Nutricia Research, Utrecht, The Netherlands.

Financial Disclosures for previous 12 months: Prof. Dr Johan Garssen is an employee of Nutricia Research, Utrecht, The Netherlands.

References

1. Stilling RM, Dinan TG, Cryan JF. Microbial genes, brain & behaviour—epigenetic regulation of the gut-brain axis. *Genes Brain Behav* 2014;13:69–86.
2. Galland L. The gut microbiome and the brain. *J Med Food* 2014;17:1261–1272.
3. Quigley EMM. Microbiota-brain-gut axis and neurodegenerative diseases. *Curr Neurol Neurosci Rep* 2017;17:94.

4. Bercik P, Collins SM, Verdu EF. Microbes and the gut-brain axis. *Neurogastroenterol Motil* 2012;24:405–413.
5. Grenham S, Clarke G, Cryan JF, Dinan TG. Brain-gut-microbe communication in health and disease. *Front Physiol* 2011;2:94.
6. Cryan JF, O'Mahony SM. The microbiome-gut-brain axis: from bowel to behavior. *Neurogastroenterol Motil* 2011;23:187–192.
7. Rodríguez JM, Murphy K, Stanton C, et al. The composition of the gut microbiota throughout life, with an emphasis on early life. *Microb Ecol Health Dis* 2015;26:26050.
8. Yatsunenko T, Rey FE, Manary MJ, et al. Human gut microbiome viewed across age and geography. *Nature* 2012;486:222–227.
9. Sender R, Fuchs S, Milo R. Revised estimates for the number of human and bacteria cells in the body. *PLoS Biol* 2016;14:e1002533.
10. O'Mahony SM, Clarke G, Borre YE, Dinan TG, Cryan JF. Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. *Behav Brain Res* 2015;277:32–48.
11. Clarke G, Grenham S, Scully P, et al. The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Mol Psychiatry* 2013;18:666–673.
12. Heijtz RD, Wang S, Anuar F, et al. Normal gut microbiota modulates brain development and behavior. *Proc Natl Acad Sci U S A* 2011;108:3047–3052.
13. Diamond B, Huerta PT, Tracey K, Volpe BT. It takes guts to grow a brain: increasing evidence of the important role of the intestinal microflora in neuro- and immune-modulatory functions during development and adulthood. *BioEssays* 2011;33:588–691.
14. Erny D, De Angelis ALH, Jaitin D, et al. Host microbiota constantly control maturation and function of microglia in the CNS. *Nat Neurosci* 2015;18:965–977.
15. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. *Cell* 2014;157:121–141.
16. Powell N, Walker MM, Talley NJ. The mucosal immune system: master regulator of bidirectional gut-brain communications. *Nat Rev Gastroenterol Hepatol* 2017;14:143–159.
17. Blander JM, Longman RS, Iliev ID, Sonnenberg GF, Artis D. Regulation of inflammation by microbiota interactions with the host. *Nat Immunol* 2017;18:851–860.
18. Carabotti M, Scirocco A, Maselli MA, Severi C. The gut-brain axis: Interactions between enteric microbiota, central and enteric nervous systems. *Ann Gastroenterol* 2015;28:203–209.
19. Forsythe P, Bienenstock J, Kunze WA. Vagal pathways for microbiome-brain-gut axis communication. *Adv Exp Med Biol* 2014;817:115–133.
20. O'Mahony L, Mccarthy J, Kelly P, et al. Lactobacillus and Bifidobacterium in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. *Gastroenterology* 2005;128:541–551.
21. Kamada N, Seo SU, Chen GY, Núñez G. Role of the gut microbiota in immunity and inflammatory disease. *Nat Rev Immunol* 2013;13:321–335.
22. Dinan TG, Stilling RM, Stanton C, Cryan JF. Collective unconscious: how gut microbes shape human behavior. *J Psychiatr Res* 2015;63:1–9.
23. Mulak A, Bonaz B. Brain-gut-microbiota axis in Parkinson's disease. *World J Gastroenterol* 2015;21:10609–10620.
24. Cassani E, Barichella M, Cancello R, et al. Increased urinary indoxyl sulfate (indican): new insights into gut dysbiosis in Parkinson's disease. *Parkinsonism Relat Disord* 2015;21:389–393.
25. Latourelle JC, Pankratz N, Dumitriu A, et al. Genomewide association study for onset age in Parkinson disease. *BMC Med Genet* 2009;10:98.
26. Olanow CW, Brundin P. Parkinson's disease and alpha synuclein: is Parkinson's disease a prion-like disorder? *Mov Disord* 2013;28:31–40.
27. Sampson TR, Debelius JW, Thron T, et al. Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. *Cell* 2016;167:1469–1480.e12.
28. Hawkes CH, Del Tredici K, Braak H. Parkinson's disease: the dual hit theory revisited. *Ann N Y Acad Sci* 2009l;1170:615–622.
29. Angot E, Steiner JA, Hansen C, Li JY, Brundin P. Are synucleinopathies prion-like disorders? *Lancet Neurol* 2010;9:1128–1138.
30. Mishima T, Fukae J, Fujioka S, Inoue K, Tsuboi Y. The prevalence of constipation and irritable bowel syndrome in Parkinson's disease patients according to ROME III diagnostic criteria. *J Parkinsons Dis* 2017;7:353–357.
31. Klingelhoefer L, Reichmann H. The gut and nonmotor symptoms in Parkinson's disease. *Int Rev Neurobiol* 2017;134:787–809.
32. Devos D, Lebouvier T, Lardeux B, et al. Colonic inflammation in Parkinson's disease. *Neurobiol Dis* 2013;50:42–48.
33. Perez-Pardo P, Dodiya HB, Engen PA, et al. Role of TLR4 in the gut-brain axis in Parkinson's disease: a translational study from men to mice. *Gut* 2019;68:829–843.
34. Rietdijk CD, Perez-Pardo P, Garssen J, van Wezel RJA, Kraneveld AD. Exploring Braak's hypothesis of Parkinson's disease. *Front Neurol* 2017;8:37.
35. Olanow CW, Wakeman DR, Kordower JH. Peripheral alpha-synuclein and Parkinson's disease. *Mov Disord* 2014;29:963–966.
36. Forsyth CB, Shannon KM, Kordower JH, et al. Increased intestinal permeability correlates with sigmoid mucosa alpha-synuclein staining and endotoxin exposure markers in early Parkinson's disease. *PLoS One* 2011;6:e28032.
37. Perez-Pardo P, Kliest T, Dodiya HB, et al. The gut-brain axis in Parkinson's disease: possibilities for food-based therapies. *Eur J Pharmacol* 2017;817:86–95.
38. Kelly LP, Carvey PM, Keshavarzian A, et al. Progression of intestinal permeability changes and alpha-synuclein expression in a mouse model of Parkinson's disease. *Mov Disord* 2014;29:999–1009.
39. Shannon KM, Keshavarzian A, Mutlu E, et al. Alpha-synuclein in colonic submucosa in early untreated Parkinson's disease. *Mov Disord* 2012;27:709–715.
40. Shannon KM, Keshavarzian A, Dodiya HB, Jakate S, Kordower JH. Is alpha-synuclein in the colon a biomarker for premotor Parkinson's disease? Evidence from 3 cases. *Mov Disord* 2012;27:716–719.
41. Hilton D, Stephens M, Kirk L, et al. Accumulation of α-synuclein in the bowel of patients in the pre-clinical phase of Parkinson's disease. *Acta Neuropathol (Berl)* 2014;127:235–241.
42. Chung SJ, Kim J, Lee HJ, et al. Alpha-synuclein in gastric and colonic mucosa in Parkinson's disease: limited role as a biomarker. *Mov Disord* 2016;31:241–249.
43. Visanji NP, Marras C, Kern DS, et al. Colonic mucosal α-synuclein lacks specificity as a biomarker for Parkinson disease. *Neurology* 2015;84:609–616.
44. Ruffmann C, Bengoa-Vergniory N, Poggiolini I, et al. Detection of alpha-synuclein conformational variants from gastro-intestinal biopsy tissue as a potential biomarker for Parkinson's disease. *Neuropathol Appl Neurobiol* 2018;44:722–736.
45. Shin C, Park SH, Yun JY, et al. Fundamental limit of alpha-synuclein pathology in gastrointestinal biopsy as a pathologic biomarker of Parkinson's disease: comparison with surgical specimens. *Parkinsonism Relat Disord* 2017;44:73–78.
46. Houser MC, Tansey MG. The gut-brain axis: is intestinal inflammation a silent driver of Parkinson's disease pathogenesis? *NPJ Parkinsons Dis* 2017;3:3.
47. Braniste V, Al-Asmakh M, Kowal C, et al. The gut microbiota influences blood-brain barrier permeability in mice. *Sci Transl Med* 2014;6:263ra158.
48. Steiner JA, Quansah E, Brundin P. The concept of alpha-synuclein as a prion-like protein: ten years after. *Cell Tissue Res* 2018;373:161–173.
49. Gao HM, Zhang F, Zhou H, Kam W, Wilson B, Hong JS. Neuroinflammation and α-synuclein dysfunction potentiate each other, driving chronic progression of neurodegeneration in a mouse model of Parkinson's disease. *Environ Health Perspect* 2011;119:807–814.
50. Guan J, Pavlovic D, Dalkie N, et al. Vascular degeneration in Parkinson's disease. *Brain Pathol* 2013;23:154–164.
51. Scheperjans F, Aho V, Pereira PAB, et al. Gut microbiota are related to Parkinson's disease and clinical phenotype. *Mov Disord* 2015;30:350–358.
52. Keshavarzian A, Green SJ, Engen PA, et al. Colonic bacterial composition in Parkinson's disease. *Mov Disord* 2015;30:1351–1360.
53. Hasegawa S, Goto S, Tsuji H, et al. Intestinal dysbiosis and lowered serum lipopolysaccharide-binding protein in Parkinson's disease. *PLoS One* 2015;10:e0142164.
54. Unger MM, Spiegel J, Dillmann KU, et al. Short chain fatty acids and gut microbiota differ between patients with Parkinson's disease and age-matched controls. *Parkinsonism Relat Disord* 2016;32:66–72.
55. Hill-Burns EM, Debelius JW, Morton JT, et al. Parkinson's disease and Parkinson's disease medications have distinct signatures of the gut microbiome. *Mov Disord* 2017;32:739–749.

56. Petrov VA, Saltykova IV, Zhukova IA, et al. Analysis of gut microbiota in patients with Parkinson's disease. *Bull Exp Biol Med* 2017;162:734–737.
57. Bedarf JR, Hildebrand F, Coelho LP, et al. Functional implications of microbial and viral gut metagenome changes in early stage L-DOPA-naïve Parkinson's disease patients. *Genome Med* 2017;9:39.
58. Hopfner F, Künstner A, Müller SH, et al. Gut microbiota in Parkinson disease in a northern German cohort. *Brain Res* 2017;1667:41–45.
59. Li W, Wu X, Hu X, et al. Structural changes of gut microbiota in Parkinson's disease and its correlation with clinical features. *Sci China Life Sci* 2017;60:1223–1233.
60. Heintz-Buschart A, Pandey U, Wicke T, et al. The nasal and gut microbiome in Parkinson's disease and idiopathic rapid eye movement sleep behavior disorder. *Mov Disord* 2018;33:88–98.
61. Minato T, Maeda T, Fujisawa Y, et al. Progression of Parkinson's disease is associated with gut dysbiosis: two-year follow-up study. *PLoS One* 2017;12:e0187307.
62. Qian Y, Yang X, Xu S, et al. Alteration of the fecal microbiota in Chinese patients with Parkinson's disease. *Brain Behav Immun* 2018;70:194–202.
63. Barichella M, Severgnini M, Cilia R, et al. Unraveling gut microbiota in Parkinson's disease and atypical parkinsonism. *Mov Disord* 2019;34:396–405.
64. Johnson ME, Stringer A, Bobrovskaya L. Rotenone induces gastrointestinal pathology and microbiota alterations in a rat model of Parkinson's disease. *NeuroToxicology* 2018;65:174–185.
65. Yang X, Qian Y, Xu S, Song Y, Xiao Q. Longitudinal analysis of fecal microbiome and pathologic processes in a rotenone induced mice model of Parkinson's disease. *Front Aging Neurosci* 2018;9:441.
66. Mertsalmi TH, Aho VTE, Pereira PAB, et al. More than constipation—bowel symptoms in Parkinson's disease and their connection to gut microbiota. *Eur J Neurol* 2017;24:1375–1383.
67. Villumsen M, Aznar S, Pakkenberg B, Jess T, Brudek T. Inflammatory bowel disease increases the risk of Parkinson's disease: a Danish nationwide cohort study 1977–2014. *Gut* 2019;68:18–24.
68. Fernández J, Redondo-Blanco S, Gutiérrez-del-Río I, Miguélez EM, Villar CJ, Lombó F. Colon microbiota fermentation of dietary prebiotics towards short-chain fatty acids and their roles as anti-inflammatory and antitumour agents: a review. *J Funct Foods* 2016;25:511–522.
69. Scott KP, Gratz SW, Sheridan PO, Flint HJ, Duncan SH. The influence of diet on the gut microbiota. *Pharmacol Res* 2013;69:52–60.
70. Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. *Cell* 2016;165:1332–1345.
71. D'Souza WN, Douangpanya J, Mu S, et al. Differing roles for short chain fatty acids and GPR43 agonism in the regulation of intestinal barrier function and immune responses. *PLoS One* 2017;12:e0180190.
72. Kelly CJ, Zheng L, Campbell EL, et al. Crosstalk between microbiota-derived short-chain fatty acids and intestinal epithelial HIF augments tissue barrier function. *Cell Host Microbe* 2015;17:662–671.
73. Bourassa MW, Alim I, Bultman SJ, Ratan RR. Butyrate, neuroepigenetics and the gut microbiome: can a high fiber diet improve brain health? *Neurosci Lett* 2016;625:56–63.
74. Arpaia N, Campbell C, Fan X, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* 2013;504:451–455.
75. Liu J, Wang F, Liu S, et al. Sodium butyrate exerts protective effect against Parkinson's disease in mice via stimulation of glucagon like peptide-1. *J Neurol Sci* 2017;381:176–181.
76. St. Laurent R, O'Brien LM, Ahmad ST. Sodium butyrate improves locomotor impairment and early mortality in a rotenone-induced Drosophila model of Parkinson's disease. *Neuroscience* 2013;246:382–390.
77. Paiva I, Pinho R, Pavlou MA, et al. Sodium butyrate rescues dopaminergic cells from alpha-synuclein-induced transcriptional deregulation and DNA damage. *Hum Mol Genet* 2017;26:2231–2246.
78. Gray MT, Woulfe JM. Striatal blood–brain barrier permeability in Parkinson's disease. *J Cereb Blood Flow Metab* 2015;35:747–750.
79. Stilling RM, van de Wouw M, Clarke G, Stanton C, Dinan TG, Cryan JF. The neuropharmacology of butyrate: the bread and butter of the microbiota-gut-brain axis? *Neurochem Int* 2016;99:110–132.
80. Zimmerman MA, Singh N, Martin PM, et al. Butyrate suppresses colonic inflammation through HDAC1-dependent Fas upregulation and Fas-mediated apoptosis of T cells. *AJP Gastrointest Liver Physiol* 2012;302:G1405–G1415.
81. Furusawa Y, Obata Y, Fukuda S, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* 2013;504:446–450.
82. Chen T, Long W, Zhang C, Liu S, Zhao L, Hamaker BR. Fiber-utilizing capacity varies in Prevotella- versus Bacteroides-dominated gut microbiota. *Sci Rep* 2017;7:2594.
83. Hoyles L, Snelling T, Umlai UK, et al. Microbiome-host systems interactions: protective effects of propionate upon the blood-brain barrier. *Microbiome* 2018;6:55.
84. Wang W, Chen L, Zhou R, et al. Increased proportions of Bifidobacterium and the Lactobacillus group and loss of butyrate-producing bacteria in inflammatory bowel disease. *J Clin Microbiol* 2014;52:398–406.
85. Gargari G, Taverniti V, Balzaretti S, et al. Consumption of a bifidobacterium bifidum strain for 4 weeks modulates dominant intestinal bacterial taxa and fecal butyrate in healthy adults. *Appl Environ Microbiol* 2016;82:5850–5859.
86. den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res* 2013;54:2325–2340.
87. Arumugam M, Raes J, Pelletier E, et al. Enterotypes of the human gut microbiome. *Nature* 2011;473:174–180.
88. Brown CT, Davis-Richardson AG, Giongo A, et al. Gut microbiome metagenomics analysis suggests a functional model for the development of autoimmunity for type 1 diabetes. *PLoS One* 2011;6:e25792.
89. Jakobsson HE, Rodriguez-Pineiro AM, Schutte A, et al. The composition of the gut microbiota shapes the colon mucus barrier. *EMBO Rep* 2015;16:164–177.
90. Schwab C, Berry D, Rauch I, et al. Longitudinal study of murine microbiota activity and interactions with the host during acute inflammation and recovery. *ISME J* 2014;8:1101–1114.
91. Tailford LE, Crost EH, Kavanaugh D, Juge N. Mucin glycan foraging in the human gut microbiome. *Front Genet*. 2015;5:81.
92. Reunanen J, Kainulainen V, Huuskonen L, et al. Akkermansia muciniphila adheres to enterocytes and strengthens the integrity of the epithelial cell layer. *Appl Environ Microbiol* 2015;81:3655–3662.
93. Corazziari ES. Intestinal mucus barrier in normal and inflamed colon. *J Pediatr Gastroenterol Nutr* 2009;48(Suppl 2):S54–S55.
94. Derrien M, Belzer C, de Vos WM. Akkermansia muciniphila and its role in regulating host functions. *Microb Pathog* 2017;106:171–181.
95. Derrien M, Van Baarlen P, Hooiveld G, Norin E, Müller M, de Vos WM. Modulation of mucosal immune response, tolerance, and proliferation in mice colonized by the mucin-degrader Akkermansia muciniphila. *Front Microbiol* 2011;2:166.
96. Desai MS, Seekatz AM, Koropatkin NM, et al. A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. *Cell* 2016;167:1339–1353.e21.
97. Cakmak YO. Provotella-derived hydrogen sulfide, constipation, and neuroprotection in Parkinson's disease. *Mov Disord* 2015;30:1151.
98. Ostojic SM. Inadequate production of H_2 by gut microbiota and Parkinson disease. *Trends Endocrinol Metab* 2018;29:286–288.
99. Linden DR. Hydrogen sulfide signaling in the gastrointestinal tract. *Antioxid Redox Signal* 2014;20:818–830.
100. Hu LF, Lu M, Tiong CX, Dawe GS, Hu G, Bian JS. Neuroprotective effects of hydrogen sulfide on Parkinson's disease rat models. *Aging Cell* 2010;9:135–146.
101. Kida K, Yamada M, Tokuda K, et al. Inhaled hydrogen sulfide prevents neurodegeneration and movement disorder in a mouse model of Parkinson's disease. *Antioxid Redox Signal* 2011;15:343–352.
102. Hou X, Yuan Y, Sheng Y, et al. GYY4137, an H2S slow-releasing donor, prevents nitrative stress and α-synuclein nitration in an MPTP mouse model of Parkinson's disease. *Front Pharmacol*. 2017;8:741.
103. Vogt SL, Peña-Díaz J, Finlay BB. Chemical communication in the gut: effects of microbiota-generated metabolites on gastrointestinal bacterial pathogens. *Anaerobe* 2015;34:106–115.
104. Hu LF, Wong PTH, Moore PK, Bian J-S. Hydrogen sulfide attenuates lipopolysaccharide-induced inflammation by inhibition of p38 mitogen-activated protein kinase in microglia. *J Neurochem* 2007;100:1121–1128.

105. Liu M, Bing G. Lipopolysaccharide animal models for Parkinson's disease. *Parkinsons Dis* 2011;2011:327089.

106. Peterson LJ, Flood PM. Oxidative stress and microglial cells in Parkinson's disease. *Mediators Inflamm* 2012;2012:401264.

107. Bik EM, Ugalde JA, Cousins J, Goddard AD, Richman J, Apte ZS. Microbial biotransformations in the human distal gut. *Br J Pharmacol* 2018;175:4404–4414.

108. Nandi R, Sengupta S. Microbial production of hydrogen: an overview. *Crit Rev Microbiol* 1998;24:61–84.

109. Jhala SS, Hazell AS. Modeling neurodegenerative disease pathophysiology in thiamine deficiency: consequences of impaired oxidative metabolism. *Neurochem Int* 2011;58:248–260.

110. Costantini A, Pala MI, Grossi E, et al. Long-term treatment with high-dose thiamine in Parkinson disease: an open-label pilot study. *J Altern Complement Med* 2015;21:740–747.

111. Haglin L, Johansson I, Forsgren L, Backman L. Intake of vitamin B before onset of Parkinson's disease and atypical parkinsonism and olfactory function at the time of diagnosis. *Eur J Clin Nutr* 2017;71:97–102.

112. Marashly ET, Bohlega SA. Riboflavin has neuroprotective potential: focus on Parkinson's disease and migraine. *Front Neurol* 2017;8:333.

113. Xin Z, Pu L, Gao W, et al. Riboflavin deficiency induces a significant change in proteomic profiles in HepG2 cells. *Sci Rep* 2017;7:45861.

114. Queipo-Ortuño MI, Seoane LM, Murri M, et al. Gut microbiota composition in male rat models under different nutritional status and physical activity and its association with serum leptin and ghrelin levels. *PLoS One* 2013;8:e65465.

115. Song N, Wang W, Jia F, et al. Assessments of plasma ghrelin levels in the early stages of Parkinson's disease. *Mov Disord* 2017;32:1487–1491.

116. Bayliss JA, Lemus M, Santos VV, Deo M, Elsworth JD, Andrews ZB. Acylated but not des-acyl ghrelin is neuroprotective in an MPTP mouse model of Parkinson's disease. *J Neurochem* 2016;137:460–471.

117. Moon M, Kim HG, Hwang L, et al. Neuroprotective effect of ghrelin in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease by blocking microglial activation. *Neurotox Res* 2009;15:332–347.

118. Winter SE, Bäumler AJ. Why related bacterial species bloom simultaneously in the gut: principles underlying the 'Like will to like' concept. *Cell Microbiol* 2014;16:179–184.

119. Cao H, Liu X, An Y, et al. Dysbiosis contributes to chronic constipation development via regulation of serotonin transporter in the intestine. *Sci Rep* 2017;7:10322.

120. Voigt RM, Summa KC, Forsyth CB, et al. The circadian clock mutation promotes intestinal dysbiosis. *Alcohol Clin Exp Res* 2016;40:335–347.

121. Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. *Science* 2012;336:1268–1273.

122. van Kessel SP, Frye AK, El-Gendy AO, et al. Gut bacterial tyrosine decarboxylases restrict levels of levodopa in the treatment of Parkinson's disease. *Nat Commun* 2019;10:310.

123. Png CW, Lindén SK, Gilshenan KS, et al. Mucolytic bacteria with increased prevalence in IBD mucosa augment in vitro utilization of mucin by other bacteria. *Am J Gastroenterol* 2010;105:2420–2428.

124. Fasano A, Visanji NP, Liu LWC, Lang AE, Pfeiffer RF. Gastrointestinal dysfunction in Parkinson's disease. *Lancet Neurol* 2015;14:625–639.

125. Tysnes OB, Storstein A. Epidemiology of Parkinson's disease. *J Neural Transm (Vienna)* 2017;124:901–905.

126. Bolnick DI, Snowberg LK, Hirsch PE, et al. Individual diet has sex-dependent effects on vertebrate gut microbiota. *Nat Commun* 2014;5:4500.

127. Odamaki T, Kato K, Sugahara H, et al. Age-related changes in gut microbiota composition from newborn to centenarian: a cross-sectional study. *BMC Microbiol* 2016;16:90.

128. Collado MC, Derrien M, Isolauri E, De Vos WM, Salminen S. Intestinal integrity and Akkermansia muciniphila, a mucin-degrading member of the intestinal microbiota present in infants, adults, and the elderly. *Appl Environ Microbiol* 2007;73:7767–7770.

129. Singh RK, Chang HW, Yan D, et al. Influence of diet on the gut microbiome and implications for human health. *J Transl Med* 2017;15:73.

130. Zhernakova A, Kurilshikov A, Bonder MJ, et al. Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. *Science* 2016;352:565–569.

131. Miller GE, Engen PA, Gillevet PM, et al. Lower neighborhood socioeconomic status associated with reduced diversity of the colonic microbiota in healthy adults. *PLoS One* 2016;11:e0148952.

132. Sekirov I, Russell SL, Antunes LCM, Finlay BB. Gut microbiota in health and disease. *Physiol Rev* 2010;90:859–904.

133. Tsuji H, Nomoto K. Yakult intestinal flora-SCAN: A novel culture-independent analytical method for detection of bacteria in the bloodstream. *Ann Nutr Metab* 2017;71:4–10.

134. Willis AW, Sterling C, Racette BA. Conjugal parkinsonism and Parkinson disease: a case series with environmental risk factor analysis. *Parkinsonism Relat Disord* 2010;16:163–166.

135. Tremlett H, Bauer KC, Appel-Cresswell S, Finlay BB, Waubant E. The gut microbiome in human neurological disease: a review. *Ann Neurol* 2017;81:369–382.

136. Hillman ET, Lu H, Yao T, Nakatsu CH. Microbial ecology along the gastrointestinal tract. *Microbes Environ* 2017;32:300–313.

137. Chen J, Chia N, Kalari KR, et al. Multiple sclerosis patients have a distinct gut microbiota compared to healthy controls. *Sci Rep* 2016;6:28484.

138. Miyake S, Kim S, Suda W, et al. Dysbiosis in the gut microbiota of patients with multiple sclerosis, with a striking depletion of species belonging to clostridia XIVa and IV clusters. *PLoS One* 2015;10:e0137429.

139. Murri M, Leiva I, Gomez-Zumaquero JM, et al. Gut microbiota in children with type 1 diabetes differs from that in healthy children: a case-control study. *BMC Med* 2013;11:46.

140. Kang DW, Park JG, Ilhan ZE, et al. Reduced incidence of Prevotella and other fermenters in intestinal microflora of autistic children. *PLoS One* 2013;8:e68322.

141. De Angelis M, Piccolo M, Vannini L, et al. Fecal microbiota and metabolome of children with autism and pervasive developmental disorder not otherwise specified. *PLoS One* 2013;8:e76993.

142. Larsen N, Vogensen FK, Van Den Berg FWJ, et al. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS One* 2010;5:e9085.

143. Malinen E, Rinttilä T, Kajander K, et al. Analysis of the fecal microbiota of irritable bowel syndrome patients and healthy controls with real-time PCR. *Am J Gastroenterol* 2005;100:373–382.

144. Sato J, Kanazawa A, Ikeda F, et al. Gut dysbiosis and detection of "Live gut bacteria" in blood of Japanese patients with type 2 diabetes. *Diabetes Care* 2014;37:2343–2350.

145. Nair AT, Ramachandran V, Joghee NM, Antony S, Ramalingam G. Gut microbiota dysfunction as reliable non-invasive early diagnostic biomarkers in the pathophysiology of Parkinson's disease: a critical review. *J Neurogastroenterol Motil* 2018;24:30–42.

146. Goldman SM, Musgrove RE, Jewell SA, Di Monte DA. Pesticides and Parkinson's disease: current experimental and epidemiological evidence. In: Aschner M, Costa LG, eds. *Advances in Neurotoxicology*. Vol 1. Environmental Factors in Neurodegenerative Diseases. New York, NY: Academic; 2017:83–117.

147. Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov A V., Greenamyre JT. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat Neurosci* 2000;3:1301–1306.

148. Norris EH, Uryu K, Leight S, Giasson BI, Trojanowski JQ, Lee VMY. Pesticide exposure exacerbates α-synucleinopathy in an A53T transgenic mouse model. *Am J Pathol* 2007;170:658–666.

149. Shehata AA, Schrödl W, Aldin AA, Hafez HM, Krüger M. The effect of glyphosate on potential pathogens and beneficial members of poultry microbiota in vitro. *Curr Microbiol* 2013;66:350–358.

150. Nalls MA, Plagnol V, Hernandez DG, et al. Imputation of sequence variants for identification of genetic risks for Parkinson's disease: a meta-analysis of genome-wide association studies. *Lancet* 2011;377:641–649.

151. Scheperjans F. The prodromal microbiome. *Mov Disord* 2018;33:5–7.

Supporting Information

Supporting information may be found in the online version of this article.

Table S1. Methodology of the 13 studies reviewed Age, disease duration, UPDRS, and H & Y shown as mean ± SD. Disease duration refers to years from onset of motor symptoms.

*Calculated from data in the original article.

A-st, advanced-stage PD patients; DG, deteriorated group; E-st, early-stage PD patients; GM, gut microbiota; iRBDp, iRBD patients (iRBD, idiopathic rapid eye movement sleep behavior disorder); mOTU, molecular operational taxonomic unit; MPD, mild PD group; MS, motor symptoms; M-st, mid-stage PD patients; MX, mixed phenotype; NMS, nonmotor symptoms; PDp, PD patients; PIGD, postural instability and gait difficulty; SG, stable group; SPD, severe PD group; TD, tremor dominant; YIF-SCAN, Yakult Intestinal Flora-SCAN.

Table S2. Altered bacterial taxa in fecal/mucosal samples of PD patients compared to controls across the studies reviewed Increased/reduced taxa refers to taxa changes in PDp compared to control participants.

[a]Change in 2 years in PDp (Minato and colleagues study).

*Only in mucosal samples (and not in fecal samples).

**In both mucosal and fecal samples.

+ iRBDp, also in RBD patients (RBD, rapid eye movement sleep behavior disorder); cl., class; DG, deteriorated group; Fi., Firmicutes; i.s., incertae sedis; incr, increased; n.c., not confirmed by ANCOM (analysis of composition of microbiomes); n.s., not significant; NM, no mention; o., order; PDp, PD patients; red, reduced; SG, stable group; uncl., unclassified; y, years.