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NEUROLOGY OF AGING (K MARDER, SECTION EDITOR)

The Microbiome in Neurodegenerative Disease

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Abstract

Purpose of Review To review recent updates in our understanding of the microbiome and its relationship to neurodegenerative disease.

Recent Findings Recognition of the microbiome's role in health and disease continues to expand. Recent techniques have focused on delineating the function and metabolism of resident organisms, which may correlate more directly with human physiology than identification of species. The role of the microbiome may be of particular importance in certain neurodegenerative diseases, including Parkinson's disease and Alzheimer's disease, among others.

Summary The microbiome influences brain function and may play a role in neurodegenerative disease. Potential mechanisms include immunologic activation and promotion/attenuation of inflammation, as well as direct effects on induction and/or exacerbation of protein aggregation. The microbiome also has increasingly well-documented effects on the metabolism of therapeutic medications. Future studies will need to work through complex methodologic issues in order to identify which changes are truly disease-specific. Nevertheless, manipulation of the microbiome may soon improve our ability to treat neurodegenerative disease.

Keywords Microbiome · Microbiota · Neurodegeneration · Parkinson's disease · Alzheimer's disease · Gut-brain axis

Introduction

Over the last several years, studies have increasingly highlighted the importance of the microbiome, or the summation of the genetic material of organisms living on and in the human body, and its relationship to human health and disease. While the microbiome has been acknowledged as instrumental for some time, this newfound appreciation derives largely from advances in high-throughput genetic sequencing that have enhanced our ability to identify and characterize bacteria and other organisms without the timely, expensive, and often ineffective process of culture growth [1•]. These studies clarify and expand our understanding of the role of the microbiome in the so-called gut-brain axis and in neurodegenerative disease, shedding new perspective on pathophysiology and potential therapeutics in many conditions.

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Expansion of Terms and Techniques

Our appreciation of the importance of this flora, a population collectively referred to as the "microbiota" (the totality of organisms) or "microbiome," (the totality of organismal genomes)—terms we will use interchangeably here, has paralleled our ability to measure it in a variety of sample types obtained from a broad range of human body sites. A full discussion of these techniques is beyond the scope of this article, but we will briefly review basic concepts below.

Although most studies have focused on *intestinal* microbiota, all body surfaces in contact with the outside environment are colonized by a wide array of microorganisms. Major sites include the mouth, respiratory tree, skin, and vagina. In addition, there is increasing evidence of colonization of sites previously considered sterile, such as blood and semen. Bacteria comprise the most numerous members of the microbiota and have been the focus of most studies to date, although viruses

("virome"), fungi ("mycobiome"), and even protozoa ("parasitome") that also reside on human surfaces also influence health and disease [2–4].

As techniques to measure microbiota expand, so does our appreciation of its scope and regional specificity. The number of bacteria living in and on the average human is approximately 4×10^{13}, with 90% of these residing in the colon [5]. Counts are three orders of magnitude lower in the small intestine and six orders lower in the highly acidic stomach, but are only one to two orders lower in the mouth [5]. Large numbers are also found on skin (10^{11}). The microbiome of the oral cavity, skin, vagina, and gastrointestinal and respiratory tracts are likely all clinically significant [6]. The ability of our epithelial surfaces to attract and harbor these organisms, along with our ability as hosts effectively to nurture the populations, is a result of a complex ecological system that has co-evolved with the microbiome [7]. An influence of the microbiome on our own genetic development over time has also been proposed [8]. Most of the interplay between host and microbiota is thought to be symbiotic (i.e., beneficial to both host and microbe). Acute changes in colonizing organisms have long been recognized as a cause of disease, but only recently has the relationship between the long-standing composition of the microbiome and chronic disease been recognized.

The technique most frequently used to study the bacterial microbiome is genetic amplification through quantitative polymerase chain reaction (qPCR) using primers for bacterial 16S ribosomal RNA (16S sequencing), a subunit that is highly conserved in bacteria, but not found in human cells [9]. These methods identify the amount and relative proportions of bacteria by using primers that can distinguish between different phyla, family, and genus, but often cannot make meaningful distinctions at the level of species. For instance, non-disease causing *Clostridium species* may not be distinguishable from *Clostridium difficile*, or non-pathogenic *Escherichia coli* species from *E. coli 157:H7* using this method [1•].

These limitations are partially solved by sequencing the entirety of a sample, through a method known as "shotgun metagenomics." This method sequences the entire genome present in a sample by fragmenting the DNA and sequencing each fragment using high-throughput methods [10]. This allows for more successful distinction between species, and by applying similar techniques to RNA sequencing (the "metatranscriptome"), we can infer details about function of the microbial population [9]. Classic molecular techniques applied to the microbiome, including analysis of the proteins transcribed and the metabolites produced ("metabolomics"), can more definitively clarify the function of various organisms and provide an important next step for developing interventional therapies [9, 11].

Ultimately, understanding microbiotal function is more important than determining species composition. Metagenomic studies have revealed that similar function may be carried out by different organisms. For example, in a study of obese and lean persons, many different organisms in the obese cohort were able to perform a similar metabolic function, such as carbohydrate metabolism, which was less frequently observed in organisms of lean patients [12]. The true differences between health and disease may lie in the functional characteristics of one's microbiome as opposed to simply the relative abundance of species [12].

Limitations of Current Techniques

As technology for measuring the microbiome rapidly expands, so does our understanding of the limitations of these techniques. Sample collection and storage often vary considerably among studies at many different steps [13]. Processing of samples, including lysis of cells and extraction of DNA, can be variably successful in different organisms, introducing bias [14]. Sequencing of microbiome DNA is more affected by errors than traditional genomic sequencing, as a given sample has fewer copies of any particular species than a homogenous human sample, thus requiring more extensive estimation of the error rate [14]. Amplification bias and primer bias are also considerations for 16S sequencing, as is false positive detection of contaminants [15••]. Biases from these processes may affect different bacterial species to different extents [15••].

In the analysis phase, we are still learning how to determine significance with these relatively new data collection methods. The restricted environment of the samples along with high-throughput sequencing places constraints on the data that require compositional data analysis, with traditional statistical estimates leading to potentially false conclusions in some studies [16].

Beyond the technical aspects of measurement, microbiome studies may be difficult to interpret because uncontrolled behavioral or other host factors may also affect the microbiome. Many of these factors are difficult to measure and may confound results if not considered in study design and analysis [17]. For example, factors such as time of day or recent diet can markedly influence the gastrointestinal microbiome [18]. Rigorous attention to study design and establishment of plausible biological mechanisms with appropriate biomarkers will advance determinations of causality from these largely correlational study designs [19].

How Do We Influence the Microbiome?

To understand the role of the microbiota in disease, we first need to understand the microbial makeup that underlies healthfulness. However, the microbiota is influenced by many factors that change over the course of a person's life. How an individual's microbiota responds to variable homeostatic stressors may be more important than its specific makeup at any one time.

Effect of Birth and Aging

Microbiota formation and differentiation begin at birth. Vaginally delivered infants have gut bacteria similar to their mothers' vaginal microbiota, while those delivered by cesarean section have organisms similar to their mothers' skin [20]. These differences largely disappear within the first 6 weeks, and the diversity among body sites increases as the microbiome becomes regionally specific [21, 22]. The skin, airway, oral cavity, and gastrointestinal tract all diversify based on the bacteria and environment with which they come into contact [6]. Significant variation can exist even between nearby sites, depending on their physical characteristics. For instance, the composition of the skin microbiota is highly variable; even within one individual, skin flora varies considerably between the forearm and post-auricular area or even between the left and right hands of an individual [6]. Understanding this level of regional specificity is important when considering microbiome changes associated with disease.

The microbiota continues to change as we age, with an "adult" profile largely established by age two [6]. For the gastrointestinal tract, diversity among individuals (beta diversity) is greatest for children, whereas the adult microbiota tends to be similar within a given population. However, microbiome diversity within an individual (alpha diversity) increases into adulthood [23] and remains relatively stable until old age. In the elderly, alpha diversity diminishes again, with changes in the relative abundances of some species [24, 25•]. Functionally, the elderly gut microbiota may more closely resemble an infant's in certain respects, such as sugar metabolism [26].

Several studies indicate that the changes seen with aging may contribute to diseases of aging. Maffei et al. examined the relationship between the microbiome and "biological age," incorporating functional measures such as mobility, cognition, and comorbidities, and found that microbiome changes correlated more closely with biological than chronological age [27•]. Microbiotal changes of aging have also been correlated with increases in peripheral cytokines and the presence of chronic inflammation [28]. Causes of age-related changes could include increased antibiotic use in the elderly, reduced gastric motility, and changes in lifestyle and diet [26]. For example, transitioning from the community to long-term care affects the microbiome [29]. Understanding the causes and effects of aging-related microbiotal changes is a key to informing interventions to maintain healthy aging [30] (Table 1).

Effect of Genetics

Differences in the microbiota among individuals may be determined in part by genetic factors. Human twin studies comparing the relative similarity of identical and fraternal twin microbiomes have reported relatively small heritability. A recent study found higher correlation of 8.8% of organisms in monozygotic compared with dizygotic twins [56]. Certain bacterial phyla may be more heritable than others, such as those related to fat metabolism [56, 57]; however, another twin study found no significant differences with respect to zygosity. Whether genetic factors influence the microbiota directly, such as through immunologic function, or indirectly, such as through genetically determined behavioral traits, is unclear.

For some diseases, the microbiome may potently mediate genetic risk. For instance, variation in the NOD2 gene that mediates risk for inflammatory bowel disease (IBD) modifies the microbiota of the terminal ileum [58]. Another risk allele for IBD, CARD9, may mediate inflammation through the microbiota's ability to metabolize tryptophan, which is reduced in carriers of the CARD9 risk allele among subjects with IBD [59]. In another example, the presence of a risk allele for metabolic syndrome produces microbiome changes similar to those seen in obese patients [60]. Understanding these interactions further is essential for expanding the potential of microbiome interventions in personalized medicine.

Effect of Environment

In contrast to genetic determinants, environmental factors play a clear role in microbiome makeup and function. Diet is perhaps the most significant contributor and can alter the microbiome within a single day [61, 62]. Changes in the microbiome induced by diet involve alterations in metabolic processes, allowing for optimal digestion of foods. Metabolomic changes mediate downstream changes in weight gain or gastrointestinal disease through metabolic byproducts [61, 62]. While plant-based versus meat-based diets have been most scrutinized, many dietary elements affect the microbiome. For instance, high-fat diets increase levels of bacteria related to inflammation, whereas high fiber increases bacterial abundance and genetic richness, associated with some reduction in pro-inflammatory cytokines [63]. Diet may be an important way to manipulate the microbiome to target disease processes.

Other demographic and behavioral characteristics correlate with microbiotal features, though these may be partially confounded by diet. Microbiomes are similar in those with common living environment, geographic location, or ethnicity, indicating either direct sharing of species or similar behavioral determinants [23, 64]. Smoking cessation causes rapid and profound shifts in microbiota, including increased bacterial diversity, increased *Firmicutes*, and decreased *Bacteroidetes* [65]. A reduced proportion of *Bacteroidetes* is a prominent difference between obese and lean people [66], suggesting that the microbiome may mediate smoking cessation-related

Table 1 Condition-specific changes identified, and proposed mechanisms suggested in the literature

Condition	Microbiota changes observed	Potential mechanisms of disease	References
Aging	• Increased *Bacteroidetes* • Rearrangement of *Clostridium* • Decreased alpha diversity (especially with "biological age")	• Increased peripheral inflammation • Need to consider: ○ Institutionalization ○ Changes in diet ○ Increased antibiotic use	[26, 27•, 28, 31]
Parkinson's disease	• Reduced *Prevotellaceae* • Increased *Lactobacillus* and *Akkermansia* • Submucosal colonic *E. coli* invasion	• Increased mucosal and peripheral inflammation • Bacterial fragments/metabolites • Increased gastric permeability • Exacerbation/induction of protein aggregation • Need to consider: ○ Primary gastrointestinal symptoms ○ Medication use	[32•, 33–39]
Alzheimer's disease	• Elevated antibodies to oral pathogens in serum and brain (e.g., *P. gingivalis*) • Decreased alpha diversity • Bacterial products in post-mortem samples of hippocampus and associated with amyloid plaques • Higher *E. coli* in brain samples	• Periodontitis leading to increased peripheral inflammation or direct transmission of bacteria • Exacerbation/induction of protein aggregation and deposition by bacterial fragments • Need to consider: ○ Post-mortem translocation of microbiota when interpreting autopsy studies	[40, 41•, 42–45, 46••, 47]
Huntington's disease	• Alterations in tyrosine, tryptophan, and purine pathways related to microbiota functions	• Reduced antioxidant effect, depletion of energy • Need to consider: ○ Systemic effects of *huntingtin* mutation	[48]
ALS	• Reduced *Firmicutes/Bacteroidetes* ratio • Low *Ruminococcus*	• Increased peripheral inflammation • Increased gastric permeability • Need to consider: ○ Reduced mobility and body mass ○ Intraintestinal feeding	[49–53]
Depression	• Alterations in alpha diversity • Reduced *Prevotellaceae*	• Altered tryptophan metabolism • Increased peripheral inflammation • Need to consider: ○ Effect of stress itself on the microbiota	[31, 54•, 55]

weight gain [65]. Exercise also affects the microbiome. Among previously sedentary subjects, intense exercise led to higher bacterial production of short chain fatty acids (SCFAs), which was reversed after cessation of exercise [67]. These metabolic products have wide-ranging effects and their potential role in disease will be discussed below.

Antibiotic use is a profound example of how environment affects the microbiome, although their effects vary by class and are not always predictable. Antibiotics may induce relative brief and specific changes to microbial makeup, or conversely, they may have broad and persistent effects [68••, 69]. For example, tetracyclines and fluoroquinolones cause widespread induction of genes related to drug metabolism, stress response, and antibiotic resistance among microbiota [68••]. Evidence of genetic changes conferring bacterial resistance can be detected years after antibiotic use in some cases [70].

The recognition that antibiotics and other environmental factors change the microbiota so significantly has prompted investigations into forms of therapeutics aimed at re-establishing or accelerating the return of a healthy microbiome. These therapies include fecal transplants, probiotics (consumed bacteria with beneficial effects meant to improve the microbiota), or prebiotics (supplements indigestible by the human host but metabolized by microbiota) [71]. Whether these strategies can influence the microbiome in various conditions is still being investigated, but to date, persistent and targeted manipulation of the microbiome remains challenging.

How Does the Microbiome Influence Us?

In addition to effects on health and disease, there is increasing recognition that the gut microbiota influences the structure and function of the central nervous system (CNS), the so-called gut-brain axis, which in turn mediates health by influencing human behavior [72•]. For example, as mentioned above, changes in the ratio of two common phyla *Bacteroidetes* and *Firmicutes* are associated with obesity in mice and humans; germ-free mice that receive fecal transplants from obese human donors develop higher body fat than mice that receive transplants from lean donors [73••]. While this may be due to in part to altered food metabolism, the gut microbiome likely contributes to taste and appetite, which in turn affects eating habits [74]. In effect, the microbiota alters our diets to provide the nutritional milieu most beneficial to its survival, suggesting a bidirectional pathway of influence [7].

Other behaviors potentially altered by the gut microbiome include social interaction, stress, and pain sensation [75, 76].

Gut microbiota may regulate behavior by inducing local gastrointestinal hormone release that circulates to the CNS or via bacterial fragments and metabolites that have direct hypothalamic effects [77]. These products may regulate brain development and alter gene expression [75]. Gut microbiota has been shown to regulate blood-brain barrier permeability and serotonin release [78]. Neurotransmitter levels in the CNS may be further regulated by polyunsaturated fatty acids and SCFAs, both products of microbiota metabolism [78]. Microbiome-regulated immune system activation also affects microglial function in the CNS [79••]. Gut bacteria can directly induce peripheral inflammation through intraluminal interactions with the host, gut permeability, or molecular mimicry [79••]. Many of these interactions involve the vagus nerve, the main autonomic regulator of the gut [80].

Medication metabolism is a recently recognized effect of the microbiome on host function. The microbiome may enhance or alter medication metabolism and absorption, influencing efficacy and toxicity [81]. Prominent examples include effects on diabetic medications [82] and response to cancer immunotherapy medications [83, 84]. In the future, evaluation/manipulation of the microbiome may become an essential step prior to treatment for many types of therapy [85].

The Microbiome and Neurodegeneration

As mechanisms of the gut-brain axis are further elucidated, the clinical evidence for the role of the microbiome in neurologic disease, and neurodegenerative disease in particular, is mounting. The microbiome may allow us to understand the pathophysiology of these conditions in new ways and provide new routes of therapy. Though the field is still relatively young, the microbiome's involvement in neurodegenerative disease is already becoming clear.

Parkinson's Disease

The gastrointestinal microbiome is altered in Parkinson's disease compared with healthy controls, and these differences may contribute to disease symptoms and pathogenesis. On the most basic level, an altered microbiome may cause gastrointestinal symptoms. Gastrointestinal dysfunction is common in Parkinson's disease. Constipation is reported in up to 80% of patients and may precede motor symptoms by years or decades [86]. On a more fundamental level, the gastrointestinal microbiome may be causally related to Parkinson's disease. Braak and other investigators have long hypothesized that Parkinson's disease may start in the gastrointestinal system [87, 88], with retrograde transport of pathogens or alpha-synuclein protein aggregates into the central nervous system [89]. Even if not the site of disease initiation, an altered microbiome may increase systemic inflammation that hastens neurodegeneration. Finally, the microbiome may affect metabolism of therapeutic drugs, influencing patients' symptoms and complications.

Animal studies support the role of gastrointestinal dysbiosis. In a mouse model of Parkinson's disease that over-expresses alpha-synuclein protein, "germ-free" mice had fewer alpha-synuclein aggregates in brain than mice raised under standard conditions with typical complex microbiota [90••]. Among those with alpha-synuclein aggregates, germ-free mice had less microglial activation, and germ-free mice or antibiotic-treated mice raised under standard conditions had less motor deficits than those with typical microbiota [90••]. Investigators implanted germ-free mice with fecal transplants from patients with Parkinson's disease or from healthy donors and tested the performance of motor tasks previously validated in the same model, such as removing nasal adhesive. Mice that received fecal transplants from Parkinson's patients were slower at these tasks than those that received transplants from healthy controls, suggesting a directly causal role of microbiome changes [91].

Studies in humans also suggest a causal role for the microbiota in Parkinson's disease. Polymorphisms in peptidoglycan recognition protein genes (PGLYRPs), which regulate the immune response to gut bacteria, were associated with an increased risk of Parkinson's disease in two independent studies [92]. Though the microbiome has not been studied in these patients, this relationship suggests a dysfunctional interaction between the microbiome and immune response. An association between *Helicobacter pylori* and Parkinson's disease has been suggested by several studies [93]. In a population-based study that prospectively identified persons through pharmacy records, prior treatment with an *H. pylori* eradication drug regimen was associated with 45% increased risk [94]. Other *Helicobacter* species have also been associated with PD and with increased CNS inflammation in a mouse model [95, 96]. In addition to a possible etiologic role, active *H. pylori* infection may impair levodopa absorption, and eradication in infected patients improved medication effect and quality of life [97]. Small intestinal bowel overgrowth (SIBO), a syndrome of high bacterial density in the small intestine, is also more common in Parkinson's disease [91, 98]. A likely consequence of intestinal dysmotility in Parkinson's disease, SIBO may impair medication absorption and diminish its effectiveness [37], as described below.

Recent techniques have allowed a more comprehensive examination of fecal microbiome differences in Parkinson's disease [32•, 33–38]. In a prominent early study, Scheperjans et al. found that abundance of the bacterial family *Prevotellaceae* was 77.6% lower in Parkinson's disease patients than age- and sex-matched controls, independent of constipation or comorbid medical disease [32•]. The

abundance of *Prevotellaceae*, which are involved in vitamin synthesis and produce SCFAs, also correlated inversely with motor severity. SCFAs are well-studied bacterial metabolic products that can affect the central nervous system [73••]. In two other studies, investigators detected non-significant reductions of *Prevotellaceae*, though found other evidence for reduced production of anti-inflammatory SCFAs [33, 35]. Altered microbiota may therefore contribute to PD progression through reduction of SCFA synthesis. On the other hand, SCFAs induced inflammation and alpha-synuclein aggregation in a mouse model of PD [90••], suggesting more complexity to this relationship.

In addition to low *Prevotellaceae*, Scheperjans found higher abundance of several other families, including *Lactobacillaceae*, which may contribute to gastrointestinal hormone release and modulation of the enteric nervous system [32•]. Other studies have replicated some of these findings. Petrov et al. found low *Prevotella* and high *Lactobacillus* in persons with Parkinson's disease [37]. Bedarf et al. found low abundance of *Prevotella* among early-onset patients who had not started medications for Parkinson's disease [39]. Others found increased abundance of *Lactobacillus*, though did not detect lower levels of *Prevotella* [34, 36, 38]. Most recently, investigators examined microbiome differences associated with idiopathic REM sleep behavior disorder (iRBD), a well-recognized prodromal feature of early Parkinson's disease [99], and found low *Prevotella* in subjects with iRBD [99]. These differences may be explained by unappreciated confounders, such as geography or diet, or functional similarity between these organisms that is not appreciated from these compositional assays. Longitudinal studies as disease progresses will be most useful to tease apart these possibilities.

Studies focusing on other areas of the gastrointestinal tract have not yet shown significant changes between patients with PD and controls. One study found modest differences in the oral microbiome, but proposed that these were likely due to differences in Parkinson's disease-related oral hygiene, though this information was not systematically collected. No differences were seen in in the nasal microbiome [100].

Other studies have investigated mechanistic theories of microbiome contributions to the pathophysiology of Parkinson's disease. *Prevotella*, in addition to SCFA production, is also involved in mucin synthesis, the depletion of which can decrease gastric permeability [32•, 39]. Consistent with this, increased colonic permeability was found in patients with Parkinson's disease in a study that measured 24-h urinary excretion of a (typically) non-absorbed orally administered sugar [101••]. Intestinal permeability correlated with greater submucosal invasion of *E. coli* and alpha-synuclein inclusions in submucosal biopsies [101••]. Interestingly, *E. coli* bacteria may be increased in PD patients with a more severe subtype [32•]. Thus, microbiome-mediated increases in colonic permeability may contribute to the protein aggregation and propagation hypothesized to underlie PD pathogenesis.

The altered microbiome may lead to higher systemic inflammation in Parkinson's disease. Functional analysis of the microbiota changes described above suggests an increase in microbial genes that can induce inflammation, including those involved in synthesis of lipopolysaccharide (LPS) [33]. LPS, also known as endotoxin, is the primary constituent of gram-negative bacterial membranes and is the basis of a well-established animal model of Parkinson's disease. CNS or systemic infusion of exceedingly small amounts of LPS causes specific degeneration of nigral dopaminergic neurons, similar to the pattern seen in PD [102]. LPS-induced neuronal degeneration is mediated by blood-brain barrier impairment, CNS immune cell activation, and cytokine release and is likely both cause and effect of increased gastrointestinal permeability [102, 103]. LPS-binding protein (LBP) is elevated in some studies of Parkinson's disease, and peripheral inflammatory markers may be associated with risk and progression [104, 105]. A gastrointestinal inflammatory basis is also suggested by a recent study that identified mutations in the LRRK2 gene that are associated with both Crohn's disease and Parkinson's disease [106].

Finally, the gut microbiota may affect the symptoms of Parkinson's disease through metabolism of levodopa, the most effective therapy for Parkinson's disease. Rats grown in a germ-free environment are unable to fully metabolize levodopa [107]. In humans, levodopa metabolites change significantly after antibiotic administration, or with administration of enteric-coated levodopa, which is metabolized in the distal gastrointestinal tract [108, 109]. Variation in gut bacteria may underlie variable absorption and fluctuating benefit from Parkinson's disease medications. Indeed, the presence of SIBO in Parkinson's disease was associated with less benefit from medications, and treatment of SIBO with antibiotics improved medication effectiveness [110]. Manipulating delivery of medications or the gastrointestinal microbiota may lead to important therapeutic advances in the near future.

These studies support a relationship between Parkinson's disease and gastrointestinal microbiota, though the specific nature of this relationship needs clarification. Future studies will need to ensure that changes in microbiota are not simply a result of constipation, reduced physical activity, or some other non-specific reactions to chronic disease [39]. Even if not the initiating cause of Parkinson's disease, the gut microbiota may have an important role in its evolution and progression. Reducing the byproducts of disadvantageous bacteria or supplementing the metabolites of bacteria that are abnormally depleted in disease may be future treatment strategies.

Alzheimer's Disease

Compared with Parkinson's disease, investigations into the role of the microbiome in Alzheimer's disease are still relatively nascent. In contrast to Parkinson's disease, the oral microbiome has received the most attention. Interest in the oral microbiome derives in part from a relatively consistent epidemiologic association between periodontal disease and Alzheimer's disease, with risk increased 1.5–3-fold [111•]. A recent retrospective longitudinal cohort study found a 1.7-fold higher risk of development of Alzheimer's disease in persons with periodontal disease for at least 10 years [112]. Furthermore, among patients with prevalent Alzheimer's disease, current periodontal disease predicted more significant cognitive decline over a 6-month period [113]. This hypothesis is supported by clinical studies that found higher levels of antibodies to periodontal pathogens in serum samples from people who subsequently developed Alzheimer's disease [40, 41•], and by a mouse model wherein oral inoculation with *Porphyromonas gingivalis* led to the detection of the bacteria in the central nervous system [42].

Several other studies have found evidence for the role of bacteria in Alzheimer's disease pathophysiology. Recapitulating some of the findings in Parkinson's disease, broad-spectrum antibiotics reduced amyloid plaque deposition, increased soluble amyloid-beta, and reduced inflammation in a mouse model of Alzheimer's disease [114]. A small study using next-generation sequencing in post-mortem brain tissue found higher reads of bacteria in the temporal cortex of patients with Alzheimer's disease compared with controls [43]. Specifically, higher levels of *Propionibacterium acnes* were found, an organism associated with pro-inflammatory changes in other studies. A primary criticism of this provocative finding, which requires replication, is the possibility of post-mortem specimen contamination by extrinsic sources of bacteria.

Several studies have found alterations in the gastrointestinal microbiome in Alzheimer's disease. Cattaneo et al. found increased *Escherichia/Shigella* and reduced *E. rectale* in fecal samples from subjects with cognitive impairment and amyloid deposition on PET imaging [44]. These differences were thought to represent a more inflammatory state and indeed correlated with higher serum levels of inflammatory cytokines. Vogt et al. found reduced within-subject microbial diversity in fecal samples from people with Alzheimer's disease compared to age- and sex-matched controls [115], as well as reduced relative abundance of *Firmicutes* and increased *Bacteroidetes*. Reduced *Firmicutes* is associated with insulin sensitivity and diabetes, established risk factors for Alzheimer's disease, and increased *Bacteroidetes* may trigger an inflammatory state through LPS secretion [48]. Interestingly, these changes correlated with CSF biomarkers of Alzheimer's pathology in both case and control subjects.

A suggested mechanism by which the microbiome induces neurodegeneration in Alzheimer's disease is through neurotoxic inflammation, perhaps mediated by LPS [116]. LPS derived from periodontal bacteria and injected into mice caused amyloid buildup and memory impairment [117]. In an autopsy study, LPS from oral pathogens, specifically *P. gingivalis*, was found in post-mortem brain samples of patients with Alzheimer's disease [45]. In a separate study, higher levels of LPS were found in the brains of Alzheimer's patients than in controls and co-localized with amyloid plaques and amyloid around vessels [118]. The same study also found a higher prevalence of *E. coli* pili in brain samples [46••]. In a separate study, LPS was again found to be more prevalent in the brains of patients with Alzheimer's disease than that of controls, more so in the hippocampus than the neocortex and more so in patients with severe disease [47]. As with all post-mortem human studies, the risk of contamination and an understanding of the potential spread of bacteria after death are important considerations.

Other Neurodegenerative Diseases

The microbiome has been only minimally studied in other neurodegenerative conditions, though the literature is expanding rapidly. Patterns of serum metabolites involved in the tyrosine, tryptophan, and purine pathways enabled investigators to accurately distinguish controls from pre-symptomatic carriers of the Huntington's mutation and pre-symptomatic carriers from disease-manifesting patients [48]. In particular, metabolites known to be produced by gut microbiota best differentiated controls from pre-symptomatic subjects, whereas metabolites related to neurodegeneration distinguished symptomatic from pre-symptomatic carriers [48]. However, direct sampling of the microbiota was not performed.

Amyotrophic lateral sclerosis (ALS) is a multifactorial and clinically diverse neurodegenerative disease that affects upper and lower motor neurons of the motor cortex and spinal cord. In a mouse model of ALS that expresses mutant superoxide dismutase ($SOD1^{G93A}$), intestinal epithelial tight junctions were found to be impaired, with higher gastrointestinal permeability [49]. In addition, mutant mice had fewer Paneth cells (specialized intestinal immune epithelial cells) and lower levels of the intestinal antimicrobial peptide defensin 5-alpha. In comparison to wild-type mice, the relative abundance of organisms was altered, including reduced levels of *Butyrivibrio fibrisolvens*, *Escherichia coli*, and *Firmicutes*, and these changes were associated with higher peripheral inflammation. In the same mouse model, treatment with butyrate, a SCFA thought to modulate inflammatory response and reduce gastrointestinal permeability, delayed disease onset and mortality [50]. Two subsequent small studies in humans, each with approximately ten subjects in total, have shown alterations in the fecal microbiomes from ALS patients. One

study reported higher relative levels of *Bacteroidetes* in ALS, while the other found a low ratio of *Firmicutes*/*Bacteroidetes* as well as elevated fecal inflammatory markers (fecal secretory IgA, calprotectin and/or eosinophilic protein X) [51, 52]. However, a large trial that rigorously controlled for confounders potentially common in ALS (e.g., dysphagia, gastrostomy, body mass index) found no differences in either the diversity or abundance of bacterial taxa or in metagenomes among ALS patients and controls [53]. More work is needed to clarify the role of the microbiome in ALS pathology.

Neuropsychiatric Disease

While not strictly considered a neurodegenerative disease, psychiatric symptoms such as depression or anxiety accompany neurodegenerative disease and are often the presenting features [119]. Early evidence suggests a role for the microbiome in development of neuropsychiatric disease. Transfer of fecal samples from people with chronic depression into germ-free rats induced behavioral changes suggestive of anhedonia and anxiety [54•]. Microbiome differences have also been reported in schizophrenia, autism spectrum disorder, and attention deficit hyperactivity disorder, spurring interest in clinical trials to alter the microbiome [55]. Whether these differences are causal or reflective of disease-associated phenomena remains to be determined [31].

Conclusion

Increasing recognition of the role of the microbiome in neurodegenerative disease has spurred many exciting new research directions. This work may provide new avenues for revealing pathophysiologic processes and for mapping out previously unappreciated interactions between the environment and the brain. Although the potential clinical implications are vast, the field is still incredibly young—everything from sample collection to analytic techniques to interpretation of results still needs standardization. The number of potential confounders and unmeasured effects is enormous, and observations in animal models require replication in well-controlled longitudinal human studies. We are still at the threshold of understanding the gut-brain axis and the role of the microbiome in neurodegenerative disease causation, treatment, and prevention.

Compliance with Ethical Standards

Conflict of Interest Caroline Tanner reports personal fees from Adamas, Neurocrine, Photopharmics, Alexza, and 23andMe and fees from Voyager, Intec, and Biotie for DMC service. Samuel Goldman declares no conflict of interest. Ethan Brown reports personal fees from Abbvie, Inc., for serving on the Fellowship Advisory Board, outside the submitted work.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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