

Gut microbiome in multiple sclerosis: The players involved and the roles they play

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ABSTRACT

The human gut contains trillions of bacteria (microbiome) that play a major role in maintaining a healthy state for the host. Perturbation of this healthy gut microbiome might be an important environmental factor in the pathogenesis of inflammatory autoimmune diseases such as multiple sclerosis (MS). Others and we have recently reported that MS patients have gut microbial dysbiosis (altered microbiota) with the depletion of some and enrichment of other bacteria. However, the significance of gut bacteria that show lower or higher abundance in MS is unclear. The majority of gut bacteria are associated with certain metabolic pathways, which in turn help in the maintenance of immune homeostasis of the host. Here we discuss recent MS microbiome studies and the possible mechanisms through which gut microbiome might contribute to the pathogenesis of MS.

ARTICLE HISTORY

Received 16 March 2017
Revised 7 June 2017
Accepted 27 June 2017

KEYWORDS

gut microbiome; host-microbe interaction; immune response; microbial metabolism; multiple sclerosis (MS); phytoestrogen; short chain fatty acids

Introduction

Multiple sclerosis (MS) and altered gut microbiota

Multiple sclerosis (MS), an autoimmune disease of the central nervous system (CNS), is characterized by demyelination, axonal damage, and progressive neurologic disability. Collective evidence suggests that disease onset might result from aberrant T cell-mediated immune responses to several myelin antigens. The etiology of MS is complex and not well understood. While certain genetic factors (e.g., certain HLA class II haplotypes) have emerged as strong candidates associated with the disease, the exact nature of environmental contributing factors remains elusive. Altered gut microbiota might be one of the major missing environmental factors contributing to MS because evidence suggests that certain gut microbiota might be linked to either disease susceptibility or protection.^{1–5}

Gut microbiome and human health

Human Microbiome Project (HMP), a National Institutes of Health initiative to catalog microbial flora in healthy individuals, has shown that large microbial

communities residing within or on the human body play a major role in both health and disease.^{6–8} Human beings could be considered a superorganism encompassing both human genome and microbiome (microbial communities, their genome, proteins, and metabolites).^{6–8} The gut microbiota functions like a bioreactor that influences nutrient uptake, food metabolism, energy homeostasis, and shaping mucosal as well as systemic immune responses (Fig. 1). A healthy gut microbiota is characterized by its diversity (species richness) and resilience (Box 1).⁸ The gut microbiota helps in keeping a healthy state in multiple ways, including maintenance of an intact intestinal barrier, inhibition of colonization by pathogenic organisms, and regulation of host physiology and immune responses.^{9–12} Alteration of the gut microbiota and subsequent changes in its metabolic network perturb this homeostasis, leading to negative consequences that may result in intestinal and systemic disorders. This observation has led to an increased emphasis for a detailed understanding of the mechanism by which the gut microbiota regulates host metabolism and immune responses. The gut microbiota consists of organisms including bacteria, viruses,

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Addendum to: Chen J, Chia N, Kalari KR, Yao JZ, Novotna M, Soldan MM, et al. Multiple sclerosis patients have a distinct gut microbiota compared to healthy controls. *Sci Rep* 2016; 6:28484.

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Box 1

Microbiota	All the microbes (archaea, bacteria, fungi, viruses, etc.) present within an ecosystem/habitat. A collective study of these microbes in the gut is called the <i>gut microbiome</i> , and at the skin, it is called <i>skin microbiome</i> .
Microbiome	Collective genomic, protein, or metabolite content of all the microbes in a given ecosystem/habitat, e.g., the microbial community in the gut is called <i>gut microbiota</i> .
Metagenome	Study of genetic material from a given ecosystem/habitat, e.g., 16S-rRNA metagenomic analysis is the study of bacteria present within a given environment through sequencing of the 16S rRNA region of the bacteria.
Healthy microbiota	A diversified microbiota present in a healthy state responsible for maintaining homeostasis of host physiology including the immune system. It is characterized by a diverse microbial community, which is stable, shows resistance and resilience, and maintains immune homeostasis by keeping a balance between pro- and anti-inflammatory responses.
Dysbiosis	Alteration of microbiota from a healthy state; it is characterized by lower resistance and resilience ability, shifting the immune balance toward an inflammatory phenotype.

archaea, and fungi. However, the majority of MS studies have focused on bacteria or bacteria plus archaea.^{1-3,13-19} The major hurdles in the analysis of viruses and fungi are the lack of well-developed tools for their analysis. In recent years, several groups including ours have evaluated a role of the gut microbiota in MS patients by analyzing 16S-rRNA metagenomic sequencing,^{1-3,15-19}

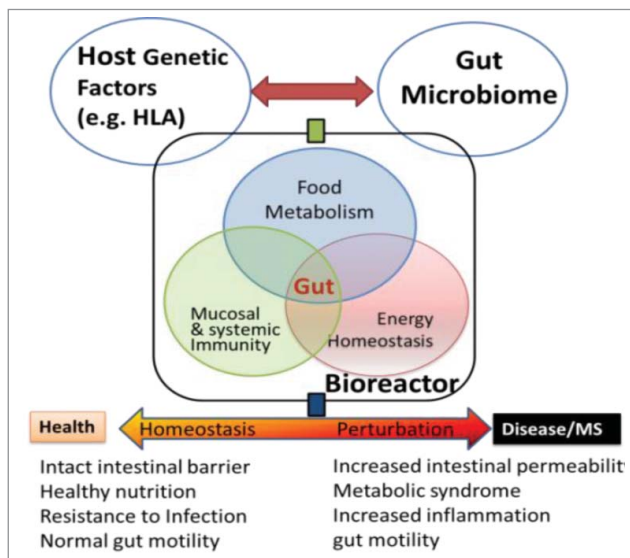


Figure 1. Role of the gut microbiota in health and disease.

phylochip analysis of fecal DNA samples,¹⁴ or RNAseq analysis of biopsies from brain white matter.¹³

Methods used for gut microbiota profiling in MS patients

The development of low-cost, culture-independent analyses with improved DNA sequencing methods has led to rapid development in the investigation of gut microbiota profiling studies in the last decade, an achievement due in large part to the HMP.²⁰ The majority of bacteria (~90%) in adult human gut belong to Firmicutes or Bacteroidetes phyla, with the remaining belonging to Actinobacteria, Proteobacteria, and few other phyla.²⁰ The majority of MS gut microbiota studies have used either sequencing of a small region within the 16S rRNA gene of bacteria or phylochip analysis, a microarray-based method that uses probes for the 16S rRNA region of known microbial taxa (~50,000) (Fig. 2). Analysis of the 16S rRNA gene is the preferred method for taxonomic classification of bacteria because it includes both conserved and 9 hypervariable regions (V1–V9) (Fig. 2). Polymerase chain reaction (PCR) primers are designed within specific conserved regions to get an amplification product comprising one or more hypervariable regions (Fig. 2). In light of current next generation sequencing technologies (Illumina and Roche), which allow sequencing of only 500 nucleotides out of the 1500 nucleotide-long 16S rRNA gene, primers are chosen for amplification products consisting of either V1–V2, V3–V5, or V6–V9 regions, each showing bias for particular taxa.^{21,22} Some groups prefer the V1–V2 region,³ which shows an increased bias for *Clostridium* and decreased bias for certain *Bacteroidetes* species, while other groups prefer the V4, V3–V4, and V3–V5 regions, which demonstrate the least biased classification of bacterial taxa.^{1,2,16-19}

Gut microbiota in MS

In the last few years, several groups including ours have profiled fecal gut microbiota from MS patients and have shown that MS patients exhibit gut microbial dysbiosis with both depletion and enrichment of certain bacteria compared with healthy controls (Table 1).^{1-3,14,16-19} Within Bacteroidetes phyla *Bacteroides*, *Prevotella*, and *Parabacteroides* are the major genera and we observed depletion of *Parabacteroides* and *Prevotella* in relapsing remitting MS (RRMS)

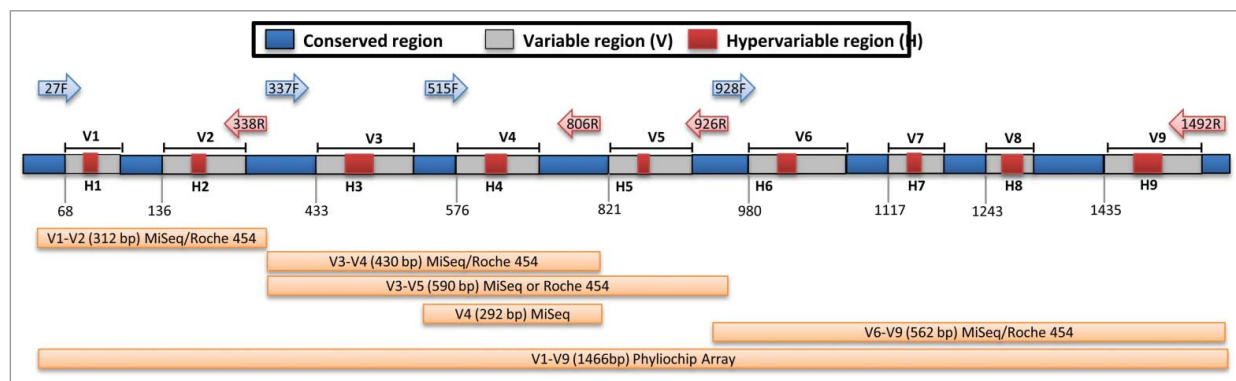


Figure 2. Illustration of conserved, variable, hypervariable regions within the 16S rRNA gene and the various primer pairs used for metagenomic sequencing. Conserved regions are represented in blue, variable regions in gray, and hypervariable regions in red. Nine hypervariable regions are not distributed uniformly, as some hypervariable regions such as H3 and H9 are longer compared to H5 or H7. Primers are designed in a conserved region to get a PCR product spanning one (V4) or more hypervariable regions (e.g., V1-2 or V3-5). Single-end (Roche 454) or double-end (Illumina) sequencing of PCR product provides data for profiling of microbiota.

patients.¹ Cekanaviciute et al. also reported a decreased abundance of *Parabacteroides distasonis* in RRMS patients compared with healthy controls.¹⁶ These observations suggest that *Parabacteroides* might be a beneficial commensal organism and may play a protecting role in RRMS. Many studies have reported either reduced abundance of *Prevotella* in RRMS patients^{1,3} or an increased abundance of *Prevotella* after treatment with disease-modifying therapies.^{2,15} We observed a reduced abundance of *Prevotella* in RRMS patients compared with age- and gender-matched healthy controls.¹ Miyake et al. demonstrated that RRMS patients had reduced abundance of *Prevotella* (*Prevotella copri*) compared with healthy controls.³ Jangi et al. showed that RRMS patients on disease-modifying therapies had increased abundance of *Prevotella* compared with untreated patients² and Castillo Alvarez et al. observed depletion of *Prevotella copri* in RRMS patients on interferon β -1b treatment.¹⁵ The reduced abundance of *Prevotella* observed across multiple MS microbiome studies in different geographical locations suggests this bacterium might have an important anti-inflammatory role in RRMS patients.

Among Firmicutes, some genera were depleted whereas others were enriched in RRMS patients. We observed an increase abundance of *Dorea* and *Blautia* in RRMS patients.¹ Although *Dorea* is considered a constituent of healthy gut flora, it has been linked with inflammatory diseases, such as Crohn's disease, where patients exhibit an abundance of *Dorea*.²³ Some discrepancy exists for *Clostridium* and *Fecalibacterium*, as we¹ and others^{2,15,16,18,19} did not observe depletion of these bacteria, whereas Miyake et al. and Cantarel et al. observed depletion of *Clostridium*³ and

Fecalibacterium,¹⁴ respectively. The discrepancies in results among studies might be due to the use of different methods for microbiome analysis. Jangi et al.² used both V4 and V3-V5, our group¹ used V3-V5, and others¹⁶⁻¹⁹ used V4-specific primers-based DNA sequencing. In contrast, Miyake et al.³ used V1-V2 specific primer-based sequencing and Cantarel et al.¹⁴ used Phylochip based analysis. Only one study has followed patients longitudinally (up to 19 months) and observed that more frequent relapses in pediatric MS patients were associated with a higher abundance of Firmicutes (Table 1).¹⁷

Within Actinobacteria phyla, we observed a lower abundance of *Adlercreutzia* (*equolifaciens*) and *Collinsella* in MS patients.¹ Although Jangi et al. did not find a difference in *Adlercreutzia*, they did report depletion of *Collinsella* and *Slackia* in MS patients.² With regard to Proteobacteria, we observed enrichment of *Mycoplana* and *Pseudomonas* in MS patients,¹; Miyake et al.³ reported higher abundance of *Bilophila* in MS patients; and Cekanaviciute et al.¹⁶ observed higher abundance of *Acinetobacter calcoaceticus*. In contrast, a few studies have reported that levels of *Sutterella* (Proteobacteria) were restored after treatment of MS patients with disease modifying drugs.^{2,15} Collectively, these studies point toward both a pro- and anti-inflammatory role of Proteobacteria in MS. Higher abundance of Proteobacteria has also been reported in other autoimmune diseases such as inflammatory bowel disease (IBD).²⁴ Based on a higher prevalence of Proteobacteria in multiple autoimmune diseases, it is suggested that Proteobacteria might

Table 1. MS Microbiome Studies.

Subjects (n, M/F) sample type (country)	Treatment	Microbiome analysis method	Change in abundance between MS vs. HC (P-Phyla, F- family, G-genus)		Change in abundance between treated and untreated MS (P-Phyla, F- family, G-genus)		Ref
			Increased in MS vs HC	Decreased in MS vs HC	Increased in MS patients	Decreased in MS patients	
RRMS (n = 31, 10M/ 21F) HC (n = 36, 14M/22F) Fecal samples (USA)		16S rRNA V3-5 Illumina MiSeq	<i>Pseudomonas</i> (G), <i>Mycoplana</i> (G), <i>Haemophilus</i> (G), <i>Blautia</i> (G), <i>Dorea</i> (G), <i>Pedobacter</i> (G) and <i>Flavobacterium</i> (G)	<i>Prevotella</i> (G), <i>Parabacteroides</i> (G), <i>Adlercreutzia</i> (G), <i>Collinsella</i> (G), <i>Lactobacillus</i> (G), <i>Coprobacillus</i> (G), <i>Haemophilus</i> (G)		No change in levels of <i>Fecalibacterium</i> (G)	1
RRMS (n = 60, 19M/41F) HC (n = 43, 6M/37F) Fecal samples (USA)	RRMS treated (disease-modifying treatment) vs untreated	16S rRNA V3-V5 Roche 454 and V4 by Illumina MiSeq	<i>Methanobrevibacter</i> (G) <i>Akkermansia</i> (G)	<i>Prevotella</i> (G) <i>Butyrivimonas</i> (G) <i>Prevotella</i> (G)	<i>Methanobrevibacter</i> (G) <i>Akkermansia</i> (G) <i>Sarcina</i> (G)	No change in <i>Butyrivimonas</i> on treatment	2
RRMS (n = 20, 6M/ 14F) HC (n = 40, 20M/ 20F) Fecal sample (Japan)		16S rRNA V1-V2 Illumina MiSeq	<i>Bifidobacterium</i> (G) <i>Streptococcus</i> (G) <i>Streptococcus</i> <i>thermophilus</i> <i>Eggerthella lenta</i>	<i>Bacteroides</i> (G) (<i>B. stercoris</i> , <i>B. coprocola</i> , and <i>B. coprophilus</i>) <i>Fecalibacterium</i> (G) <i>Prevotella</i> (G) (<i>P.copri</i>) <i>Anaerostipes</i> (G) <i>Clostridium</i> (G) <i>Sutterella</i> (G) (<i>S. wadsworthensis</i>)			3
RRMS (n = 7) HC (n = 8) no gender data Fecal samples (USA) RRMS (n = 30) HC (n = 14) no gender data Fecal samples (UK)	# GA treated vs. untreated # vitamin D supplementation in MS patient vs. HC Treated with interferon β -1b (n = 15) vs. untreated (n = 15)	Amplification of whole V1- V9 region of 16S rRNA followed by Phylochip analysis No data	<i>Ruminococcus</i> (G)	<i>Fecalibacterium</i> (G)	<i>Bacteroidaceae</i> (F) <i>Ruminococcus</i> (G) <i>Lactobacillaceae</i> (F) <i>Clostridium</i> (G) <i>Prevotella copri</i> DSM 18205	Increase in <i>Akkermansia</i> (G) <i>Fecalibacterium</i> (G) <i>Coproccoccus</i> (G) genera after Vitamin D supplementation Firmicutes, Actinobacteria and Lentisphaerae differed between untreated MS patients, vs treated and HC	14 15

Treatment Naive MS (n = 64) no gender data Fecal samples (No data)	16S rRNA V4 Illumina MiSeq	<i>Acinetobacter calcoaceticus</i> <i>Akkermansia muciniphila</i>	<i>Parabacteroides distasonis</i>	16 <i>A. calcoaceticus</i> and <i>A. muciniphila</i> induce proinflammatory response <i>P. distasonis</i> induce Tregs
Pediatric RRMS (n = 18, 8M/10F) HC (n = 17, 8M/9F) Fecal samples (USA)	16S rRNA V4 Illumina MiSeq	<i>Biophila</i> (G), <i>Desulfovibrio</i> (G), Christensenellaceae (F)	Lachnospiraceae (F) Ruminococcaceae (F)	17 Bacteroidetes was inversely associated with Th17 for RRMS but not controls. Fusobacteria correlated with Tregs in HC
Pediatric RRMS (n = 15, 7M/8F) HC (n = 9, 2M/7F) Fecal samples (USA)	16S rRNA V4 Illumina MiSeq			18 A shorter time to relapse was associated with absence of Fusobacteria and higher abundance of Firmicutes and Archaea Euryarchaeota
Pediatric RRMS (n = 17, 7M/10F) Fecal samples (USA)	16S rRNA V4 Illumina MiSeq			19 A shorter time to relapse was associated with absence of Fusobacteria and higher abundance of Firmicutes and Archaea Euryarchaeota
P-MS (n = 5), RRMS (n = 4), SPMS (n = 14) Non MS Controls (n = 21) Brain biopsies (Canada)	RNASeq analysis	Proteobacteria (P) (RRMS) Actinobacteria (P) (P-MS)	Bacteriophages with Proteobacteria	13

contribute to autoimmune diseases by promoting pro-inflammatory responses.²⁴ Finally, 2 studies had also reported an increased abundance of *Akkermansia*, belonging to the Verrucomicrobia phylum, in MS patients.^{2,16} However, the role of *Akkermansia* in inflammatory diseases is not clear, as it has been reported to be decreased in IBD patients.

In addition to the gut, one study has also reported the presence of bacteria in brain biopsies.¹³ Biopsy samples of brain white matter from RRMS patients showed a higher abundance of *Fusobacterium*,¹³ a Gram-negative anaerobic bacteria of the phyla Fusobacteria. *Fusobacterium* has been previously reported in colon cancer tissue and ulcerative colitis. Currently, it is unclear whether *Fusobacterium* is pathogenic or if it grows in an inflammatory environment.

Because the majority of these studies profile fecal samples at a single point, it is hard to conclude whether the changes in the gut microbiota are a cause or consequence of the disease. Future studies analyzing the temporal changes in the gut microbiota will be able to answer whether the disease onset and/or relapses are associated with a shift in the gut microbiota.

Functional significance of bacteria positively or negatively associated with MS

In a healthy individual, the gut consists of a diversified bacterial community that is responsible for maintaining the balance between pro- and anti-inflammatory immune responses. RRMS is an inflammatory disease in which the immune balance is tilted toward a pro-inflammatory state. Therefore, it is reasonable to hypothesize that gut dysbiosis can be characterized by depletion of bacteria responsible for induction/maintenance of anti-inflammatory responses and/or enrichment of bacteria with the ability to induce pro-inflammatory responses. Major immune cells associated with anti-inflammatory response are CD4⁺CD25⁺FoxP3⁺T cells (Tregs), IL-10-producing CD4⁺ T cells (Tr1), tolerogenic dendritic cells, suppressive macrophages, and regulatory B cells. In contrast, immune cells associated with pro-inflammatory responses are CD4 T cells of Th1 or Th17 phenotype, inflammatory dendritic cells, monocytes, and B cells. Discussion of these inflammatory and regulatory immune cell populations can be found elsewhere.²⁵

Significance of gut bacteria negatively associated with MS (anti-inflammatory bacteria)

As mentioned previously, gut bacteria exhibit a symbiotic relationship with the host (human), which provides them space and nutrients. In turn, bacteria help in maintaining a healthy state of the host by performing several physiologic functions such as digestion of food, immune system development, maintenance of the gut barrier, suppression of colonization of pathogens, etc. Because the gut microbiota is a community structure, some bacteria directly feed on host-provided nutrients, whereas other bacteria feed on bacterial by-products, a process called *cross-feeding*. Unsurprisingly, diet is one of the major factors determining our gut microbiota. Specifically, gut microbiota help with digestion/metabolism of several compounds including starches/fibers, phytoestrogens, bile acids, and tryptophan. Recent studies show that metabolism of food by microbiota have a strong influence on the development and function of the immune system.²⁶ Metabolism of starch/complex sugars by gut bacteria leads to the production of short-chain fatty acids (SCFAs), which are one of most studied bacterial metabolites. Firmicutes, such as *Clostridium*, have received much attention because certain species can produce SCFAs and aid in the maintenance of regulatory FoxP3⁺ CD4 T cells. However, some Bacteroidetes species can also produce SCFAs.²⁷ Among the bacteria showing lower abundance in MS patients, *Prevotella*, *Parabacteroides*, and *Lactobacillus* have the ability to induce SCFAs production.^{28,29} We have recently shown that *Prevotella histicola*, a member of the *Prevotella* genus, can suppress disease in experimental autoimmune encephalomyelitis (EAE), a preclinical murine model of MS (Mangalam et al. *Cell Reports*, in press). *P. histicola* induced CD4⁺FoxP3⁺ regulatory T cells, tolerogenic dendritic cells and suppressive macrophage (Mangalam et al. *Cell Reports*, in press). *Parabacteroides distasonis* had been also shown to convert naïve human CD4 T cells into IL-10 producing CD4⁺CD25⁺ regulatory T cells.¹⁶

One of the metabolic pathways used by *Prevotella* and *Parabacteroides* as well as *Adlercreutzia* (which is also depleted in MS patients) is metabolism of phytoestrogens.³⁰⁻³² It is important to note that estrogens have been shown to possess disease-suppressive properties in MS as evidenced by

several studies in animals as well as in MS patients.³³ While SCFAs have been investigated extensively, the importance of phytoestrogen metabolism in regulation/maintenance of immune responses has not been explored in detail.

Significance of gut bacteria positively associated with MS (pro-inflammatory bacteria)

In our study, we observed a higher abundance of *Dorea*, *Blautia*, *Pseudomonas*, and *Mycoplana* in MS patients¹ (Table 1). Although *Dorea* is thought to be a constituent of healthy gut microflora, its higher abundance in MS and IBD patients suggests a pro-inflammatory role for this bacterium. Recently, Schirmer et al. have shown that ceratin species of *Dorea* might be pro-inflammatory because they can induce IFN γ ,³⁴ metabolize sialic acids and degrade mucin.³⁵ Thus, *Dorea* might be an example of a bacterium that exhibits either pro or anti-inflammatory roles depending on the surrounding gut bacteria and/or available nutrients. Indeed, as *Blautia* utilizes gases produced by *Dorea*, the increased abundance of *Dorea* in patients with MS might promote the growth of *Blautia*. Jangi et al.² and Cekanaviciute et al.¹⁶ reported a higher abundance of another mucin-degrading bacterium *Akkermansia* among MS patients. Both studies suggested that *Akkermansia muciniphila* can promote the expansion of pro-inflammatory cytokines. It is possible that *Dorea*, which showed a higher abundance in our study, and *Akkermansia*, which showed higher abundance in other MS studies, can utilize a common pathway such as mucin degradation to induce proinflammatory responses resulting in predisposition/chronic inflammation in MS. Cekanaviciute et al. also reported that *Acinetobacter calcoaceticus* showing higher abundance in MS patients, was able to suppress differentiation of regulatory CD4 T cells and induce differentiation of pro-inflammatory Th1 cytokine.¹⁶ *Pseudomonas aeruginosa*, a member of the *Pseudomonas* genus, is a Gram-negative opportunistic pathogen and has been linked with several diseases, including MS. *Pseudomonas aeruginosa* has been shown to have amino acid homology with myelin basic protein, a major component of myelin and anti-sera against myelin basic protein (residues 110–124) were shown to be reactive against *Pseudomonas* peptide from carboxymuconolactone decarboxylase.³⁶ Additionally, higher abundance of *Pseudomonas aeruginosa* was reported in mice on a high-fat diet, suggesting a role in high-fat diet-induced obesity

and inflammation.³⁷ The role of *Mycoplana* in health or disease is unknown.

Conclusion

It is difficult to say with certainty whether changes in gut microbiota is a cause or consequence of MS because MS patients have immunological and microbial changes months to years before clinical onset of the disease. Colonization of germ-free mice with gut bacteria positively or negatively associated with the disease might offer more insight into the significance of these bacteria in susceptibility versus protection from MS. In summary, the major conclusions from MS microbiome studies are that compared with healthy controls, MS patients have i) gut dysbiosis; ii) reduced Bacteroidetes phylum with lower abundance of certain genera such as *Prevotella*, *Parabacteroides*, and *Bacteroides* (which can induce Tregs); iii) higher abundance of certain Firmicutes such as *Akkermansia* and *Dorea* (which can metabolize mucin and induce pro-inflammatory cytokines); iv) depletion of ceratin Actinobacteria such as *Adlercreutzia*, *Collinsella*, and *Slackia* (anti-inflammatory) and Proteobacteria such as *Sutterella*; and v) higher abundance of certain Proteobacteria such as *Acinetobacter calcoaceticus*, *Pseudomonas*, and *Mycoplana*. These data suggest that gut microbiota might sustain a healthy state of the host by maintaining immune homeostasis, and subsequent changes that perturb this homeostasis can lead to negative consequences such as inflammatory diseases. Altogether, MS microbiome studies suggest that in MS patients, there is depletion of bacteria with the ability to induce immuno-regulatory cells and enrichment of bacteria with the ability to induce pro-inflammatory responses. Further research is needed to determine a role of gut microbiota and their metabolites in the susceptibility to and protection from MS.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

The authors to acknowledge funding support from National Multiple sclerosis Society (RG 5138A1/1T), the Carver College of Medicine University of Iowa (Pathology pilot grant), and the Mayo Clinic Center for Microbiome.

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