

The gut microbiome in neurological disorders

John F Cryan, Kenneth J O'Riordan, Kiran Sandhu, Veronica Peterson, Timothy G Dinan



Summary

Research into the role of the gut microbiome in modulating brain function has rapidly increased over the past 10 years, albeit chiefly in animal models. Increasing clinical and preclinical evidence implicates the microbiome as a possible key susceptibility factor for neurological disorders, including Alzheimer's disease, autism spectrum disorder, multiple sclerosis, Parkinson's disease, and stroke. Cross-sectional clinical studies are bolstering the concept of altered microbial composition contributing to the pathophysiology of such diseases. However, the field is nascent, and interpretation of such data is often difficult given that the composition of the microbiome is influenced by various factors such as diet and exercise. Longitudinal studies and randomised controlled trials in humans are needed to find out if targeting the microbiome can yield novel therapeutic strategies. Systems biology approaches will also be important in integrating such data with genomic and metabolomic datasets from clinical cohorts with neurological disease to help guide individual treatment selection.

Introduction

The disciplines of neurology and microbiology have evolved along distinct trajectories with little overlap, except in cases of bacterial or viral infections of the CNS, prion infections, Guillain-Barré syndrome, and septic or hepatic encephalopathies. However, over the past two decades, a revolution has occurred in biomedicine with the realisation that the gut microbiota (ie, the trillions of microorganisms that reside within the gut) and the microbiome (ie, the genetic material of the microbiota) have a role in maintaining homeostasis and in regulating almost every major body system, including the CNS.

Studies in animals have been instrumental in showing the crucial role of the microbiota in key aspects of neurodevelopment, neuroinflammation, and behaviour. A growing body of research has focused on illuminating the bidirectional communication pathways between gut bacteria and the CNS: the microbiota–gut–brain axis.^{1,2} Over the past 5 years, dysregulation of this axis has been increasingly implicated in the pathophysiology of neurological disorders such as Alzheimer's disease,³ autism spectrum disorder,^{4,5} brain injury,⁶ multiple sclerosis,⁷ Parkinson's disease,^{8,9} and stroke.¹⁰ In this Review, we provide an update on the link between the microbiota and brain function in the context of neurological disorders.

Microbiota–gut–brain axis

Two of the main reasons that the microbiota has emerged as such an exciting area in neurology are advances in, and reduced costs of, sequencing and metabolomics technologies. The human organism is more than 99% microbial in terms of genes,¹¹ and is made up of more microbial cells than human cells.¹² Although bacteria are the most abundant and best-studied microorganisms within the gut, it also hosts a multitude of archaea, yeasts, single-celled eukaryotes, helminth parasites, and viruses, including bacteriophages.¹³

Colonisation of the gut is thought to largely commence at birth when the infant becomes exposed to the maternal microbiota during delivery.¹⁴ Various factors in early life can influence this colonisation, including mode of delivery,

breastfeeding, prematurity, environment, host genetics, antibiotic exposure, and maternal infection, stress, or obesity.^{11,15} Throughout life, diet has perhaps the greatest influence on the composition of the microbiota.^{16,17} A growing number of cross-sectional studies have investigated the microbiota composition in individuals with a specific neurological disorder versus healthy age-matched individuals. However, these studies provide just a snapshot in time and longitudinal cohort studies are needed. Experimental models have been essential for moving research in the human microbiota–gut–brain axis forward (figure 1).² In tandem, a large experimental effort has been directed towards attempting to dissect the various pathways of communication between the gut and the brain at key times throughout the lifespan, especially in ageing and in early life where several major potential pathways have been identified.

Microbiota and neurodevelopment

Increasing attention is being paid to understanding the role of the microbiota–gut–brain axis in neurodevelopmental processes (figure 2).^{18,26} However, there have only been a few studies in infants, most of which have been cross-sectional. In one study of 89 infants, cognitive function at 2 years of age (as assessed with the Mullen Scales of Early Learning) significantly correlated with the composition of the microbiota 1 year earlier.²⁷ Microbiota α -diversity (a statistic describing within-sample variability) was also associated with functional connectivity between the supplementary motor area and the inferior parietal lobule in a cohort of 39 infants.²⁸ Importantly, this functional connectivity was also related to cognitive outcomes at 2 years of age.²⁸ The strongest evidence for a role of the microbiota in neurodevelopment comes from research in germ-free mice (ie, mice that are entirely devoid of microbiota).²⁹ In these models, fundamental neural processes such as development, myelination, neurogenesis, and microglia activation have been shown to be crucially dependent on the composition of the microbiota (figure 3). However, germ-free mice are an extreme situation with an inherently defective immune system development and

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APC Microbiome Ireland
(Prof J F Cryan PhD,
K J O'Riordan PhD, K Sandhu PhD,
V Peterson PhD,
Prof T G Dinan DSc),
Department of Anatomy and
Neuroscience (Prof J F Cryan,
V Peterson), and Department of
Psychiatry and
Neurobehavioural Science
(Prof T G Dinan), University
College Cork, Cork, Ireland

Correspondence to:
Prof John F Cryan, Department of
Anatomy and Neuroscience,
University College Cork, Western
Gateway Building,
Cork T12 XF62, Ireland
j.cryan@ucc.ie

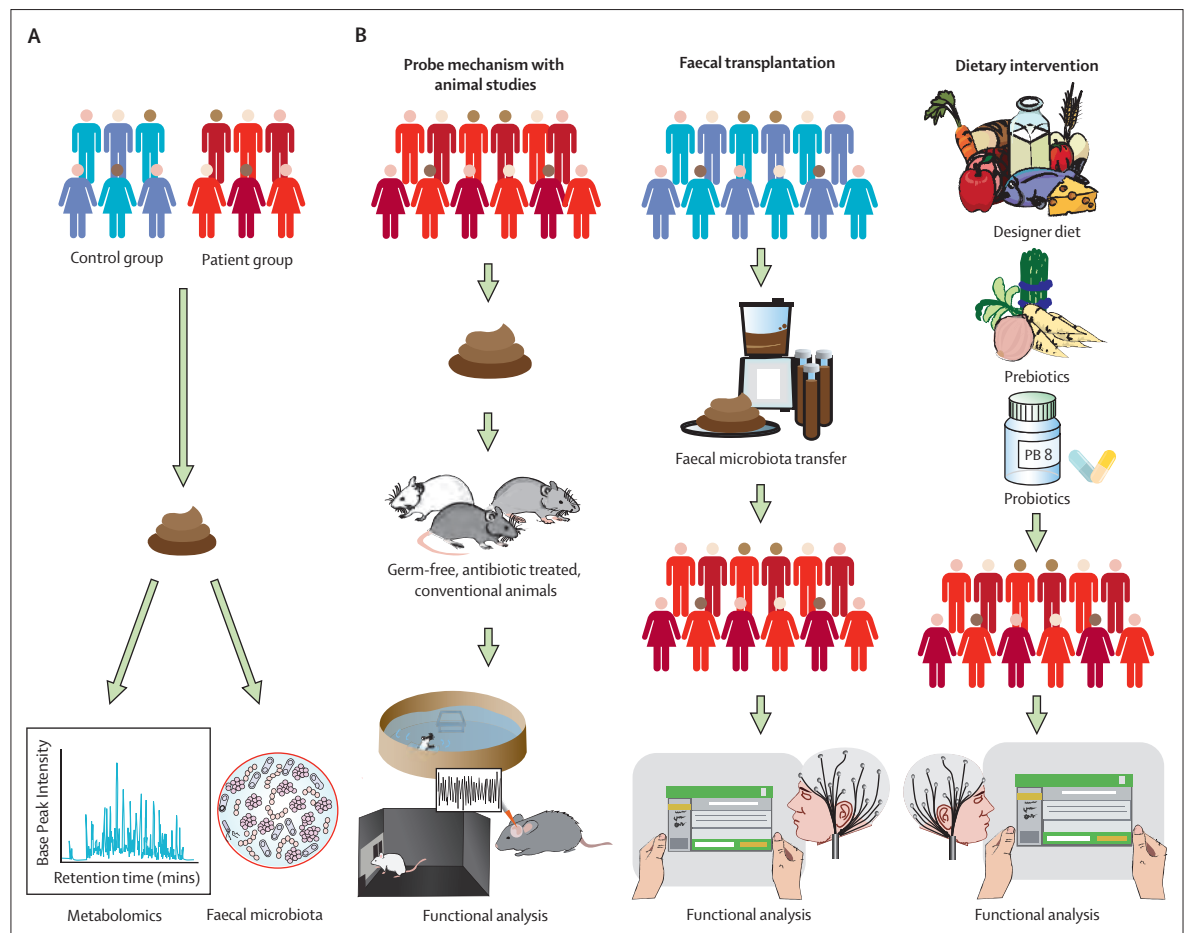


Figure 1: Potential ways to harness the human microbiome

(A) The microbiome from patient and control groups is assessed and differences in the composition (what bacteria are there) and the metabolome (what they are doing) are identified. Note that stool samples are commonly used for microbiome analyses, and colonic bacteria are not a reliable surrogate for the microbiome of the small intestine. (B) Three potential ways to use the human microbiome in preclinical and clinical trials to study mechanisms of disease and effects of potential treatments. Probe mechanism with animal studies: animals can be humanised by faecal microbiota transplantation, where faecal matter is taken from humans and used to repopulate the rodent gut with a composition similar to that of the human donor. In doing so, reconstituting a phenotype in the rodent similar to that of the donor human is possible, offering a preclinical way to study mechanisms that would otherwise be very difficult (if not impossible) to study in humans. Functional analysis can be done through a battery of animal behavioural testing, and in-vitro testing done with molecular and imaging techniques.^{18–20} Faecal transplantation: if differences in microbiome composition are present, faecal microbiota transplantation could be done from control individuals, or a defined bacterial consortia. Promising clinical studies have been reported, including in people with autism spectrum disorder.⁴ Dietary intervention: selective diets, prebiotics, or probiotics could be implemented to target the microbiome. However, this has been studied mostly in animal models and validation of such effects of targeting the microbiome with selective diets, prebiotics, or probiotics in humans is now warranted.²¹

little translation into humans, although they have been useful in pushing the field forward to establish whether the microbiota is involved in specific brain processes.²⁹

Microbiota and ageing

The relationship between the gut microbiota and the ageing brain is also receiving much attention because many neurological disorders occur in older people. The Irish ELDERMET study¹⁶ of 178 individuals aged more than 65 years showed that the composition of the gut microbiota correlated with overall global indices of health, frailty, and immune function. This study showed that the greater the diversity of the microbiota, the better the health outcomes. Of note, it was the diversity of diet that

correlated with diversity of the gut microbiota, with people eating processed, bland food (often in nursing homes) having reduced diversity of their microbiota, and individuals with a diet rich in fruit and vegetables having more gut microbiota diversity.¹⁶ Thus, the diversity of the gut microbiota is a potential hallmark of healthy ageing. A small 12-week, double-blind, randomised, placebo-controlled study with no follow-up assigned 36 healthy Korean people (20 men and 16 women; aged 60–75 years [SD 4.14]) to either a probiotic diet (n=26) or placebo (n=10).⁴³ This small study showed that administration of a specific bacterial strain (*Lactobacillus helveticus* IDCC3801) improved performance in cognitive fatigue tests compared with individuals who received the placebo.⁴³

Post-hoc tests showed statistically significant differences in the number of correct responses on a rapid visual information-processing task and in error response on the Stroop colour-word test between people in the placebo and probiotic groups. However, because the sample size was small, the findings should be considered with caution. More intervention studies are needed to test the robustness of microbial-based interventions in promoting healthy brain ageing. Mouse studies have shown that age-related behavioural deficits are coincident with changes in the microbiota,⁴⁴ and that age-associated neuroinflammation can be ameliorated by a dietary intervention (prebiotic inulin) that targets the microbiota.⁴⁵ Furthermore, the microbiota has been shown to regulate microglia activation, which has a key role in ageing and neurodegeneration.³⁸

Microbiota and neurological disorders

In the following section, we review the evidence for the role of the microbiota in neurological disorders in which a role for gut microbiota has been shown to be key in clinical or animal studies. The table summarises human studies on the microbiota and its role in neurological disorders, including inherent limitations of the studies. We also frame the relationship that the microbiota has with each disorder within the context of how the microbiota affects key brain processes involved in healthy neurodevelopment and ageing. We begin with disorders where there is most evidence, concluding with descriptions of nascent fields.

Multiple sclerosis

Given that the gut microbiota is essential for the development and maturation of the immune system, it is not surprising that the microbiota is implicated in the pathogenesis of multiple sclerosis, an immune-mediated neurological disorder. Cross-sectional studies have largely shown that subtle, discrete taxonomic changes, rather than large-scale differences in α -diversity or β -diversity (a statistic describing the variability between different samples) of the gut microbiota, are seen in children with multiple sclerosis within 2 years of disease onset compared with healthy children without autoimmune disorders up to age 18 years (table).⁷ Two studies have transplanted the microbiota from patients with multiple sclerosis into two different models of experimental autoimmune encephalomyelitis, a well-validated animal model of multiple sclerosis.^{19,20} Mechanistically, these studies highlighted the importance of interleukin IL10-producing CD4 T cells in the immunomodulatory effects of the gut microbiota.^{19,20} Early studies in germ-free mice have also shown that these mice were particularly resistant to developing experimental autoimmune encephalomyelitis, which was reversed by faecal microbiota transplantation from normal mice.^{19,73,74} Furthermore, the presence of specific Gram-positive segmented filamentous bacteria in the gastrointestinal tract, which activate

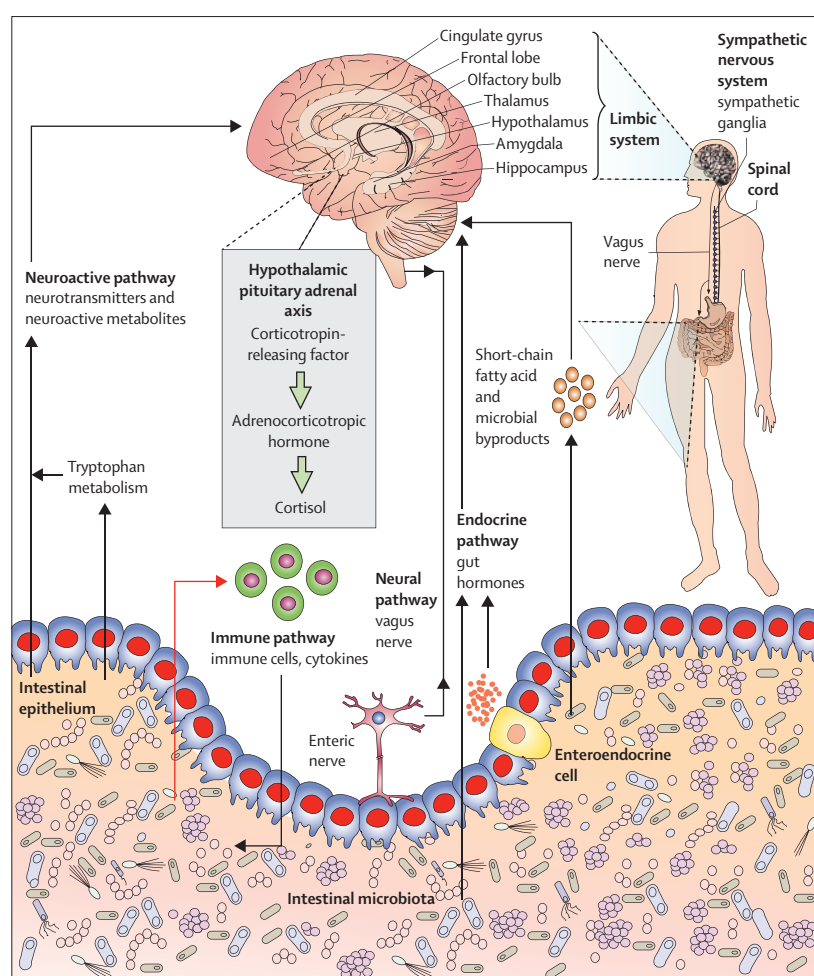


Figure 2: Pathways of communication between the microbiota and the brain

Multiple direct (eg, vagus nerve) and indirect (eg, short-chain fatty acids, cytokines, and key dietary amino acids, such as tryptophan, tyrosine, and histidine²³) pathways exist through which the gut microbiota can modulate the gut-brain axis. They include the neuroactive pathway, including neurotransmitters and neuroactive metabolites; the immune pathway (including cytokines); short-chain fatty acids and microbial metabolites; the neural pathway (vagus nerve, enteric nervous system, and spinal nerves²³); the hypothalamic-pituitary-adrenal axis; and the endocrine pathway. Microbes can synthesise neurotransmitters (ie, GABA, noradrenaline, and dopamine) locally in the gut in both animals and humans,²⁴ which is an important avenue of communication. Neuroactive bacterial metabolites,²⁵ as well as metabolites from the diet, can modulate the brain and behaviour in ways that are still being elucidated, including affecting epithelial cells to change gut-barrier function, enteroendocrine cells to release hormones, and dendritic cells to modulate immune and microglial function, which play a fundamental role in ageing and neurological disorders. However, the exact molecular signalling pathways involved have not been defined yet. Red arrow indicates immune system stimulation by the luminal contents, producing a negative effect (host immune activation).

Th17 cells, significantly affected the severity of experimental autoimmune encephalomyelitis.¹⁹

Multiple sclerosis is a demyelinating disorder and converging data from germ-free mice and antibiotic preclinical studies have implicated the microbiota in regulating myelin production in mouse prefrontal cortex.^{20,33} Loss of integrity of the blood-brain barrier is also a hallmark of multiple sclerosis, and germ-free mice studies have shown that the microbiota is crucial in regulating the blood-brain barrier.³⁹ Moreover, dietary administration of short-chain fatty acids, or of bacteria that produce short-chain

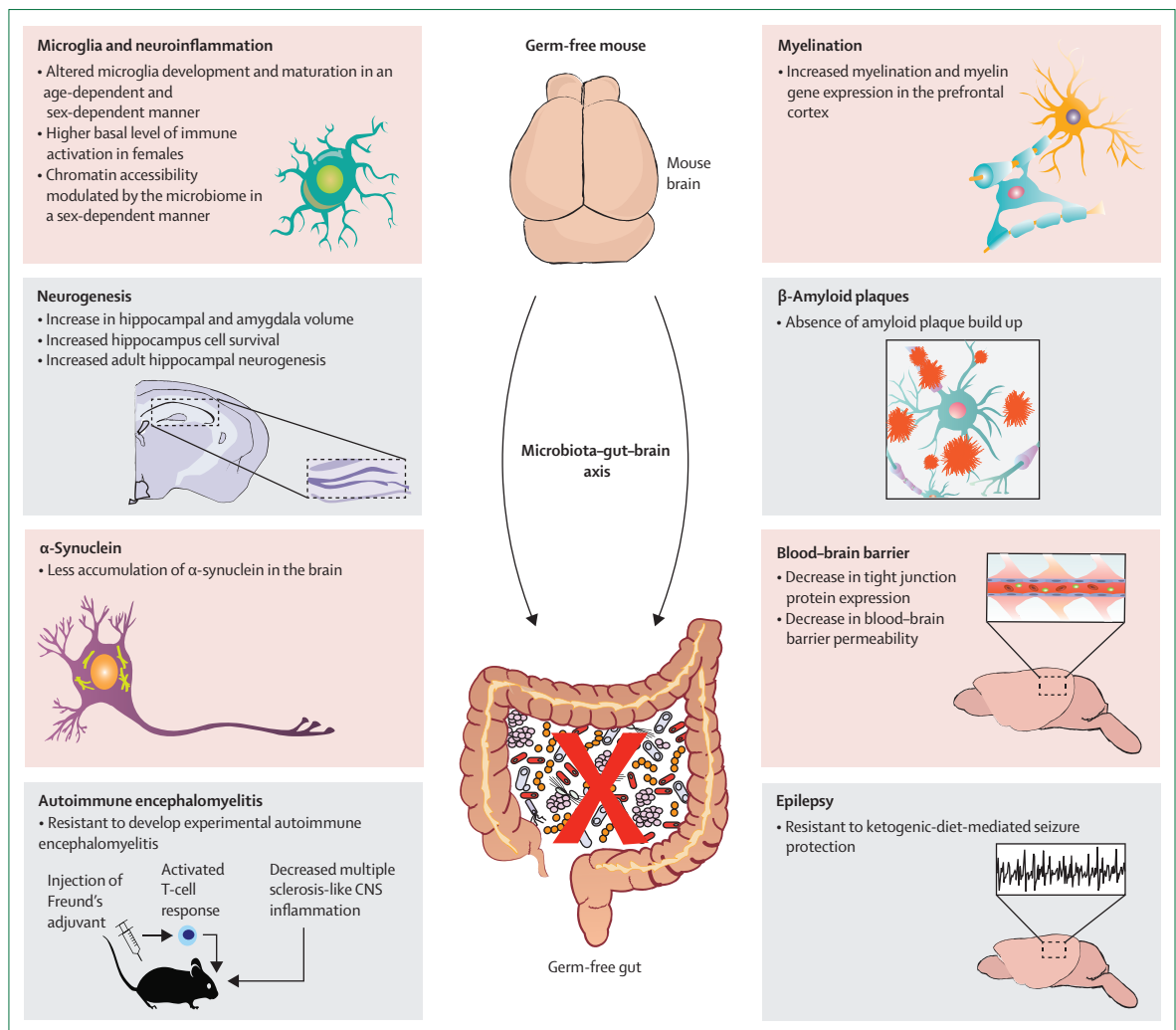


Figure 3: Microbiota, neurodevelopment, and neuroimmune function in animal models

The strongest evidence for a link between the microbiota and brain function comes from studies in germ-free mice.^{29,30} The brains of germ-free mice fail to develop normally; absence of a microbiota in germ-free mice results in many neurobiological changes that are relevant to various neurological disorders, including strain-dependent mouse alterations in anxiety-like behaviour, reduced hippocampal volume in germ-free mice, and reduced myelination.^{31,32} Studies have reported increased myelination in the prefrontal cortex,³³ increased neurogenesis in the hippocampus,³⁴ changes in various neurotransmitter concentrations,³⁵ and alterations in dendritic arborisation.³² Functionally, germ-free mice have shown the involvement of microbiota with brain signalling in sociability, visceral pain, immune function, stress sensitivity, and fear and anxiety responses.^{29,31,35–37} Microglia, important regulators of neuroinflammation, from germ-free mice display an immature phenotype and fail to respond effectively to bacterial-associated molecular patterns (ie, lipopolysaccharide).³⁸ Germ-free mice also show increases in the permeability of the blood-brain barrier.³⁹ A reduction in the integrity of the blood-brain barrier might allow the translocation of immune cells and bacterial components into the brain and influence neuroinflammation. Germ-free mice are also resistant to the effects of experimental autoimmune encephalomyelitis,³⁹ transgenic-mediated β -amyloid plaque formation,⁴⁰ α -synuclein formation,¹⁸ and ketogenic diet-induced protection of seizures. Studies using antibiotics in young (eg, post-natal days 4–13) rats also show a key role for the microbiota in shaping neurodevelopmental trajectories relevant to aggression⁴¹ and visceral pain.⁴²

fatty acids, reversed loss of integrity of the blood-brain barrier. Diet-induced changes in microbiota composition have also been implicated in experimental autoimmune encephalomyelitis manifestation.⁷⁵ A growing body of evidence supports the concept that the microbiota is a key regulator of neuroinflammation;^{20,52,76,77} however, further research is needed to understand how this relationship could contribute to the pathophysiology of multiple sclerosis.

Taken together, human and animal studies have suggested that the microbiota might be involved in many

aspects of the pathology of multiple sclerosis. The question remains as to how the microbiota can be successfully targeted as an intervention strategy to prevent relapse and to minimise symptoms in patients. In a pilot study, a multispecies probiotic (containing *Lactobacillus* species, *Bifidobacterium* species, and *Streptococcus* species administered twice daily for 2 months; table) reversed microbiota changes and was shown to have anti-inflammatory properties, suggesting that such a microbiota-targeted strategy is worth pursuing,⁵² but much larger trials are needed to confirm the results.

	Participants and intervention	Results	Limitations
Multiple sclerosis			
Double-blind, randomised, placebo-controlled ⁴⁶	25 women with multiple sclerosis (mean age 34.4 years [SD 9.2]) treated for 12 weeks with probiotic containing <i>Lactobacillus acidophilus</i> , <i>Lactobacillus casei</i> , <i>Bifidobacterium bifidum</i> , and <i>Lactobacillus fermentum</i> ; control group: five men and 25 women (mean age 33.8 years [SD 8.9]) who took placebo; the control group was matched for disease severity on the basis of EDSS, relapses, sex, type of medications, body-mass index, and age	Significant increase in depression scores on BDI, multiple sclerosis scores on EDSS, diet scores on DHQ, and scores on DASS; significant changes in concentrations of high-sensitivity C-reactive protein, plasma nitric oxide metabolites, and malondialdehyde; significantly increased quantitative insulin sensitivity check index and HDL cholesterol; significantly decreased serum insulin homoeostasis model of assessment-estimated insulin resistance, β -cell function, and total and HDL cholesterol in patients with multiple sclerosis compared with placebo	Small sample size; all female treatment group; faecal bacteria loads were not evaluated either before or after probiotic supplementation; alternative inflammatory indicators were not assessed; cognitive impairment was not evaluated
Double-blind, randomised, placebo-controlled ⁴⁷	40 patients with multiple sclerosis aged 18–55 years, 20 treated for 12 weeks with probiotic containing <i>L. acidophilus</i> , <i>L. casei</i> , <i>B. bifidum</i> , and <i>L. fermentum</i> , and 20 received a starch placebo	Significantly reduced expression of IL-8, TNF α , and mRNA from peripheral blood mononuclear cells; no change in IL-1, LDL-receptor, or PPAR- γ expression in patients with multiple sclerosis compared with controls	Small sample size; no analysis of microbiota composition characterisation over the duration of the study; gene expression analysis limited to a few genetic markers rather than a full comparison screen; no protein level analysis done
Case-controlled, cross-sectional, observational ⁵⁰	71 patients with relapsing–remitting multiple sclerosis who had not received treatment for at least 3 months before sample collection; control group: 71 healthy individuals with no autoimmune disorders; a subset of 79 individuals were used as a time-control group	PBMCs from patients with multiple sclerosis, when compared with 71 healthy control individuals, showed an impaired ability to differentiate or expand CD25-positive, FoxP3-positive regulatory T-cell populations; significant increase in acinetobacter and akkermansia; significant decrease in parabacteroides (mostly <i>Parabacteroides distasonis</i>)	Cross-sectional study design; in-vivo studies using monoclonalised mice do not completely replicate in-vitro results from human cells
Case-controlled, cross-sectional, observational ⁷	18 children (ten girls, eight boys; mean age 12.5 years [SD 4.44]) within 2 years of relapsing–remitting multiple sclerosis onset; control group: 17 age-matched, healthy individuals (nine girls, eight boys; mean age 13.5 years [SD 3.08]); age range for all participants 4–18 years	β -diversity significantly differed by immunomodulatory drug exposure; significantly increased relative abundance of bilophila, desulfovibrio, and Christensenellaceae; significantly decreased relative abundance of Lachnospiraceae and Ruminococcaceae in patients with relapsing–remitting multiple sclerosis compared with healthy control individuals	Cross-sectional study design, small sample size, single-centre study; use of immunomodulatory drugs or systemic corticosteroids was included and potential effects of this not controlled for; asthma and eczema were allowed in the control population; stool samples collected at home; unorthodox β -diversity metric used
Case-controlled, cross-sectional, observational ⁴⁸	19 male and 41 female (mean age 49.7 years [SD 8.50]) patients with relapsing–remitting multiple sclerosis; control group: six healthy men and 37 healthy women (mean age 42.2 years [SD 9.61])	Significant increase in abundance of methanobrevibacter (Archaea) and akkermansia in all patients with multiple sclerosis compared with controls; significant reduction or tendency towards reduction in abundance of prevotella and sutterella in patients with multiple sclerosis who were not treated compared with patients who were treated; significant increase in abundance of methanobrevibacter, akkermansia, prevotella, and sutterella in patients with multiple sclerosis who were treated relative to patients who were not treated; significantly lower abundance of butyricimonas in patients with multiple sclerosis compared with healthy control individuals, significantly lower abundance of sarcina in patients with multiple sclerosis on therapy compared with patients who were not treated	Cross-sectional study design; largely female participants; associational findings only, no control for medications or diet; variability in time of sample collection and variable interval of previous dietary intake before collection; samples collected only after disease onset, none before
Case-controlled, cross-sectional, observational ⁴⁹	Ten male and 21 female patients with relapsing–remitting multiple sclerosis (mean age 42.9 years [SD 10.6]); control group: 14 men and 36 women (mean age 40.3 years [SD 7.3]) including an age-matched and sex-matched cohort with no known disease symptoms	β -diversity differed as a function of relapse status; significant increase in abundance of pseudomonas, mycoplasma (Proteobacteria), haemophilus, blautia, and dorea (Firmicutes); significantly lower abundance of parabacteroides, and adlercreutzia (Actinobacteria) in patients with multiple sclerosis compared with controls	Cross-sectional study design; small sample size; female-skewed
Case-controlled, cross-sectional, observational ⁵⁰	Six male and 14 female patients with relapsing–remitting multiple sclerosis (mean age 36.0 years [SD 7.2]); control group: 23 healthy men and 27 healthy women (mean age 27.2 years [SD 9.2])	Significantly lower abundance of faecalibacterium, prevotella, and anaerostipes, Clostridia clusters XIVa and IV, and several Bacteroidetes; significant increase in abundance of <i>Eggerthella lenta</i> in patients with multiple sclerosis compared with control individuals	Cross-sectional study design; single-centre study; small sample size; female-skewed; no control for medications or diet; control group from a different location

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Autism spectrum disorder

Although genetics is one of the key factors in the pathogenesis of autism spectrum disorder, there is also a very strong gene–environment interaction involved, with estimates that more than 50% of the neurobiology is driven by non-heritable factors.⁷⁸ An important and often under-recognised feature of autism spectrum disorder is

the marked comorbidity with gastrointestinal symptoms.⁷⁸

Many cross-sectional studies have shown alterations in the composition of the microbiota in autism spectrum disorder (table).^{4,55,78} However, most of these studies were relatively small and heterogeneous, did not always take diet into account, and could not monitor changes over the progression of disease. Animal studies have helped to

Participants and intervention		Results	Limitations
(Continued from previous page)			
Open-label, interventional, single groups assignment, non-randomised ⁵¹	Vitamin D3 supplementation study with 15 women aged 29–51 years, five with multiple sclerosis treated with glatiramer acetate and two with untreated multiple sclerosis (median age 42 years; range 30–48), and eight healthy individuals (median age 38 years; 29–51)	Significant increase in abundance of ruminococcus; significantly lower abundance of faecalibacterium and Bacteroidaceae; abundance of faecalibacterium was significantly lower in patients with multiple sclerosis, which significantly increased in patients with untreated multiple sclerosis only, after vitamin D3 supplementation; abundance of Akkermansia and Coprococcus also significantly increased after vitamin D3 supplementation in untreated patients with multiple sclerosis	Small sample size; all female participants; only one sample was available per subject before and after supplementation; did not control for vitamin D3 insufficiency at baseline; race and ethnicity were self-reported; no metagenomic approaches were used
Controlled, interventional ⁵²	Nine patients with relapsing–remitting multiple sclerosis (mean age 50 years [SD 10]) and 13 healthy control participants (mean age 35 years [SD 14]); treated with <i>Lactobacillus paracasei</i> , <i>Lactobacillus plantarum</i> , <i>L. acidophilus</i> , <i>Lactobacillus delbrueckii bulgaricus</i> , <i>Bifidobacterium longum</i> , <i>Bifidobacterium infantis</i> , <i>Bifidobacterium breve</i> , and <i>Streptococcus thermophilus</i> twice daily for 2 months	Significant increase in abundance of lactobacillus, known to be reduced in patients with multiple sclerosis; significant decrease in abundance of akkermansia, dorea, and blautia associated with dysbiosis; significant decrease in the abundance of several Kyoto Encyclopedia of Genes and Genomes pathways in both control individuals and patients with multiple sclerosis after administration of treatment	Small sample size; unable to calculate FDR for stool metabolomics, FACS, gene expression studies, and correlational studies
Autism spectrum disorder			
Controlled, interventional ⁵³	Nine male and one female patients with autism spectrum disorder (aged 2–9 years), seven male and two female siblings (aged 5–17 years) without autism spectrum disorder, and ten male control participants without autism spectrum disorder (aged 2–11 years): all treated with dietary supplementation with three strains of <i>Lactobacillus</i> (60%), two strains of <i>Bifidobacterium</i> (25%), and one strain of <i>Streptococcus</i> (15%), three times a day for 4 months	In children with autism spectrum disorder and their siblings compared with controls: significantly greater gastrointestinal dysfunction; strong positive correlation between gastrointestinal symptoms and autism spectrum disorder severity; significant increase in Bacteroidetes:Firmicutes ratios back to healthy control values, significant decrease in Firmicutes abundance, and significant decrease in bifidobacterium and desulfovibrio after probiotic treatment; correlation between severe autism spectrum disorder and increase in <i>Clostridia</i> and desulfovibrio, and a greater Bacteroidetes:Firmicutes ratio; correlation between TNFα concentrations and gastrointestinal symptoms, decreased by probiotic supplementation	Small sample size; patient heterogeneity in terms of sex; no female non-sibling control individuals were recruited; self-reporting of gastrointestinal symptoms
Cross-sectional, observational ⁵⁴	39 male and ten female patients with autism spectrum disorder (mean age 11.4 years); 26 were receiving probiotic therapy and 17 had gastrointestinal disease; control group: 29 male and seven female (mean age 10.2 years) individuals	Significantly lower plasma concentrations of myeloperoxidase and plasma copper with probiotic therapy in patients with autism spectrum disorder compared with controls	Patient heterogeneity in terms of age and gastrointestinal symptoms; unable to differentiate effect of different types of probiotics; individual patients were not followed up
Interventional (single group assignment), non-randomised, open-label ⁴	18 patients with autism spectrum disorder with moderate-to-severe gastrointestinal problems (aged 7–16 years); 10-week oral and rectal microbiota transfer therapy (14 days oral vancomycin, followed by 12–24 h fasting with bowel cleansing, then repopulating gut bacteria with high initial dose of standardised human gut microbiota); control group: 20 age-matched and sex-matched neurotypical individuals without gastrointestinal disorders	Marginally lower fibre consumption in children with autism spectrum disorder who were breastfed for a significantly shorter time than were neurotypical children; no differences in antibiotic administration, or carbohydrate, fat, protein, and calorie intake; significantly reduced abdominal pain, indigestion, diarrhoea, and constipation with microbiota transfer therapy; significant improvement in behaviour related to autism spectrum disorder; significantly reduced bacterial diversity; significantly increased bifidobacterium, prevotella, and desulfovibrio abundances with microbiota transfer therapy in children with autism spectrum compared with before treatment	Small sample size; not placebo-controlled, blinded, or randomised; patient heterogeneity in terms of age, sex, gastrointestinal symptoms, diet, and supplements; short time scale
Follow-up ⁵ of the interventional (single group assignment), non-randomised, open-label trial ⁴	18 patients with autism spectrum disorder (aged 9–18 years)	Significant (58%) reduction in GSRS, and in proportion of days of abnormal stools (26%; daily stool record); 47% lower on CARS; 35% lower on ABC; maintained higher gut microbiota diversity; significantly increased median relative abundances of bifidobacteria and prevotella	Small sample size; not placebo-controlled, blinded, or randomised; patient heterogeneity in terms of age, sex, gastrointestinal symptoms, diet, and supplements; 12 (67%) of 18 participants made one or more changes to their medications, nutritional supplements, and diets between the end of the original trial and the 2-year follow-up after stopping treatment

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provide a mechanistic understanding of how the microbiota could have a role in autism spectrum disorder. Germ-free mice have deficits in social behaviour and increased repetitive behaviour,^{29,79} suggesting that appropriate composition of the microbiota is required for

normal social development. Transplantation of gut microbiota from human donors with autism spectrum disorders into germ-free mice revealed that colonisation with microbiota from people with autism spectrum disorder was sufficient to induce autistic behaviours in

Participants and intervention		Results	Limitations
(Continued from previous page)			
Cross-sectional, observational ⁵⁵	25 male and five female patients with autism spectrum disorder (aged 2·5–18 years; mean age 4·43 years [SD 1·47]) with 16 male and four female healthy individuals (mean age 4·28 years [SD 1·00])	Significantly increased incidence of constipation; short-chain fatty acids acetic acid and butyrate were significantly decreased, and valeric acid significantly increased; reduced species diversity and evenness; the abundance of Firmicutes was significantly decreased and Acidobacteria, Enterobacteriaceae, Pseudomonadaceae, Veillonellaceae, and megamonas significantly increased in patients with autism spectrum disorder compared with controls	Small sample size; patient heterogeneity in terms of age, sex, bowel habits, and dietary habits
Cross-sectional, observational ⁵⁶	111 strains of <i>Clostridium perfringens</i> examined: 49 isolates from 23 male and six female patients who were diagnosed with autism spectrum disorder between ages 3·5 and 18 years, 30 isolates from 17 healthy individuals, and 32 isolates from 24 young people who were obese	Significant upregulation of the <i>cpb2</i> gene in <i>C perfringens</i> from faeces from patients with autism spectrum disorder compared with both control groups	Cross-sectional study design; patient heterogeneity in terms of sex; small sample size; small number of strains studied
Double-blind, randomised, placebo-controlled, parallel-design ²¹	31 male and ten female patients with autism spectrum disorder (aged 4–11 years; mean age 7·7 years) enrolled; 11 withdrew during the baseline period because of difficulties associated with collecting samples; the remaining 30 patients included children whose diet was not restricted (n=18) and children on an exclusion diet (n=12), mainly gluten and casein free; all were randomly assigned to receive either placebo or prebiotic galacto-oligosaccharide for 6 weeks; four patients dropped out during the trial	Significant improvement in social behaviour symptoms and sleep with prebiotic galacto-oligosaccharide compared with placebo	Small sample size; patient heterogeneity in terms of sex; unable to control for medication, diet, or lifestyle, but they were recorded
Parkinson's disease			
Cross-sectional, observational ⁵⁷	66 sigmoid mucosal biopsy samples, 65 faecal samples from 24 male and 14 female patients with Parkinson's disease (mean age 61·6 years [SD 9·4]), and 18 male and 16 female healthy individuals (mean age 45·1 years [SD 14·4])	Significantly increased diversity of mucosal-associated and faecal microbiota; significant increase in faecal Bacteroidetes, Proteobacteria, and Verrucomicrobia; significant decrease in mucosal Lachnospiraceae, significant increase in Oxalobacteraceae, and significant decrease in faecal Firmicutes in patients with Parkinson's disease compared with healthy control individuals	Cross-sectional, single-centre study design; uncontrolled for age, body-mass index, medication, race, and duration of Parkinson's disease; only examined colonic mucosa and faecal bacteria; samples collected at home
Randomised, cross-sectional, observational ⁵⁸	21 male and 31 female patients with Parkinson's disease (mean age 68·9 years [SD 6·8]); 21 male and 15 female spouses of patients with Parkinson's disease (mean age 68·4 years [SD 9·7])	Stool frequency was negatively correlated with disease duration and UPDRS2, and positively correlated with MMSE, MoCA-J, and FAB; significant decrease in serum leptin and lipopolysaccharide-binding protein; significant increase in lactobacillus; significantly decreased <i>Clostridium coccoides</i> group, <i>Clostridium leptum</i> subgroup, and <i>Bacteroides fragilis</i> ; significantly decreased hydrogen-producing faecal bacteria in patients with Parkinson's disease compared with spousal healthy control individuals	Patient heterogeneity in terms of sex; no comprehensive microbiome analyses were done (sequencing nor metabolomics); serum lipopolysaccharides were not measured; detailed medical history was not available or recorded; controls were cohabitants of patients with Parkinson's disease; unmatched sexes; intestinal mucosal biopsy samples were not obtained
Case-controlled, observational ⁵⁹	37 male and 35 female patients with Parkinson's disease (mean age 65·3 years [SD 5·5]); sex-matched and age-matched (within 5 years) control group with no signs of parkinsonism or potential premotor symptoms: 36 men and 36 women (mean age 64·5 years [SD 6·9])	Significant decrease in Prevotellaceae in faeces of patients with Parkinson's disease compared with control individuals	Case-controlled design; studied population came from a geographically restricted region; no information about the dietary habits of individuals was recorded; stool sample collection done at home
Cross-sectional, observational ⁶⁰	24 male and ten female patients with Parkinson's disease (mean age 67·7 years [SEM 8·9]); control group: 18 male and 16 female age-matched, healthy individuals (mean age 64·6 years [SEM 6·6])	Significant decrease in <i>Faecalibacterium prausnitzii</i> , Lactobacillaceae, Enterococcaceae, and Bacteroidetes Prevotellaceae; significant increase in <i>Akkermansia muciniphila</i> , bifidobacterium, and Enterobacteriaceae; significantly decreased short-chain fatty acids (acetate, propionate, and butyrate) in patients with Parkinson's disease compared with healthy controls	Small sample size; self-reported diet, recent antibiotic use, probiotic, or prebiotic usage; collection of stool samples at home; target sequencing rather than whole faecal microbiome sequencing

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the mice.³⁰ Animal models of epidemiologically validated environmental risk factors for autism spectrum disorder (including maternal exposure to the anticonvulsant valproate, maternal exposure to inflammation in pregnancy, and maternal obesity) reported an altered composition of the microbiota.^{80,81} Administration of a single bacterial strain, either *Bacteroides fragilis*⁸⁰ or *Lactobacillus*

reuteri,^{23,81} could reverse many of the behavioural and gastrointestinal changes reported in both human studies and animal models of autism spectrum disorder. Moreover, the presence of some gut microbial communities that are susceptible to vancomycin that promote a pro-inflammatory status has been associated with autism spectrum disorder.⁸² Finally, administration of other

Participants and intervention		Results	Limitations
(Continued from previous page)			
Cross-sectional, observational ⁶¹	31 male patients (mean age 64.8 years [SD 9.5]) with Parkinson's disease were compared with 28 male age-matched individuals without Parkinson's disease (mean age 65.6 years [SD 10.4])	Significant increase in Verrucomicrobiaceae (genus akkermansia), unclassified bacteria (of the classified prokaryotes), and Firmicutes; significant decrease in Prevotellaceae (genus prevotella) and Erysipelotrichaceae (genus eubacterium); significant increase in microbiota richness in patients treated with monoamine oxidase inhibitors in combination with amantadine compared with patients treated with other drugs and medication-free patients; five bacterial families (Burkholderiaceae, Propionibacteriaceae, Enterococcaceae, Actinomycetaceae, and Enterobacteriaceae) differed between statin-treated and untreated patients; OTU richness differed markedly for patients taking monoamine oxidase inhibitor and amantadine	Cross-sectional study design; small cohort for testing confounders; monoamine oxidase inhibitor and amantadine might influence assessed microbiota richness; only men were recruited; patients with atypical or secondary parkinsonism were excluded; no detailed dietary plan was requested before faeces collection
Cross-sectional, observational ⁶²	132 male and 65 female patients with Parkinson's disease (mean age 68.4 years [SD 9.2]) were compared with 51 male and 79 female individuals without Parkinson's disease (mean age 70.3 years [SD 8.6]); 54 of the case-control pairs were spouses	Patients with Parkinson's disease were more likely to have been born by caesarean section; significant decrease in bifidobacterium and Lachnospiraceae blautia; Ruminococcaceae significantly increased with length of Parkinson's disease symptoms in patients with Parkinson's disease; overall 13 taxa at the OTU level, eight at the genus level, and seven at the family level were associated with Parkinson's disease compared with control individuals	Cross-sectional study design; control group self-reported absence of neurodegenerative disease; stool samples collected at home and not frozen immediately; high number of variables considered as confounders; large discrepancy in geographical location of samples and how far they had to travel at room temperature; patients' spouses were recruited; unequal sex ratio; no date recorded for sample collection; self-reported height and weight of patients and control group
Two complementary observational, epidemiological studies; study 1: longitudinal; study 2: cross-sectional ⁶³	Study 1: 257 057 male and 297 590 female individuals with appendectomy (mean age 32.45 years) and Parkinson's disease (mean age 74.94 years) were compared with 614 883 male and 537 470 female individuals without an appendectomy and Parkinson's disease (mean age 76.27 years); study 2: 39 male and 15 female patients at mean age of 60.67 years at Parkinson's disease onset	Appendectomy was associated with a lower risk of Parkinson's disease and delayed age of Parkinson's disease onset when comparing patients with Parkinson's disease with an appendectomy vs without an appendectomy	Strong heterogeneity between the two sample datasets used; genetic screening of patients in the Parkinson's Progression Markers Initiative dataset did not include all known Parkinson's disease risk alleles; low Parkinson's disease diagnosis accuracy with Swedish National Patient Registry dataset; in the Parkinson's Progression Markers Initiative dataset, appendectomy surgery is based on patient recollection
Blind, retrospective, cross-sectional, observational ⁹	117 upper and lower gastrointestinal tissue samples from 62 patients with Parkinson's disease aged 46–85 years (mean 68 years) at diagnosis and from 161 histologically normal control individuals aged 44–90 years (mean 71 years) at time of biopsy; for each included patient, at least one age-matched and sex-matched control individual was identified from the histopathology computerised database	Significant accumulation of α -synuclein in the bowel of patients in the preclinical phase of Parkinson's disease compared with healthy controls	Archival biopsy samples used for analysis were extensively examined for other purposes, leaving little tissue for α -synuclein assessment; many of the samples contained tumours and adenomas; unreliable knowledge of previous adverse motor symptomatology
Cross-sectional, observational ⁶⁴	Assessment of microbiota from flash-frozen stool and nasal wash samples from 76 patients (50 men and 26 women) with Parkinson's disease (mean age 68.0 years [SD 9.7]), 21 patients (12 men and nine women) with idiopathic rapid-eye-movement sleep behaviour disorder (mean age 66.1 years [SD 7.9]), and 78 healthy control individuals (46 men and 32 women; mean age 68.4 years [SD 6.7])	Differential abundance of Bacillaceae (possibly attributed to levodopa treatment rather than the disease) in patients with Parkinson's disease receiving levodopa (comparisons were done between patients with Parkinson's disease receiving levodopa, dopamine agonists, catechol-O-methyl transferase inhibitor, or monoamine oxidase type B inhibitors vs patients who were treatment-naïve); differential relative abundances and enrichment of anaerotruncus, clostridium XIVb, several Bacteroidetes, akkermansia, and Verrucomicrobiaceae in patients with Parkinson's disease compared with healthy control individuals; 41 differentially abundant OTUs identified in patients with idiopathic rapid-eye-movement sleep behaviour disorder vs the control group; nine of these 41 OTUs were also among the 48 differentially abundant OTUs in the gut microbiota of patients with Parkinson's disease vs the control group; 30 other differentially abundant OTUs in patients with idiopathic rapid-eye-movement sleep behaviour disorder or Parkinson's disease vs the control group showed the same directions of change in the comparisons to the control group	Single-centre study; patient sex heterogeneity

(Table continues on next page)

probiotics (usually live microbial cultures that impart a health benefit to the host) or prebiotics (non-digestible foodstuffs, including fibre, which convey a beneficial

effect for the host or microbiota) has been shown to modulate the social behaviour of animals.⁸³ These studies are very provocative, and if they could be translated

Participants and intervention		Results	Limitations
(Continued from previous page)			
Randomised, double-blind, placebo-controlled ⁶⁵	120 patients with idiopathic Parkinson's disease were randomly assigned to receive either probiotic and prebiotic treatment (41 men and 39 women; mean age 71.8 years [SD 7.7]) or placebo (24 men and 36 women; mean age 69.5 years [SD 10.3])	Probiotic and prebiotic treatment resulted in a significant increase in complete bowel movements in patients with Parkinson's disease compared with patients in the placebo group	Single-centre study; no recording of symptom burden or other drug use; laxatives were not forbidden and no record taken of use; no prebiotic control group; symptoms were self-reported; bowel habits were characterised by individual variability
Alzheimer's disease			
Double-blind, randomised, controlled ⁶⁶	Six men and 24 women (aged 60–95 years; mean 77.67 years [SEM 2.62]) treated for 12 weeks with a probiotic containing <i>L. acidophilus</i> , <i>L. casei</i> , <i>B. bifidum</i> , and <i>L. fermentum</i> and were compared with six men and 24 women (aged 60–95 years; mean 82 years [SEM 1.69]) matched for disease severity, sex, age, and body-mass index who were given milk; all participants had Alzheimer's disease	Significantly higher MMSE scores and significant decrease in malondialdehyde in the probiotic supplementation group compared with control group; significant change in blood lipid profile and carbohydrate metabolism factors and significantly different VLDL and HDL baseline concentrations between groups	Measurement of faecal bacteria before and after probiotic supplementation was not done; cognition of patients with Alzheimer's disease was only measured with MMSE test; participants heavily skewed towards women
Blind, cross-sectional, observational ³	20 male and 20 female patients who were cognitively impaired and amyloid-positive (mean age 71 years [SD 7]); 16 male and 18 female patients who were cognitively impaired and amyloid-negative (mean age 70 years [SD 7]); four male and six female patients who were cognitively healthy and amyloid-negative (mean age 68 years [SD 8])	Significant association only between <i>Pseudomonas aeruginosa</i> and body-mass index across all groups; cognitively impaired amyloid-positive patients had significant decrease in abundance of <i>B. fragilis</i> and <i>Eubacterium rectale</i> , significant increase in abundance of <i>Escherichia</i> and <i>Shigella</i> , significant upregulation of NLRP3, CXCL2, IL-6, and IL-1 β , and downregulation of IL-10; cognitively impaired amyloid-positive and cognitively impaired amyloid-negative patients had significant upregulation of TNF- α , blood NLRP3, CXCL2, and IL-1 β correlated with <i>E. rectale</i> , all compared with cognitively healthy control individuals	Cross-sectional study design; current standards not used for microbiome assessment (next generation sequencing); no dietary habits were recorded; cognition in patients with Alzheimer's disease was only measured with MMSE test
Blind, cross-sectional, observational ⁶⁷	Eight male and 17 female patients with Alzheimer's disease (mean age 71.3 years [SD 7.3]), and seven male and 18 female age-matched and sex-matched control individuals without Alzheimer's disease (mean age 69.3 years [SD 7.5])	Significant decrease in richness and α -diversity and β -diversity of microbiome; significantly decreased abundance of Firmicutes (Ruminococcaceae, Turicibacteraceae, Peptostreptococcaceae, Clostridiaceae, and Mogibacteriaceae), and the genera SMB53 (family Clostridiaceae), dialister, clostridium, turicibacter, cc115 (family Erysipelotrichaceae), and Actinobacteria (Bifidobacteriaceae at the family level and bifidobacterium and adlercreutzia at the genus level); significant increase in abundance of genus bilophila in the phylum Proteobacteria, and Bacteroidetes (Bacteroidaceae and Rikenellaceae at the family level, and bacteroides and alistipes at the genus level) in patients with Alzheimer's disease compared with control individuals	Cross-sectional study; self-reporting of gastrointestinal difficulties; stool collection was done at home
Cross-sectional, observational ⁶⁸	23 male and 20 female patients with Alzheimer's disease (mean age 70.12 years [SD 8.78]); control group: 23 male and 20 female age-matched and sex-matched individuals with normal cognition (mean age 69.72 years [SD 9.24])	Significantly increased frequency of apolipoprotein $\epsilon 4$ carriers, and CDR and ADL score; significantly lower MMSE score; significantly decreased abundance of Bacteroidetes, Verrucomicrobia, Negativicutes, Bacteroidia, Lachnospiraceae, Bacteroidaceae, and Veillonellaceae; significant increase in Actinobacteria, Bacilli, Ruminococcaceae, Enterococcaceae, and Lactobacillaceae in patients with Alzheimer's disease compared with the control group	Cross-sectional study; no diet information was collected

(Table continues on next page)

to humans, they might offer novel microbiota-based strategies for management of autism spectrum disorder. Large-scale, targeted intervention studies are needed to test whether any of the symptoms of autism spectrum disorder are modifiable in humans. One small-scale pilot study of microbiota transfer therapy of defined microbial consortia has shown promising results in patients with autism spectrum disorder (table).⁴ This clinical trial showed that children with autism spectrum disorder had lower fibre consumption and less breastfeeding in infancy than neurotypical children.⁴ The same study showed that, compared with before treatment, children with autism spectrum disorder who had microbiota transfer therapy

had significantly reduced abdominal pain, indigestion, diarrhoea, and constipation, and significant improvements in behaviour related to autism spectrum disorder, which persisted for at least a further 2 years from the point of microbial transfer.⁵ The authors also noted that after treatment, patients had significantly reduced bacterial diversity, and significantly increased abundance of bifidobacterium, prevotella, and desulfovibrio.⁵

Parkinson's disease

15 years have passed since seminal research postulated that the cause of Parkinson's disease might begin in the gut.⁸⁴ α -Synuclein, the protein aggregate that is the hallmark of

Participants and intervention		Results	Limitations
(Continued from previous page)			
Mild cognitive impairment			
Cross-sectional, observational ⁶⁹	43 adults (aged 50–85 years) divided into those performing ≥ 1 SD below normative cognitive performance (mean age 64.06 years [SD 9.37]) compared with normal cognitive performance (mean age 64.08 years [SD 6.49])	Significant correlations between microbiome differences and cognitive test performance; significant increase in Verrucomicrobia that correlated with better scores on HVLT-R total learning; significantly higher abundance of Firmicutes correlated with higher scores on the CFT immediate and delayed recall task; significant increase in Bacteroidetes that correlated with poorer performances on the CFT immediate; significant increase in Proteobacteria correlated with poorer scores on HVLT-R recognition and discrimination, FAB, and FAS; all in cognitively impaired individuals compared with healthy control individuals	Cross-sectional study; small sample size; self-reported measures to assess dietary habits and physical activity
Stroke and brain injury			
Case-controlled, cross-sectional, observational ⁶	220 male and 112 female patients who have had an acute ischaemic stroke or transient ischaemic attack (median age 61 years), compared with 130 male and 101 female asymptomatic individuals (median age 56 years)	Significantly increased phylogenetic and α -diversity, richness, and evenness; significant increase in Proteobacteria and decrease in bacteroides, prevotella, and faecalibacterium; significantly lower blood trimethylamine N-oxide concentrations in stroke and transient ischaemic attack groups compared with the asymptomatic control group	Cross-sectional study design; heterogeneity in terms of patient age and sex; patient and control groups were recruited from different locations; no diet information was collected
Epilepsy			
Cross-sectional, interventional ⁷⁰	11 male and three female children with epilepsy (mean age 1.95 years), and 15 male and 15 female age-matched healthy infants (aged <3 years; mean age 2.33 years) received a ketogenic diet for 1 week	After a week of treatment, nine (64%) of 14 of infants with epilepsy showed a 50% decrease in seizure frequency compared with healthy infants; significantly lower abundance of Actinobacteria, bacteroides, prevotella, and bifidobacterium; significant increase in Proteobacteria, and cronobacter were reported in all patients with epilepsy after the treatment compared with healthy control individuals	Cross-sectional study design; small sample size; no reporting of gastrointestinal issues
Interventional, observational ⁷¹	12 children with therapy-resistant epilepsy (aged 2–17 years; mean age 7.67 years [SD 4.51]) received 3 months of ketogenic diet; 11 healthy parents of the children included in the cohort acted as control individuals (one parent per child); the control group had a normal dietary intake and did not make any substantial changes in their diet during the study period	Significantly reduced α -diversity and relative abundance of bifidobacteria, Actinobacteria, as well as <i>E rectale</i> and dialister and increased relative abundance of <i>Escherichia coli</i> in the children with therapy-resistant epilepsy compared with the control group	Small sample size; no reporting of gastrointestinal issues; heterogeneity of this epilepsy cohort; no age-matched control group
Amyotrophic lateral sclerosis			
Cross-sectional, observational ⁷²	12 male and 13 female patients with amyotrophic lateral sclerosis (mean age 57.56 years [SD 11.24]), and 16 male and 16 female age-matched and sex-matched healthy controls (mean age 56.00 years [SD 11.65])	Significant increase in interindividual variability in relative abundances (OTUs) of faecal taxa in both patients with amyotrophic lateral sclerosis and the control group; no significant changes in the abundance of OTUs and taxa in either group	Cross-sectional study design; patients of a single ethnicity
BDI=Beck Depression Inventory. EDSS=Expanded Disability Status Scale. DHQ=Diet History Questionnaire. DASS=Depression Anxiety Stress Scale. IL=interleukin. PBMCs=peripheral blood mononuclear cells. FDR=false discovery rate. FACS=fluorescence-activated cell sorting. GRS=Gastrointestinal Symptom Rating Scale. CARS=Childhood Autism Rating Scale. ABC=Aberrant Behaviour Checklist. UPDRS2=Unified Parkinson's Disease Rating Scale 2. MMSE=Mini Mental-State Exam. MoCA-J=Japanese Montreal Cognitive Assessment. FAB=Frontal Assessment Battery. SEM=standard error of the mean. OTU=operational taxonomic unit. CDR=Clinical Dementia Rating. ADL=activities of daily living. HVLT-R=Hopkins Verbal Learning Test-Revised. CFT=Complex Figure Test. FAS=a verbal fluency test.			
Table: Clinical studies assessing the microbiota–gut–brain axis in neurological disorders			

the pathology of Parkinson's disease in the brain, has also been identified in the mucosal and submucosal nerve fibres and ganglia^{8,9} of patients with parkinsonian syndrome, with some preclinical evidence even suggesting that α -synuclein in the gut can be transported to the brain via the vagus nerve.⁸⁵ The relationship between gut proteins and cognitive health is receiving increased attention because amyloid-like proteins can be produced by bacteria and have been shown to increase α -synuclein pathology in older rats.⁸⁶ Furthermore, functional gut symptoms, such as constipation, often occur prodromal, years before any motor symptoms emerge.⁸⁷

The vagus nerve is particularly well placed to be the conduit for signals from the gut to the brain, either

through the transport of small or large molecules such as the prion-like translocation of α -synuclein, or neuronally via electrical signalling. One of the most intriguing findings linking the vagus nerve and Parkinson's disease comes from epidemiological studies using Danish and Swedish patient registries that have shown that truncal vagotomy is protective against Parkinson's disease.^{88,89} Moreover, truncal vagotomy in mice prevented gut-to-brain propagation of α -synucleinopathy, along with related neurodegeneration and behavioural deficits.⁹⁰

A growing number of studies are showing alterations in the composition of the microbiota in patients with Parkinson's disease (table).^{57,59,64,91} However, a consensus has not been reached on whether a specific microbial

signature exists for Parkinson's disease itself. Although there has been much excitement in the field, caution is needed when examining the available data because they are largely derived from small cohorts and do not provide a longitudinal perspective. More mechanistic studies are needed to understand how changes in the microbiota can moderate both the motor and non-motor symptoms of Parkinson's disease.^{92,93} When mice were colonised with the microbiota of patients with Parkinson's disease via faecal microbiota transplantation, they developed motor deficits and neuroinflammation, two hallmark symptoms of Parkinson's disease.¹⁸ Additionally, behavioural symptoms improved when the mice were treated with antibiotics. This study implicated short-chain fatty acids as drivers of the neuroinflammatory processes in animal models of Parkinson's disease.¹⁸ Whether this implication is translatable to humans is unclear because data from human studies showed a reduction in short-chain fatty acids in patients with Parkinson's disease,⁶⁰ and beneficial effects of short-chain fatty acids on behaviour have been reported in mouse studies.⁹⁴

Another way in which the gut microbiota might affect neurological disorders is by interacting with pharmacological agents used in their treatment.⁹⁵ Evidence has shown that gut microbial tyrosine decarboxylases in rats can attenuate plasma concentrations of levodopa used in the treatment of Parkinson's disease.⁹⁶ More research is warranted to understand the effects such interactions might have on the safety and efficacy of this drug.

Alzheimer's disease

Despite much disappointment in drug discovery for Alzheimer's disease over the past decade, there has been some excitement with the possibility that gut microbes have a role in the disease. Although not a new concept, several studies^{86,97} suggest a possible microbial origin for Alzheimer's disease. The concept that amyloid might act as an antimicrobial peptide in the brain has been an intriguing one, backed up by seminal experimental evidence.⁹⁸ However, proving that there is an infective cause to the neuroinflammation and neurodegeneration seen in patients with Alzheimer's disease is logistically and ethically challenging in humans. As in Parkinson's disease, the relationship between gut proteins and cognitive health has received increased attention, showing that amyloid-like proteins can be produced by bacteria⁹⁹ and increase α -synuclein pathology in vagotomised older rats.⁸⁶ However, confirmation in patients with Parkinson's disease is outstanding.

Cross-sectional studies have identified that the *Escherichia* and *Shigella* bacterial taxa, which are associated with mediating inflammation, are increased in faecal samples from patients with Alzheimer's disease compared with healthy individuals (table).³ Moreover, the microbiota changes in patients with Alzheimer's disease were associated with pro-inflammatory cytokine concentrations in unstimulated and non-centrifuged blood from these

patients. The increased abundance of pro-inflammatory *Escherichia* and *Shigella*, and a reduction in the abundance of anti-inflammatory *Escherichia rectale* being possibly associated with a peripheral inflammatory state in patients with cognitive impairment and brain amyloidosis, suggest a link between dysregulation of the microbiota and systemic inflammation, which might initiate or exacerbate the neurodegeneration that occurs in the brain of patients with Alzheimer's disease. However, it is important to note that these results are from small studies and that longitudinal research is needed in larger cohorts to assess microbiota involvement in the progression of, and its causal relationship with, Alzheimer's disease.

In parallel, transgenic mouse models of Alzheimer's disease have been shown to have altered microbiota.^{40,100,101} Seminal studies in germ-free mice showed that there is a marked absence of amyloid plaque build-up and neuroinflammation when microbes are not present.⁴⁰ Similarly, chronic treatment of transgenic mice with an antibiotic cocktail reduced microglia and astrocyte accumulation around amyloid plaques in the hippocampus, and decreased insoluble amyloid β plaques.^{101,102} Together, these studies highlight that the microbiota has a role in regulating key molecular components of Alzheimer's disease.

Stroke and brain injury

Systemic and peripheral risk factors can exacerbate the local pathophysiological response to stroke and brain injury, including neuroinflammation.¹⁰³ Thus, the relationship between stroke or brain injury and the microbiota in terms of susceptibility and moderation of outcome is a growing area of research (table). For example, atherosclerosis and hypertension, both of which are known risk factors in cardiovascular disease and stroke, are associated with microbiota richness and diversity.^{10,104,105} Cross-sectional studies have reported microbiota composition dysregulation in patients with stroke compared with healthy, asymptomatic control individuals.⁶ The gut bacterial metabolite trimethylamine N-oxide is associated with an increased risk of major adverse cardiovascular events,¹⁰⁶ gestational diabetes,¹⁰⁷ and Alzheimer's disease,¹⁰⁸ indicating the possibility of therapeutic advances in disease treatment through modulation of the gut microbiota.

In preclinical models, cerebral ischaemia is associated with an altered microbiota composition and functional gastrointestinal effects of motility and barrier permeability.^{103,109} Moreover, faecal microbiota transplantation from a stroke model into germ-free mice¹⁰³ or from patients who have had a stroke into antibiotic-treated mice¹¹⁰ exacerbated the ischaemia-induced cerebral lesion volume and associated functional deficits. Administration of broad-spectrum antibiotics before ischaemic injury is associated with significantly worse outcomes in mice.¹¹¹ Antibiotic-induced microbiota dysregulation also resulted in a reduction in the trafficking of pro-inflammatory IL-17 $\gamma\delta$ T cells and IL-17-associated chemokine expression.¹¹² Thus, the

gut microbiota appears to influence the magnitude of neuroinflammation after a stroke by modulating intestinal T-cell trafficking to the brain. Administration of a specific bacterial strain, *Clostridium butyricum*, is neuroprotective in an animal model of cerebral ischaemia–reperfusion injury.¹¹³

There is also a growing interest in the microbiota as a factor that influences outcomes after traumatic brain injury.¹¹⁴ Alterations in microbiota composition have been shown following traumatic brain injury,¹⁰⁹ and *C butyricum* was shown to have neuroprotective effects in a mouse model of such injuries.¹¹⁵ Some emphasis has been placed on developing diets that are enriched with prebiotics or probiotics to counter some of the comorbidities associated with traumatic brain injury,¹¹⁶ but more clinical trials are needed to understand the therapeutic potential of such interventions.

The role of the microbiota in mediating susceptibility and moderating stroke and brain injury outcomes is just beginning to be understood. Although animal models have provided very intriguing data, more research is needed to determine whether, and to what extent, such effects translate to clinical practice.

Epilepsy, amyotrophic lateral sclerosis, and Huntington's disease

Much less evidence implicates the microbiota in epilepsy, amyotrophic lateral sclerosis, and Huntington's disease compared with other disorders. However, researchers and clinicians in these fields are also becoming focused on the potential of the microbiota to regulate physiology and behaviour in these disorders. Because of the use of the ketogenic diet to treat epilepsy, it is perhaps surprising that there has not been more focus on the relationship between epilepsy and the microbiota. Germ-free mice studies have implicated the microbiota in synaptic changes in key brain areas involved in epileptogenesis,^{31–33,117} and the ketogenic diet has been shown to alter the microbiota in infants (table)^{70,71} and animals¹¹⁸ with epilepsy. Studies in germ-free mice have also shown that the beneficial effects of the ketogenic diet are dependent on the microbiota.¹¹⁹ It is likely that ensuing research will place an increased emphasis on the potential role of the microbiota as a mediator of epilepsy.

Mouse models of amyotrophic lateral sclerosis have implicated alterations in the gut microbiota in the pathogenesis of this disease. For example, these models have a lower relative abundance of butyrate-producing bacteria than healthy mice, which was associated with alterations in gut permeability.¹⁰² However, cross-sectional studies in humans have not yet found any relationship between the microbiota and disease progression of amyotrophic lateral sclerosis (table).⁷²

Data on microbiota changes in patients with Huntington's disease are scarce, perhaps because this disease is viewed predominantly as a genetic disorder. However, intrinsic factors (eg, changes to protein homeostasis, mitochondrial

dysfunction, and uncontrolled corticostriatal input) and extrinsic environmental factors (eg, ethnicity, geographic region, tea consumption, and alcohol and tobacco use) can moderate progression of Huntington's disease. One metabolomic study showed that altered metabolites derived from the gut microbiota were found in the serum of a cohort of patients who were premorbid and in patients with early-stage Huntington's disease compared with individuals in a control group.¹²⁰ A preclinical study claims to be the first to provide evidence of gut dysbiosis in a transgenic mouse model of Huntington's disease.¹²¹ However, more research is warranted to fully understand the ramifications of the gut microbiota and its metabolites in terms of onset, progression, and severity of the behavioural manifestations in Huntington's disease.

Conclusions and future directions

There has been an explosion of basic research that suggests the microbiota is important for the normal development and maintenance of brain function. Evidence is also accumulating from both clinical and animal studies implicating the microbiota in neurological disorders. The strongest evidence in support of the role of the microbiota is in Parkinson's disease,^{9,18,63,85,90,96} multiple sclerosis,^{7,19,20,48,49} and autism spectrum disorder,^{4,5,23,30,53,55,56} with a growing appreciation of its role in Alzheimer's disease and stroke. However, it is still very much early days and caution is needed to avoid overinterpreting such data. Most of the studies are underpowered, often with participant-selection bias, differing sampling and sequencing protocols, bioinformatics pipelines, statistical methods, and confounders (table). As a result, to understand the intricate processes behind the microbiota–gut–brain axis involvement in neurological disorders, more well controlled and well designed studies are needed.

To move from purely correlational observational studies towards causative and functional outcomes, more emphasis is required on interventional approaches using probiotic strains, prebiotics, and, potentially, faecal microbiota transplantation therapies. These studies should be longitudinal in design, and not just cross-sectional, to provide a temporal factor in identifying the microbiota as a potential biomarker of disease. Already, human brain imaging and EEG studies have examined the effect of microbiota changes on brain function in healthy volunteers.^{122–124} These studies use targeted microbiota interventions that support good cognitive health to improve targeting of the microbiota–gut–brain axis and are paving the way for the development of novel therapeutics. Further work is needed to understand to what extent any of these studies translate to neurological populations.

Currently, definition of a healthy microbiome is perhaps one of the biggest conundrums in microbiome-based medicine, compounded by the fact that interindividual differences in microbiome composition can be large, making a one-size-fits-all approach to target the microbiome challenging. However, targeting the microbiome

Search strategy and selection criteria

We searched PubMed, Google Scholar, and Web of Science for articles published in English using the terms “microbiota”, or “microbiome”, or “microbes”, or “gut”, and “neurological”, or “neurodegenerative”, or “Parkinson’s”, or “Alzheimer’s”, or “Huntington’s”, or “ageing”, or “neurodevelopment”, or “autism”, or “stroke”, or “brain injury”, or “amyotrophic lateral sclerosis”, or “epilepsy” between Jan 1, 2012, and June 30, 2019. The final reference list was generated on the basis of the relevance to this Review.

also offers opportunities, because it might be the conduit for future personalised-medicine approaches. More research is needed to fully understand the internal and external factors that constrain the microbiota from being modified by diet or other interventions. Better description of the microbial composition, delving deeper than just the genus, down to the strain level, using metagenomic and multiomic approaches rather than 16S rRNA gene sequencing is also required. Furthermore, expansion beyond the bacteriome is needed, especially in terms of the virome and bacteriophage fields, to appreciate fully the importance of the microbiome in regulating brain function. Understanding the crosstalk between host genetics and the microbiome is understudied and will be very important in parsing the biological mechanisms of neurological disorders. Systems biology approaches will be key in integrating such multiomic data. Even more important will be the interpretation of the molecular mechanisms involved in the bidirectional microbiota–gut–brain communication, identifying and understanding the roles of the metabolites generated and their potential interactions with the host.

Diet is perhaps one of the greatest factors influencing microbiota composition.¹⁷ Because many neurological disorders affect appetite, swallowing, and nutrition in general, it is essential to have good dietary data for all human studies. This will enable a closer understanding of the relationship between diet, microbiota composition, and the brain, which has shown to be crucial in both early life and ageing.¹⁶ Where research has shown that there are clear links between diet and vulnerability or protection for neurological diseases, assessing what role, if any, the microbiome has in terms of causality will be important. The effects of both dietary components and microbial-generated metabolites on host physiology and health are gaining attention, which will be important for moving therapeutic approaches forward.

Many patients are prescribed multiple medications and understanding of the relationship between the microbiota and drug action is growing.^{25,105} An in-vitro study showed that 284 (25%) of 1053 different non-antibiotic drugs tested affected the microbiota.¹²⁵ Thus, the effect of drugs on the microbiota need to be investigated. A lot has been learnt in the past 5 years and, evidently, the next 5 years

will allow for a better understanding of to what extent the microbiota can translate into therapies for neurological disorders.

Contributors

JFC and TGD did the writing, created the concepts, and prepared the manuscript. KJOR edited the text and figures, and created the table. VLP created the figures and panel, wrote the legends, and input the citations. KS created the figures and edited the manuscript.

Declaration of interests

TGD has been an invited speaker at meetings organised by Servier, Lundbeck, Janssen, and AstraZeneca, and has received research funding from Mead Johnson, Cremo, Suntory Wellness, Nutricia, and 4D Pharma. JFC has been an invited speaker at meetings organised by Mead Johnson, Yakult, Alkermes, Ordesa, and Janssen, and has received research funding from Mead Johnson, Cremo, Suntory Wellness, Nutricia, Pharmavite, Dupont, and 4D Pharma. All other authors declare no competing interests.

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