

The Gut Microbiome in Human Neurological Disease: A Review

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Almost half the cells and 1% of the unique genes found in our bodies are human, the rest are from microbes, predominantly bacteria, archaea, fungi, and viruses. These microorganisms collectively form the human microbiota, with most colonizing the gut. Recent technological advances, open access data libraries, and application of high-throughput sequencing have allowed these microbes to be identified and their contribution to neurological health to be examined. Emerging evidence links perturbations in the gut microbiota to neurological disease, including disease risk, activity, and progression. This review provides an overview of the recent advances in microbiome research in relation to neuro(auto)immune and neurodegenerative conditions affecting humans, such as multiple sclerosis, neuromyelitis optica spectrum disorders, Parkinson disease, Alzheimer disease, Huntington disease, and amyotrophic lateral sclerosis. Study design and terminology used in this rapidly evolving, highly multidisciplinary field are summarized to empower and engage the neurology community in this “newly discovered organ.”

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Despite extensive clinical and biomedical research, the etiology, progression, and optimal treatment of prominent neurological disorders remain largely unknown. The etiopathology of these conditions is likely multifactorial. Recently, the human microbiota has been proposed as a key component (Figs 1 and 2). Studies have established complex and varied interactions between the gut microbiota and the central nervous system (CNS; see Fig 1).¹ These bidirectional interactions form the gut microbiota–brain axis.^{2,3} Epidemiological studies mark the first steps to assess whether, and to what extent, the gut microbiota–brain axis informs human neurological disease. This review highlights the nascent studies involving human subjects, which examine the association between the gut microbiota and chronic neurodegenerative and neuro(auto)immune conditions.

Search Strategy and Selection Criteria

References for this review were identified by searching PubMed for journal articles published in English between

January 1, 2010 and July 1, 2016 using the following terms (and alternative spellings): “multiple sclerosis”, “Alzheimer’s”, “Parkinson’s”, “amyotrophic lateral sclerosis”, “Huntington’s”, “neuromyelitis optica”, “neuromyelitis optica spectrum disorders”, “microbiome”, and “microbiota”. The total number of publications resulting from this type of broad search can be seen in Figure 3 (2010–2016). In addition, the reference lists of articles were reviewed along with the authors’ own files, and the most relevant articles were included within this review. The primary focus (selection criteria) was for peer-reviewed journal articles involving humans (original observational case–control, cohort or intervention studies, or other reviews of original work) and the association between at least one of the neurological conditions above and the gut microbiome/microbiota, through direct interrogation of the human microbiota. Studies involving other microbiomes (eg, lung, nasal, mouth) were a secondary focus due, in part, to the limited literature. Case reports and case series were excluded. Select studies

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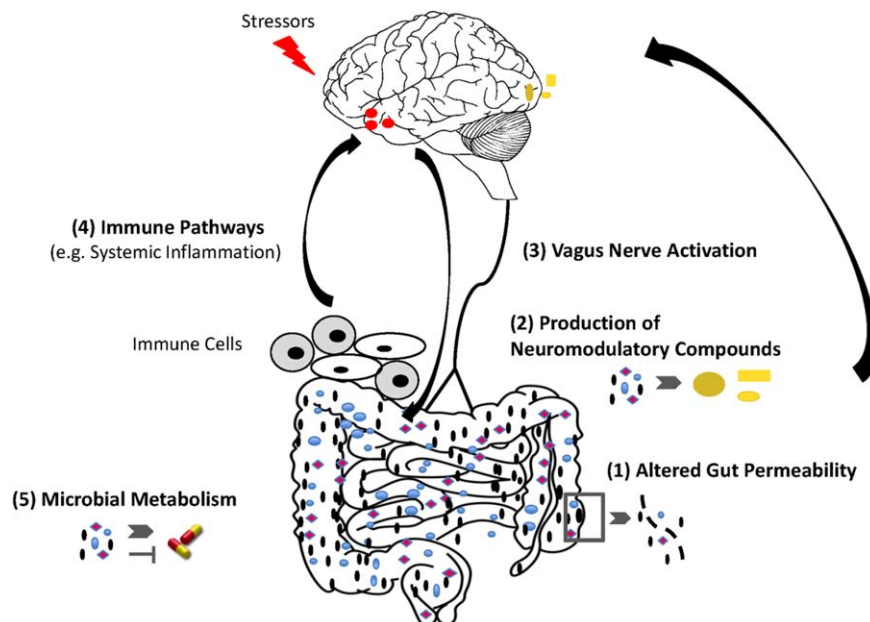


FIGURE 1: Gut–brain interactions. Proposed mechanisms suggest bidirectional communication. There is a growing body of work indicating interactions between the host, the brain, and the microbiome,^{1–3} facilitated by the following. (1) Increased gut permeability enables either microbes or microbial metabolites to enter the bloodstream. (2) Gut microbes produce neuromodulatory metabolites (eg, short chain fatty acids), as well as induce the production of host-derived vitamins (B12), neurotransmitters (eg, serotonin), and hormones (peptide YY) that may impact neurological and host health. (3) Bidirectional interactions may occur directly via innervation of the vagus nerve, providing a direct line of communication between the enteric and central nervous system. (4) In addition, gut–brain interactions can occur through immune-mediated inflammatory pathways, such as (a) microbial-driven systemic inflammation linked to the progression of neurodegenerative diseases, and (b) stressors that can alter the gut via inflammatory pathways. (5) Finally, gut microbes can metabolize xenobiotics, impacting neurological function. Furthermore, alterations of the gut microbiota have been linked with comorbidities, such as depression,¹³⁶ which are common in neurological conditions such as multiple sclerosis and Parkinson disease and may contribute to (or even result from) these interactions (see also Fig 2).^{137,138} [Color figure can be viewed at wileyonlinelibrary.com]

involving animal models of these neurological conditions or distal biomarkers of the microbiome (rather than direct interrogation) and older studies representing landmark advances were included, as necessary, to place current findings in context. A Glossary of Terms Used in Microbiome Research is available online as a supplementary file. The first time such a term is introduced into the text it appears underlined and in italics.

Who's There? Identifying the Gut Microbiota

Trillions of microorganisms colonize humans at birth,⁴ with the mode of delivery (Caesarean section or vaginal) influencing, at least over the short term, early colonization of the gut.⁵ Predominantly composed of nonpathogenic bacteria,^{4,6} these host-associated microbes (human *microbiota*) and their genomic potential (human *microbiome*) have been conventionally examined by anatomical location, notably skin, mouth, respiratory, urogenital, and gastrointestinal (GI) tract.^{7,8} The GI tract forms the largest human–microbial interface,⁹ reaching the highest microbial density within the colon.^{4,10,11} The majority of “gut” bacteria belong to either the Bacteroidetes or

Firmicutes phyla.¹² The adult microbiome is estimated to contain >100 times more genes than the human genome.^{4,10} The microbiome contributes to digestive, immune, metabolic, and various neurological functions.^{1,2,12,13} Alterations of the gut microbiota (the dysbiotic microbiota) have been linked with various diseases and disorders, including obesity,¹² gastric disorders,¹⁴ diabetes,¹⁵ autoimmune disease,¹⁶ asthma,¹⁷ and recently, neurological conditions.¹⁸

Roughly the same weight as the human brain,¹ the gut microbiota modulates development and homeostasis of the CNS through immune, circulatory, and neural pathways (see Fig 1).^{2,3} The CNS, in turn, shapes the gut microbial community via stress and endocrine responses.^{3,19} These collective bidirectional interactions, termed the gut microbiota–brain axis, likely influence the etiopathology of complex CNS conditions (see Fig 2).³

16S rRNA, High-Throughput Sequencing and Sequence Identification

The gut microbiota is largely anaerobic and uncultivated,^{20,21} and its identity and vast regulatory potential have been enabled, in part, by advances in high-

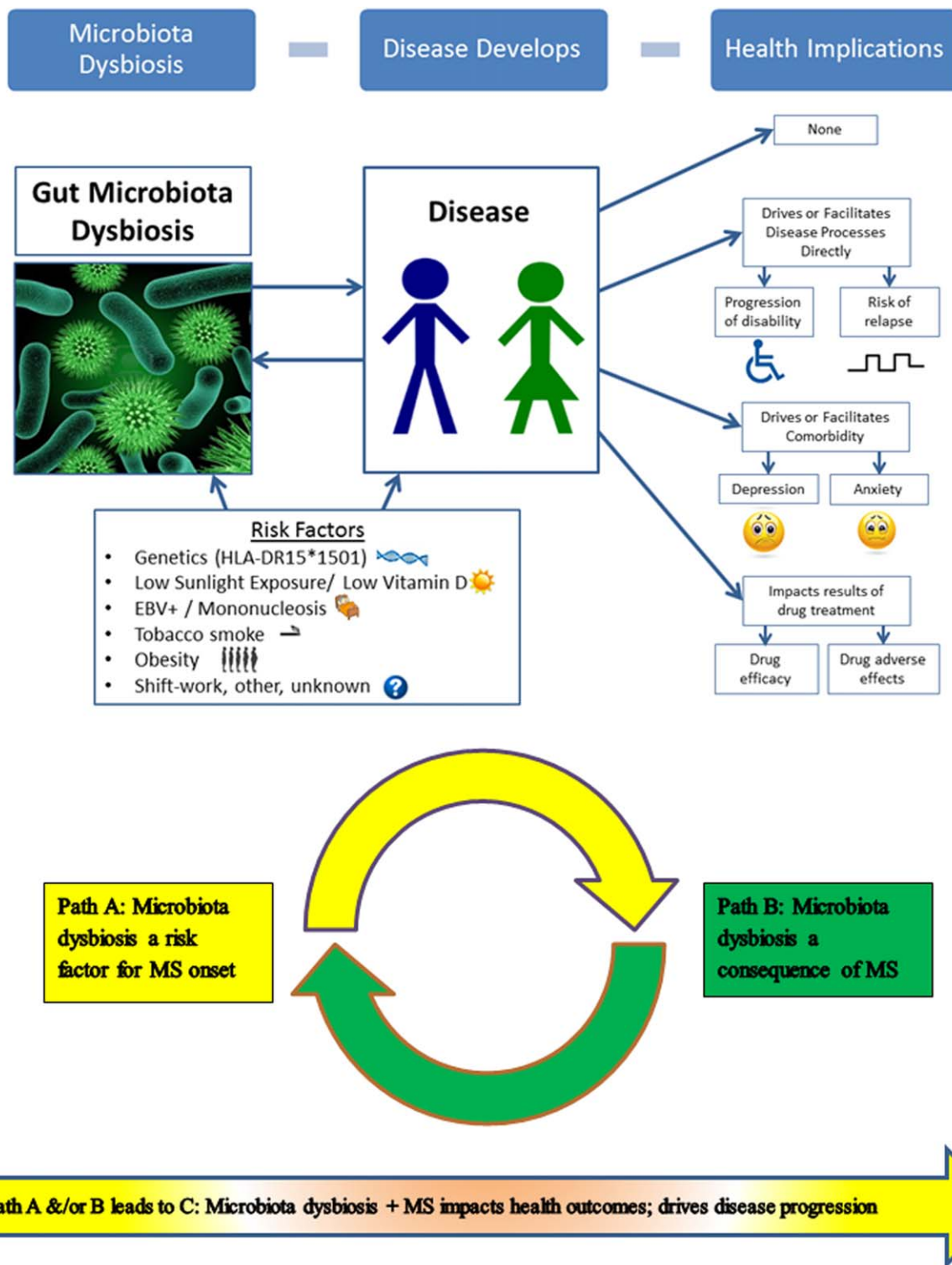


FIGURE 2: The potential role(s) of microbiota dysbiosis in disease, using multiple sclerosis (MS) as a model of neurological disease. The potential role of gut microbiota dysbiosis in facilitating the onset of MS (Path A) or driving disease progression (Path B) and related health outcomes (Path C) are depicted. The 3 paths are not necessarily mutually exclusive; elements of each or none could occur. Gut microbiota dysbiosis may contribute to disease onset (A), and also result from presence of disease (B). Either Path A or B could result in C. Path A: Gut microbiota dysbiosis as a risk factor for the development of neurological disease. The gut microbiota composition, combined with the presence of other risk factors, may play a role in disease causation. The gut microbiota could represent a component cause, that is, one of several possible risk factors that when combined would cause disease. The gut microbiota may or may not be a necessary cause (eg, if a necessary cause, then every individual would need to experience gut dysbiosis to develop disease). Path B: Gut microbiota dysbiosis as a consequence of disease. Gut microbiota dysbiosis may be (further) altered by the presence of disease and in some individuals contribute to future disease activity. Path C: Gut microbiota dysbiosis in the presence of MS may impact health outcomes and disease activity or progression, including risk of comorbidity (eg, mental health, depression, and anxiety) and response to drug treatments. EBV = Epstein–Barr virus. [Color figure can be viewed at wileyonlinelibrary.com]

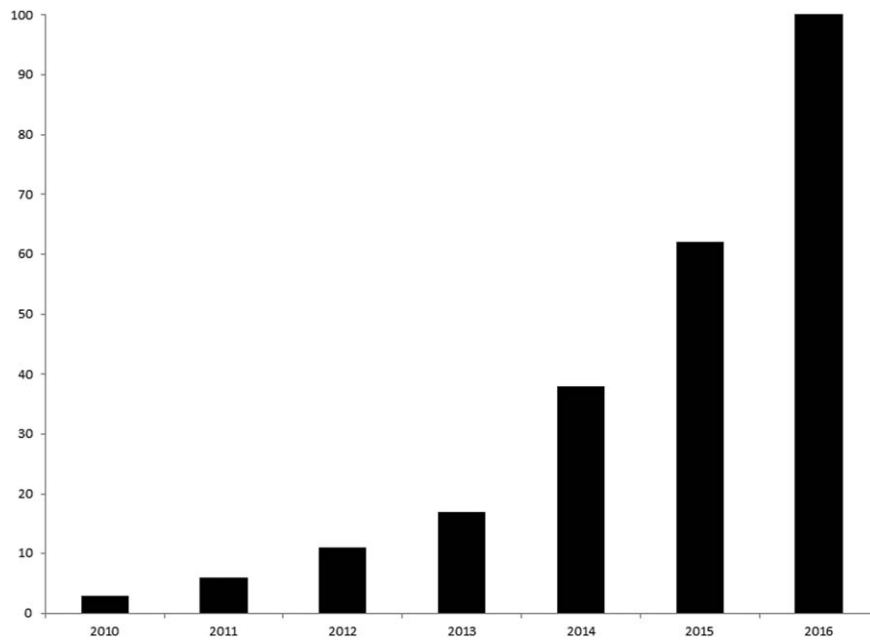


FIGURE 3: Total number of microbiome-related publications in PubMed, by year, including each neurological condition covered in this review (2010–2016). Search terms used: microbiome and multiple sclerosis OR microbiome and Alzheimer’s OR microbiome and Parkinson’s OR microbiome and amyotrophic lateral sclerosis OR microbiome and Huntington’s OR microbiome and neuromyelitis optica. For completeness, the search was performed for each entire year (2010–2016). The y-axis includes the crude total number of articles identified in PubMed (preselection). The x-axis shows the year articles first appeared on PubMed.

throughput sequencing techniques. Initial techniques included 454 pyrosequencing (Roche, Indianapolis, IN) and Ion Torrent, which are now being replaced with the widely adopted Illumina (San Diego, CA) platform.^{22,23} A limitation of these high-throughput sequencing techniques is the inability to unambiguously assemble large and/or repetitive genomic structures, due to short read lengths. The recently developed Pacific Biosciences (Menlo Park, CA) and Oxford Nanopore Technologies (Oxford, UK) MinION sequencing platforms enable rapid run times of much longer read lengths (~10–100kbp), albeit with higher error rates.^{24,25} Hybrid methods that combine these long-read sequencing techniques with the Illumina platform to more accurately assemble bacterial genomes have yielded promising results.^{24,25} It is anticipated that such advancements in deep sequencing technologies will inform future analyses of the microbiome and neurological disease. The field has also been shaped and advanced by the technologies and pipelines developed in relation to investments (2008–2012) into large collaborative efforts such as the Human Microbiome Project and Metagenomics of the Human Intestinal Tract, supported by the USA’s National Institutes of Health and the European Commission, respectively.

Identification of the *16S ribosomal RNA1* (rRNA) marker using DNA primers is the most commonly used

approach in the sequencing of bacteria and archaea (prokaryotes). First proposed in the late 1970s,²⁶ the technique capitalizes on a highly conserved region within the ribosomal subunit.^{27,28} In eukaryotes (eg, fungi), the 18S rRNA region or *ITS1* (internal transcribed spacer 1) gene have been used.²⁹ These contain hypervariable regions that are unique species-specific genomic “signatures.”^{30,31} The 16S rRNA sequence contains 9 hypervariable regions (V1–V9)³⁰; V2 and V4 are reported as having lower error rates when assigning *taxonomy*.³² However, the optimal hypervariable region(s) to target and amplify (using universal primers) for gut microbial analyses remains debated.³² “Primer bias” (eg, over- or underrepresentation of specific *taxa*) can affect findings and comparisons between studies,³² necessitating careful consideration of the primer used. An alternative to 16S rRNA sequencing is chip-based platforms, which are preloaded with a fixed number of known taxa. Benefits include good probe depth, meaning that if one of these known taxa is present, it is unlikely to be missed. However, the inability to detect unique or novel microbes, combined with relatively high cost and qualitative results based on fluorescence, rather than the quantitative data obtained with 16S rRNA sequencing, results in limited usage.³³

Following high-throughput sequencing, 16S rRNA sequences are clustered by sequence similarity into

operational taxonomic units (OTUs), the most utilized unit of microbial diversity, and identification is determined using one of the published and freely available 16S ribosomal databases (eg, Greengenes, SILVA).^{34,35} The threshold to discriminate OTUs is technically arbitrary, with the cutoff commonly set at 97% sequence similarity.^{36,37} Although it is a convenient and powerful technique to report the biological and ecological niche of largely unculturable gut microbes, the extent to which 16S rRNA clustering recapitulates the “true” microbial phylogeny remains debated. Choices throughout the analytical pipeline, including which hypervariable region to amplify, the threshold level used (eg, 97%), the database version, and the clustering algorithm selected (eg, UCLUST³⁸), can subtly influence findings,^{36,37,39} highlighting the need for standardization within a study and caution when comparing across studies.

Techniques such as whole genome shotgun sequencing are required to identify all the genes within the microbiota (ie, the microbiome), including microbial entities such as the *mycobiome* (fungi) and *virome* (viruses), as well as bacteria. In contrast to 16S rRNA sequencing, whole genome sequencing utilizes random hexamer primers rather than 16S universal primers.⁴⁰ This approach generates vast quantities of information, and computational challenges. Whether sequencing using a targeted 16S rRNA gene marker approach or more complex metagenomics, powerful, open source bioinformatics software, such as mothur⁴¹ and Quantitative Insights Into Microbial Ecology (QIIME; pronounced “chime”),⁴² can greatly facilitate several key steps of the bioinformatics pipeline. These steps include the generation of raw sequencing data through to basic statistical analyses and visualization of results.

It is worth noting that most studies included in this review have utilized 16S rRNA sequencing, which typically allows most identified bacteria (>80%) to be named at the level of phylum through to family (>80%), but often few species.

What Are They Doing? Functionality of the Microbiota

In 2012, the Human Microbiome Project Consortium reported striking interindividual variation of the human microbiota, but relatively stable functional profiles across individuals for a given body site (eg, the gut).⁸ Nonetheless, perturbations of the gut microbiota composition (eg, with extreme changes in diet) can rapidly alter these metabolic and functional profiles.⁴³ Various microbes can perform similar roles or contribute to the same metabolic pathways. Conversely, the same species of *Escherichia coli*, for example, can perform highly diverse roles, from

pathogen to beneficial producer of vitamins K and B12, depending on the strain.⁴⁴ Although functional studies have revealed vast interactions between the human gut microbiota and brain,² much remains unknown. Many microbial genes (“microbial dark matter”) remain unannotated.⁴⁵ A functional, or “omics”-based, approach can include: (1) metagenomics (sequencing techniques) and (2) metatranscriptomics, metaproteomics, and *metabolomics*, that is, interrogation of microbiome genomic expression, protein abundance, and metabolite production, respectively.⁴⁶ The former can help address “What can they do?” and the latter, “Who is doing what?” A practical challenge when conducting metabolomics-related studies involving stool samples from patients includes the need for prompt processing or freezing (eg, to -80°C) to prevent sample degradation.⁴⁶

Validated algorithms such as Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt)⁴⁷ can be employed to enable *metagenomic* predictions from 16S rRNA sequencing data, allowing inferences to be made about the potential functional capacity of the microbiota in neurological conditions. To date, this has been reported in multiple sclerosis (MS)^{48,49} and Parkinson disease (PD).⁵⁰

The Microbiome and Neuroimmune Conditions

MS

Compelling evidence for the microbiome’s role as either a trigger of neuroimmune disease or a driver of neuroimmune disease activity has largely emerged from animal models of MS (reviewed elsewhere^{51,52}). Briefly, mice raised in a “germ-free” environment were highly resistant to developing experimental autoimmune encephalomyelitis (EAE),^{53–55} an animal model of MS, unless receiving a fecal transplant from mice colonized with a gut microbiota.⁵⁵ Furthermore, reduced disease activity (lower clinical scores) was observed in germ-free mice when EAE was induced.^{54,55} Circumstantial evidence includes the overlap between many of the potential risk factors for MS and the gut microbiota; environmental factors associated with MS onset such as obesity,⁵⁶ smoking,⁵⁷ viruses,^{58,59} and vitamin D/sunlight/season⁶⁰ can also profoundly impact the gut microbiota (see Fig 2), as can host genetics (human leukocyte antigen),⁶¹ and a person’s age and sex.^{62,63} An increased prevalence of “leaky gut” (measured via an oral lactulose/mannitol test) was also recently suggested in a pilot study of 22 relapsing–remitting MS cases compared to controls.⁶⁴

Differences in the Gut Microbiota between MS Cases and Healthy Controls

Studies are emerging involving both adult and pediatric MS subjects (Supplementary Table).^{48,49,65–68} All included those with relapsing–remitting onset and showed differences, at some level, between the MS subjects' and controls' gut microbiota composition. Most were cross-sectional, case–control studies,^{48,49,65–68} with individual subjects sampled either very close to MS symptom onset⁴⁹ or after having disease for many years.⁶⁵ Whether observed differences in the gut microbiota between cases and controls resulted from or preceded MS remains unknown (see Fig 2, pathways A and/or B may apply). Overall, at the community composition level, *diversity* of the gut microbiota (*alpha* or *beta*; both are measures of the number or types of microbes present; see supplementary online Glossary) typically did not differ significantly between MS cases and controls, although modest differences might be missed in these small studies (see Supplementary Table). A high alpha diversity (ie, indicating many different microbes within a sample) is generally associated with “good” health.⁸ Differences in beta diversity (a measure assessing how distinctive the microbiota are between two groups of individuals⁶⁹ between cases and controls, when found, appeared related to MS disease-modifying drug exposure rather than MS itself.^{49,67}

At the taxon level, the relative abundance of specific groups of microbes differed significantly between cases and controls.^{48,49,65–68} These preliminary findings suggest subtle, discrete taxonomic enrichments and depletions rather than large differences in the community composition related to MS. Although direct comparisons across studies are challenging due to differences in study design, consistent patterns can be observed.⁴⁹ These include enrichment and depletion of microbial genera suggestive of a proinflammatory milieu. Overlap with other (auto)immune inflammatory conditions have been reported, including inflammatory bowel disease (eg, Crohn disease)^{49,70,71} and conditions not considered as traditionally gut-related, such as rheumatoid arthritis.⁷² Whether there is a specific gut microbiota signature of MS remains to be determined. Some researchers have highlighted or pursued specific groups of microbes. For example, the enrichment of Archaea (genus *Methanobrevibacter*) in some MS subjects was reported relative to controls⁶⁵ as well as depletion of members from the Firmicutes (eg, *Clostridium* genera) and Bacteroidetes phyla.^{49,66–68} Although not a focus of the current review, single biomarkers such as serum levels of a lipopeptide (Lipid 654) thought to be derived from oral or GI bacteria (Bacteroidetes) were found to be lower in primarily

disease-modifying drug–treated MS cases (n = 17) relative to 12 healthy controls.⁷³

No studies were found where the functional capacity of the gut microbiota in MS had been comprehensively assessed, although 2 explored the predicted metagenome using PICRUSt and found significant differences between cases and controls for pathways involving fatty acid metabolism, lipopolysaccharide biosynthesis, and glycolysis/glutathione metabolism.^{48,49} Individuals exposed to an MS disease-modifying drug also exhibited a predicted enrichment in pathways involved with immune responses compared to non–MS drug-exposed individuals.^{48,49}

Gut Microbiota and MS Disease Activity

One small cross-sectional study reported differences in the gut microbiota community composition, measured as beta-diversity, by proximity to a relapse (samples collected within 1 month of a relapse were compared to those collected at other times).⁴⁸ However, demographic and MS disease-modifying drug exposure differences between the two groups may have contributed to this observation.⁴⁸ Nonetheless, the concept that the microbiota might contribute to disease activity is intriguing. One small longitudinal study involving pediatric MS subjects found that the gut microbiota profiles (assessed at the phylum level) were associated with future relapse risk.⁷⁴ Specifically, in 17 California-based children, absence (depletion) of *Fusobacteria* was associated with a 76% risk (95% confidence interval [CI] = 55–90%) of an earlier relapse (hazard ratio [HR] = 3.2, 95% CI = 1.2–9.0, $p = 0.024$ adjusted for age and MS disease-modifying drug exposure).⁷⁴

Other Microbiomes and MS

Few published studies were found assessing the microbiome in body sites other than the gut or for kingdoms other than Bacteria and Archaea, and none fulfilled criteria for inclusion in this review. Briefly, researchers are actively pursuing these areas, for example, by sampling from the mouth, nasal passages, or autopsied brain tissue.⁷⁵ Aspects of the Fungi microbiome (the *mycobiome*) in MS have been explored; higher odds of serum antigen presence (vs absence) to specific *Candida* spp. in MS participants compared to blood donors have been reported.⁷⁶ Finally, parasitic gut helminths, implicated in MS risk or progression, are being explored by some groups in the context of interactions with the gut microbiota, but are beyond the scope of this review.⁷⁷

Neuromyelitis Optica Spectrum Disorders

A potential role for the gut microbiota in neuromyelitis optica (NMO) spectrum disorders was initially observed

indirectly, through either the presence of antibodies against GI antigens,⁷⁸ or peripheral blood T cells cross-reactive with both aquaporin-4 and a marker found in the gut microbiota (the *Clostridium* adenosine triphosphate-binding cassette transporter).⁷⁹ However, findings might not be entirely specific to NMO, as GI antibodies were also found in MS subjects and healthy controls⁷⁸ and most NMO patients were rituximab-exposed at the time of stool collection.^{78,79} Nonetheless, further work is warranted to investigate whether findings indicate a role for gut microbiota in NMO pathogenesis or reflect a consequence of treatment.

POST-LITERATURE REVIEW UPDATE. One group used a chip-based platform, preloaded with specific taxa, including a probe for *Clostridium perfringens*, to interrogate the gut microbiota from 16 largely treated NMO patients who were aquaporin-4 positive (8 were exposed to rituximab and 7 to a cytotoxic immunosuppressant). These were compared to 16 healthy controls, and 16 rituximab-treated and untreated MS controls.⁸⁰ Differences in the gut microbiota community composition were observed between NMO cases and healthy controls (beta diversity, weighted UniFrac, Adonis test, $p < 0.001$) and possibly also between the rituximab-treated individuals (NMO vs MS). Differences in the relative abundance (measured as fluorescence) of several taxa (see Supplementary Table) included higher *C. perfringens* in the NMO cases ($p = 5.24 \times 10^{-8}$). The authors concluded that the role of *C. perfringens* in NMO pathogenesis should be investigated in drug-naïve patients.⁸⁰

The Microbiome and Neurodegenerative Conditions

Neurodegenerative disorders such as Alzheimer disease (AD), PD, and amyotrophic lateral sclerosis (ALS) share several features^{81,82}: (1) accumulation of misfolded proteins (amyloid- β and hyperphosphorylated tau in AD, α -synuclein in PD, and TDP-43 in ALS), (2) evidence for a prionlike spread of pathology with misfolded proteins,⁸³ and (3) neuroinflammation. However, it remains unclear which factors initiate or maintain these processes. A complex combination of host and environmental factors is likely, with emerging evidence placing the microbiome at this interface.

PD

Premotor symptoms of PD such as constipation, hyposmia, sleep disorders, and depression are now recognized to occur years, even decades before motor symptoms appear⁸⁴ and will affect the vast majority of PD patients at some point.^{85–87} Strikingly, changes in either the gut

or the olfactory bulb occur in close proximity to mucosal surfaces that are densely populated by microbes.

Converging lines of evidence support the concept that pathological changes in PD involving α -synuclein deposition can initiate in the nervous system of the gut and the olfactory bulb.⁸⁷ Deposits can be demonstrated even in the premotor phase,^{88,89} although the finding is not necessarily specific to PD.⁹⁰ Within the GI tract, synuclein deposits show a rostrocaudal gradient with the highest concentrations in the submandibular gland and lower levels in the oesophagus.⁹¹ This gradient might reflect the potential role of a yet unknown orally or nasally ingested agent.⁹² The vagus nerve may then facilitate access and spread to the brainstem and subsequent ascent toward the cortex.⁸⁷ A Danish cohort study reported that a full, but not partial, truncal vagotomy was associated with a decreased risk of PD compared to controls in individuals followed up for >20 years (adjusted HR = 0.53, 95% CI = 0.28–0.99).⁹³ Increased total intestinal permeability has been associated with PD,⁹⁶ which in turn correlated to increased mucosal staining for *Escherichia coli* (Enterobacteriaceae family) and α -synuclein as well as lower lipopolysaccharide-binding protein in plasma, suggesting higher endotoxin exposure.^{95,96} Recent intriguing work in an α -synuclein over-expressing (ASO) mouse model of PD⁹⁴ demonstrated that mice raised in a germ-free environment developed little PD-related pathophysiology (motor dysfunction, neuroinflammation, and α -synuclein pathology), akin to that observed in the mouse models of MS. When germ-free ASO mice were either colonized with feces from wild-type mice or orally fed bacterial metabolites (short chain fatty acids) without colonization, this effect was reversed.⁹⁴ Depletion of the microbiota with antibiotics in young ASO mice prevented the development of later parkinsonian symptoms, pathology, and microglia activation. Colonization of germ-free ASO mice with feces from human subjects with PD (relative to feces from healthy donors) resulted in worse motor symptoms. Although this elegant series of experiments demonstrates the key role of the gut microbiota in the development of parkinsonian symptoms, pathology, and microglia activation in a specific PD mouse model, replication by others is needed, and use of additional models would be insightful. Nonetheless, this study presents an important step toward the development of treatment studies in (presymptomatic) human subjects. Evidence for the gut as an initial site of PD pathology and the establishment of the gut–brain axis provided the framework for 3 recent cross-sectional, moderately sized studies on the gut microbiota in PD.^{50,95,97} All observed differences

between the microbiota in established PD compared to controls (see Supplementary Table).

A Finnish study reported differences in beta but not alpha diversity and a lower abundance of Prevotellaceae (family) in the feces of subjects with PD compared to controls.⁹⁷ Low levels of Prevotellaceae (relative abundance $\leq 6.5\%$) exhibited 86.1% sensitivity, but only 38.9% specificity for PD.⁹⁷ When combined with severity of constipation, Prevotellaceae, Lactobacillaceae, Bradyrhizobiaceae, and Clostridiales IV abundance could be used to identify PD cases with 66.7% sensitivity and 90.3% specificity. Furthermore, postural instability and gait symptoms were associated with the relative abundance of Enterobacteriaceae.⁹⁷ In a United States–based study, the fecal samples and sigmoid mucosal biopsies from PD subjects exhibited lower abundance of bacteria with presumed anti-inflammatory properties and a higher abundance of the putative proinflammatory Proteobacteria.⁵⁰ In addition, a predicted enrichment in genes involved in lipopolysaccharide synthesis in PD subjects relative to controls was reported (using PICRUSt).⁵⁰ In general, more of the differences between cases and controls were observed in the stool samples relative to the mucosal biopsies.⁵⁰ Finally, a Japanese study reported counts of 19 previously cultured microbial groups in stool samples from PD cases and spousal controls.⁹⁵ Lower bacterial counts were observed in PD cases relative to controls, as were lower serum levels of lipopolysaccharide-binding protein.⁹⁵ Both the U.S. and Japanese studies found nonsignificant reductions for Prevotellaceae in PD subjects compared to controls.^{50,95} Loss of Prevotellaceae may contribute to impaired gut barrier function in PD.^{95,97} Together, these cross-sectional studies suggest a proinflammatory gut milieu in PD, which when combined with other measures, might lead to clinically useful microbial biomarkers for PD.^{50,95,97} Longitudinal studies, especially those beginning in the premotor phase,⁹⁸ are needed to inform the potential for causality and the underlying mechanisms involved.

POST-LITERATURE REVIEW UPDATE. A fourth case-control study from Germany compared 34 PD subjects with 34 age- and sex-matched controls.⁹⁹ Taxon-level findings were largely confirmatory of earlier studies (see Supplementary Table). Absolute concentrations of fecal short chain fatty acids (acetate, propionate, and butyrate) were lower in PD subjects relative to controls ($p < 0.01$, Mann–Whitney U test), and did not appear related to constipation.⁹⁹

AD

An infectious origin of AD has long been postulated; etiological hypotheses include chronic infection with various

bacteria, viruses, parasites, and fungi.^{100,101} Human studies investigating the potential role of the microbiome in the pathogenesis of AD are beginning to emerge, with a focus on the oral cavity (see Supplementary Table). Although none explored the gut microbiome, a lower gut microbiota diversity has been associated with aging, frailty, and markers of inflammation,¹⁰² of potential relevance in AD as a disorder associated with aging. Furthermore, in a male mouse model of AD, the gut microbiota community diversity was shown to regulate host innate immunity and impact $A\beta$ amyloidosis,¹⁰³ and fewer such plaques developed when mice were raised in a germ-free environment.¹⁰⁴

Oral Microbiome and AD

Poor dental status has been linked to AD or early signs of AD (reduced cognitive function), with tooth loss being a risk factor for dementia in a Swedish twin study¹⁰⁵ and in a longitudinal study of aging American nuns.¹⁰⁶ Severe clinical periodontitis was associated with lower cognitive function in nondemented probands.^{107,108} Irregular tooth brushing was associated with higher risk of dementia in a prospective study of 4,883 residents (70% were female) of a Californian retirement community (reaching significance in the larger group of women only; adjusted odds ratio = 1.7, 95% CI = 1.1–2.6 for < 1 daily brushing vs 3 times daily).¹⁰⁹ Although subjects with established AD are known to have poorer dental hygiene,¹⁰⁸ periodontal disease has been associated with increased brain amyloid load, measured via positron emission tomography scans, even in cognitively normal subjects.¹¹⁰

A small preliminary cross-sectional study was performed using DNA sequencing (16S rRNA) of bacteria from subgingival plaques.¹¹¹ There was a trend for a lower relative abundance of Fusobacteriaceae and higher abundance of Prevotellaceae in the 5 demented subjects relative to the 5 nondemented subjects (see Supplementary Table). Oral health (eg, gingivitis score) was not consistently associated with the oral microbial community composition.¹¹¹

Three other USA-based studies used a biomarker approach and examined antibodies to bacteria associated with periodontitis. Raised serum IgG antibody levels were associated with AD or future development of AD and related dementia, providing indirect evidence for a role of the oral microbiome.^{112–114} Periodontitis has been linked to increased systemic inflammatory markers,¹¹⁵ such as tumor necrosis factor- α levels,¹¹² supporting the hypothesis that a chronic oral infection may drive systemic inflammation and possibly AD.¹¹⁶ It would be of value for future studies to directly

interrogate the oral microbiome, examining its functional capacity and potential to prevent or delay onset of AD and related dementias.

Huntington Disease, ALS, and the Microbiome

Although no human studies directly interrogating the microbiome were found for either Huntington disease (HD) or ALS, intriguing changes in premanifest HD subjects have been observed, indicating a potential role for the microbiome.¹¹⁷ Distinct metabolomic serum profiles were found in 52 premanifest subjects as well as 102 early symptomatic HD subjects compared to 140 controls, and were thought to stem from gut microbe-derived metabolites.¹¹⁷ A better understanding of these perturbations may lead to much needed clinically useful biomarkers for the onset, progression, and phenotypic variability in HD.¹¹⁷

The potential for gut microbiome involvement in ALS progression was demonstrated in a transgenic mouse model.¹¹⁸ A defective intestinal tight junction structure and related protein expression were found in the G93A superoxide dismutase mouse model, leading to increased gut permeability, compared to wild-type mice.¹¹⁸ Normal epithelial Paneth cells, which impact the gut microbiome and help tune the innate immune response, were also decreased in number and function, with functionality measured as levels of specific antimicrobial proteins secreted into the gut. Shifts in the gut microbiome included a lower relative abundance of butyrate-producing bacteria such as *Butyrivibrio fibrisolvens*, demonstrated by 16s rRNA real-time polymerase chain reaction.¹¹⁸ There is potential for these observed changes to translate into a biomarker of progression or therapeutic target in ALS; work in human subjects is warranted.¹¹⁸

Study Design and Sample Collection

A comprehensive overview covering all aspects of conducting a microbiome study, from sample collection to sequencing, bioinformatics, and statistical modeling,¹¹⁹ is beyond the scope of this review. However, key elements related to study design and subject selection are important to consider. A prospective cohort study theoretically may be the optimal approach to investigate the microbiome's potential to trigger or facilitate the onset of neurological diseases. This has been successful in relatively common conditions such as childhood onset asthma or allergy, allowing groups of children to be regularly sampled soon after birth and followed until onset or markers of disease.¹⁷ However, many neurological conditions have a long latency period and manifest in adulthood after a lifetime of exposures, making this approach challenging,

labor intensive, and costly. Case-control studies offer opportunities, but also require care and caution, especially when selecting subjects, particularly controls.¹²⁰ There are no gold standards for the ideal control group in a gut microbiota study, and appropriate selection will, in part, depend on the study question. A common principle is that controls should be selected from the same source ("at risk") population as cases.¹²⁰ However, many microbiome studies included in this review either did not specify the source of controls,⁴⁸ or selected controls from a different source population than cases, for example, a different setting or geographical area (see Supplementary Table). Household controls (eg, siblings or a spouse) can be useful in certain situations, potentially helping to minimize variations in diet, lifestyle, or ethnicity. However, they may be a suboptimal choice in other circumstances,¹²¹ for example, spouses with similar lifestyles, such as smokers, tend to cohabit, are often of the opposite sex, and may not be at the same risk of disease. This may mask the impact of important exposures. Sibling controls for childhood onset neurological diseases such as pediatric MS can also be problematic. Recruitment challenges may be insurmountable due to the current trend for smaller family sizes. Sex and age differences between siblings can impact findings; the gut and skin microbiota have been shown to evolve and shift substantially as boys and girls develop.^{62,63,122–124} Most gut microbiota studies made attempts from the outset to minimize heterogeneity within their samples by, for instance, excluding recent antibiotic or corticosteroid use or overt bowel disease. Although most studies were too small to consider or formally test many potential confounders, many did, to some degree, but much remains unknown. Virtually all studies made attempts to consider or correct for multiple comparisons. Other aspects of relevance to future neurologic metagenomic studies include issues with contamination from reagents and commercial extraction kits, which has proved problematic in low biomass samples, such as cerebrospinal fluid, creating possible false signals.¹²⁵

Discussion, Future Perspectives

Despite much circumstantial evidence that microbiota may be involved in the onset or subsequent course of several neurological disorders, causality remains unproven. Based on the preliminary studies conducted to date in humans, there is potential for the microbiome to be harnessed as a clinically useful biomarker of neurological disease onset, phenotypic variability, and disease activity. Tantalizing opportunity exists to establish therapeutic targets to benefit disease-specific (eg, MS relapses) or perhaps related outcomes, including common comorbidities,

ultimately benefiting host health (see Fig 2). However, demonstrating a role of the microbiota as a necessary, sufficient, or contributory factor in facilitating onset of new neurological disease is extremely challenging.

Microbial influence on immune development in childhood has been broadly demonstrated,¹⁷ setting the stage for future disease. A permanent shift in the immune system in early life and the potential to facilitate the onset of immune-mediated neurological disease would be consistent with the current evidence surrounding risk factors for onset of diseases such as MS or NMO (see Fig 2). It is highly possible that no single causative microbe will be found; rather, perturbations in the collective microbiome community may influence health. General “inflammaging”¹²⁶ related to the aging process has the potential to play a major role in degenerative neurological diseases by modifying gut or oral health, the microbiome, and in turn, the host.¹²⁶

Prebiotics, Probiotics, Fecal Microbial Transplants, and Neurological Disease

Currently, manipulation of the microbiome for the purpose of having a beneficial impact in neurological disease lacks evidence. Although excellent response rates (>90%) have been reported in relation to fecal microbial transplants for antibiotic-unresponsive *Clostridium difficile*,¹²⁷ it remains unclear whether such success will apply to other chronic conditions.¹²⁸ Although *pre- or probiotic* interventions (see supplementary online Glossary), also termed “psychobiotics,” have shown promise, primarily in animal studies of anxiety and depression,^{129,130} substantial work is needed before any targeted intervention can be rationally recommended to prevent or ameliorate neurological diseases.

Specific Gaps and Opportunities

The need for standardized protocols and pipelines for fecal collection, transport, and analyses to facilitate comparisons between microbiome studies is critical.¹³¹ Large, longitudinal studies are necessary to elucidate the dynamic relationship between the gut microbiota and neurological disease to determine the microbiome’s role in the initiation of disease, ongoing disease activity, or later disease progression. Moreover, these studies are needed to gain a better understanding of confounders or effect modifiers (eg, sex, age, ethnicity, diet, exercise, lifestyle, mode of delivery, comorbidity, drug exposure, gut motility). Meta-analyses of studies, when possible, may also prove insightful. For instance, the much-publicized apparent relationship between the gut microbiota and obesity was reassessed in a recent study.¹³² Authors combined results from 10 independent studies of human subjects, concluding that no association could be found for

the relationship between the Bacteroidetes to Firmicutes ratio and obesity. Although a statistically significant association was found with gut (alpha) diversity, this was modest at best and of questionable clinical relevance. Small samples sizes were highlighted as a problematic feature, present across studies.¹³²

From the limited human studies conducted to date in the neurological conditions covered in this review, drug exposure was the most consistent factor (and most commonly assessed) that might confound some findings, specifically immunomodulating MS drugs^{49,67} and possibly the catechol-O-methyl transferase inhibitors for PD, which also cause broader adverse intestinal effects.⁹⁹ Even vitamin D supplementation may shift the gut microbiota composition.⁶⁷ How these changes might impact the host remains to be determined. Assessing the microbiome’s role in conversion to disease in high-risk individuals would be a pragmatic approach and of potential value, for example, early cognitive decline to AD, radiologically isolated syndrome to first MS event, premotor phase to PD, or in presymptomatic carriers of the HD gene. Advancements in the understanding of the functional capacity of the microbiome may also be key to its clinical application in neurology. Challenges include the vast quantity of data microbiome studies generate (especially for metagenomics), the complex bioinformatics involved, and limitations (eg, validity and completeness) of open access databases, especially for less studied or understood microbes, such as viruses^{133,134} and Fungi. Opportunity exists for strong interdisciplinary research teams to address some of these issues, and for microbiome studies to be incorporated into ongoing initiatives in well-defined sizable cohorts, such as Harvard’s Nurses’ Health Study, the USA/Canadian pediatric MS networks, or the Parkinson’s Progression Markers Initiative, and become part of routine collection in clinical trials. Success of these teams, and the ability for rapid knowledge translation across disciplines, underscore the need for the human microbiome to be rapidly incorporated into education curriculums and for granting agencies to develop solid mechanisms to facilitate multidisciplinary research.

Concluding Remarks

Neurological disease is a global problem, contributing to 92 million disability-adjusted life years in 2005 and projected to increase by 12% to 103 million in 2030.¹³⁵ This is at a time when human lifestyles have undergone radical changes, such as the introduction and rapid uptake of antibiotics, and other practices that deplete or eradicate microbes that have coevolved with humans for millennia. A closer look at our microbial communities

may provide a useful approach to better understanding neurological disease.

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Author Contributions

All authors contributed to each of the following tasks: study concept and design, data acquisition and analysis (ie, interpretation of studies included in the review), and drafting the manuscript and figures.

Potential Conflicts of Interest

H.T. is the Canada Research Chair for Neuroepidemiology and Multiple Sclerosis. She currently receives research support from the National Multiple Sclerosis Society, the Canadian Institutes of Health Research, the Multiple Sclerosis Society of Canada, and the Multiple Sclerosis Scientific Research Foundation. In the past 5 years, she has received research support from the Multiple Sclerosis Society of Canada (Don Paty Career Development Award); the Michael Smith Foundation for Health Research (Scholar Award), and the UK MS Trust; and speaker honoraria and/or travel expenses to attend conferences from the Consortium of MS Centres (2013), the National MS Society (2012, 2014, 2016),ECTRIMS (2012, 2013, 2014, 2015, 2016), the Chesapeake Health Education Program, U.S. Veterans Affairs (2012), Novartis Canada (2012), Biogen Idec (2014), American Academy of Neurology (2013, 2014, 2015, 2016). All speaker honoraria are either declined or donated to an MS charity or to an unrestricted grant for use by her research group. K.C.B. serves as the Program Reporter for the Canadian Institute for Advanced Research (CIFAR): Humans and the Microbiome Program and is a recipient of a Four Year Doctoral Fellowship at University of British Columbia. S.A.-C.'s professorship is supported by the Pacific Parkinson's Research Institute. She has received research support from the Parkinson Society Canada/Parkinson Society British Columbia, the Pacific Parkinson's Research Institute, the Charros Foundation, the National Parkinson Foundation, and the Djavad Mowafaghian Centre for Brain Health. In the past 2 years, she has received speaker or consulting honoraria or travel support from Merz, Allergan, and Ipsen. E.W. is the recipient of research grants from the NIH, National Multiple Sclerosis Society, and Race to Erase MS. She volunteers on an advisory board for a clinical trial by Novartis.

She is site principal investigator of trials by Genentech, Roche, and Novartis.

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