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Review article

The multiple sclerosis gut microbiota: A systematic review

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ABSTRACT

Background: To systematically review and synthesize the literature on the multiple sclerosis (MS) gut microbiota composition as compared to persons without MS.
Methods: We systematically searched MEDLINE, EMBASE, and Web of Science databases for relevant published articles (2008–2018).
Results: Of 415 articles identified ten fulfilled criteria. All studies used a case-control design, six sourced participants from the US, two Germany, one Italy, and one Japan. Nine focused exclusively on adults and one on children, totaling 286 MS and 296 control participants. Over 90% of cases had relapsing-remitting MS; disease duration ranged from 10.6 ± 6.5 months to 15.3 ± 8.6 years (mean ± SD). Nine studies examined stool and one evaluated duodenal mucosa. Diverse platforms were used to quantify microbes: Illumina MiSeq, Roche 454, microarray, and fluorescence in situ hybridization. None of eight studies reported a significant alpha-diversity differences between cases and controls. Two of seven studies reported a difference in beta-diversity ($P \leq 0.002$). At the taxa-level, ≥2 studies observed: lower relative abundance of *Prevotella, Faecalibacterium prausnitzii, Bacteroides coprophilus, Bacteroides fragilis*, and higher *Methanobrevibacter* and *Akkermansia muciniphila* in MS cases versus controls. Exposure to an immunomodulatory drug (IMD), relative to no exposure, was associated with individual taxonomic differences in three of three studies.
Conclusion: Gut microbiota diversity did not differ between MS cases and controls in the majority of studies. However, taxonomic differences were found, with consistent patterns emerging across studies. Longitudinal studies are warranted to elucidate the relationship between IMD exposure and differences in the gut microbiota composition.

1. Introduction

A growing body of evidence points to the gut microbiota playing a role in immune-mediated, neurological disease, such as multiple sclerosis (MS) (Rooks and Garrett, 2016; Tremlett et al., 2017). An important initial step to understanding the relationship between the gut microbiota and MS is to survey the gut microbial community. Recent studies on MS have focused on surveying the gut bacterial (and archaeal) communities. A common goal has been to investigate whether there are differences in the MS gut microbiota composition between MS cases and controls, measured as the overall microbiota composition (diversity) and relative abundance of individual resident microbes. Some studies also assessed potential effect modifiers (or confounders), such as exposure to an immunomodulatory drug (IMD) used to treat MS.

We conducted a systematic review in order to comprehensively collate the body of evidence surrounding the relationship between the gut microbiota and MS. Our objective was to include published articles in which the gut microbiota profiles had been compared between individuals with and without MS.

2. Methods

Our systematic review was designed to address the following

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specific questions: (1) Does the gut microbiota composition differ between MS cases and controls (participants without MS), as assessed by (a) diversity metrics and/or by (b) taxa-level relative abundances? (2) What are the main effect modifiers (confounders) identified to date in studies evaluating the gut microbiota in MS?

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines (Moher et al., 2010). The systematic review protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO) database (registration no. CRD42018089173) and the protocol is in accordance with the PRISMA-P (2015) guidelines (Shamseer et al., 2015).

2.1. Eligibility criteria

Original research articles assessing the potential gut microbiota differences in diversity or taxonomic relative abundance between MS cases and controls (individuals without MS) were eligible for inclusion. We included any of the following study designs: cohort, cross-sectional, case-control, and comparative cohort studies. Other study designs were excluded, such as intervention studies (unless pre-intervention samples were available for cases/controls) and studies without a control group (e.g., case series). No restrictions were imposed with respect to the age of study participants, geographical location or setting (e.g., community or hospital), or disease course(s) under study (e.g., relapsing-onset, primary progressive).

2.2. Literature search strategy

We performed a systematic literature search of MEDLINE, EMBASE, and Web of Science databases for original research articles published in English between January 1st, 2008 and February 8th, 2018, which we subsequently updated to June 19th, 2018. At the time of publication, a final update to August 24th, 2019 was included and reported as an addendum. The search strategies for each database are shown in Fig. 1A. We did not include conference abstracts, unpublished work or the grey literature (e.g., presentations, posters, websites or dissertations).

2.3. Eligibility assessment, data extraction, and quality assessment

The literature search results were uploaded to Mendeley for screening. Titles and abstracts were screened based on the study inclusion and exclusion criteria by two independent reviewers (AM and JF). Any disagreement was resolved between the reviewers. The full texts of all screened-in abstracts were then retrieved and assessed for eligibility and relevant information retrieved were extracted by one reviewer (AM), as outlined in Appendix 1. Two independent reviewers (AM and JF) assessed the risk of bias in individuals studies specific to our current systematic review-related questions using the US's National Institutes of Health (NIH) tool for Quality Assessment of Case-Control Studies (NIH National Heart, Lung, and Blood Institute). No study was excluded based on the risk of bias (rated as good, fair, and poor), in part because a study could be assigned as 'poor', inferring a high risk of bias, if the microbiota quantification platform was not valid to address our study question(s).

3. Results

3.1. Study and participant characteristics

Of 415 articles identified (based on titles and abstracts), ten fulfilled criteria for inclusion (Cantarel et al., 2015; Miyake et al., 2015; Chen et al., 2016; Cree et al., 2016; Tremlett et al., 2016; Jangi et al., 2016; Cosorich et al., 2017; Swidsinski et al., 2017; Berer et al., 2017; Cekanaviciute et al., 2017). A flow chart outlining the screening process

A. Literature Search Strategies

MEDLINE search - Ovid interface

1 exp Multiple Sclerosis/
2 multiple sclerosis.mp.
3 1 or 2
4 microbiota/ or gastrointestinal microbiome/ or microbial consortia/
5 (microbiome or microbiota).mp.
6 ((gut or intestin*) adj3 (microbi* or flora or microflora)).mp
7 4 or 5 or 6
8 3 and 7
9 8 not (animals/ not humans.sh.)
10 limit 9 to english language
11 limit 10 to last 10 years
12 review.pt.
13 11 not 12

EMBASE search - Ovid interface

1 exp Multiple Sclerosis/
2 multiple sclerosis.mp.
3 1 or 2
4 microflora/ or bacterial flora/ or feces microflora/ or exp intestine flora/ or exp microbiome/
5 (microbiome or microbiota).mp.
6 ((gut or intestin*) adj3 (microbi* or flora or microflora)).mp
7 4 or 5 or 6
8 3 and 7
9 8 not ((exp animal/ or nonhuman/) not exp human/)
10 limit 9 to english language
11 limit 10 to last 10 years
12 review.pt.
13 11 not 12

Web of Science

(TS=("multiple sclerosis") AND TS=((microbiome OR microbiota) OR ((gut or intestin*) NEAR/3 (microbi* OR flora OR microflora)))) AND LANGUAGE: (English) AND DOCUMENT TYPES: (Article)
Indexes=SCI-EXPANDED, ESCI Timespan=2008-2018

B. PRISMA 2009 Flow Diagram

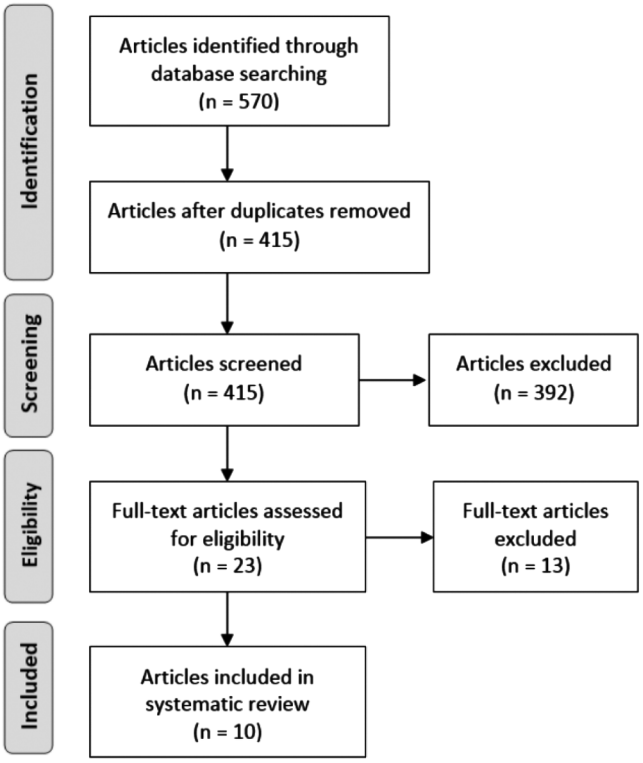

Fig. 1. Literature search strategies and PRISMA flow diagram.
A. Systematic literature search strategies of MEDLINE, EMBASE, and Web of Science databases for original research articles. B. Selection of articles for inclusion in the systematic review of the multiple sclerosis microbiota (2008–18).

is provided in Fig. 1B (Moher et al., 2010). All ten studies used a case-control design for a total of 286 MS cases and 296 controls. Nine studies enrolled adults, of which one focused solely on twins discordant for MS, and one enrolled children. Nine studies collected stool samples from

Table 1
The multiple sclerosis gut microbiota: summary of the included studies.

First Author, Year of Publication, Country	Study Design	Biological Sample Type	Number of Participants (MS / Control)	Age (Years) Near Time of Sample Collection (MS / Control)	Race & Ethnicity (MS / Control)	Body Mass Index (kg/ m^2, MS / Control)	Diet Assessement	NIH Quality (or 'Risk of Bias') Assessement
Cantarel, 2015, United States	Intervention, case-control, pilot study	Stool	7 / 8	Median 42 (30–48) / 38 (29–51)	White: 7 (100%) / 8 (100%) Hispanic: 0 (0%) /0 (0%)	MS and controls combined range: 18 - 30	NA	Fair
Miyake, 2015, Japan	Case-control	Stool	20 / 40	36.0 ± 7.2 / 28.5 ± 9.8	NA	NA	NA	Fair
Chen, 2016, United States	Case-control	Stool	31 / 36	42.9 ± 10.6 / 40.3 ± 7.3	NA	28.0 ± 6.3 / 27.8 ± 4.7	NA	Fair
Cree, 2016, United States	Case-control	Stool	16 / 16	54 ± 11.8 / 54 ± 15.8	White: 13 (81%) / 11 (69%) Black: 0 (0%) / 2 (13%) Asian: 1 (6%) / 2 (13%) Other: 2 (13%) / 1 (6%)	23.3 ± 3.59 / 23.8 ± 4.10	Yes; Block Dietary Data Systems (NutritionQuest©)	Fair.
Tremlett, 2016, United States	Case-control, pilot study	Stool	18 / 17	12.5 ± 4.44 / 13.5 ± 3.08	White: 9 (50%) / 13 (77%) Hispanic: 8 (44%) / 6 (35%)	22.2 ± 5.66 / 22.8 ± 7.10. Missing 1	Yes; Block Kids Food Screener (NutritionQuest©)	Fair
Jangi, 2016, United States	Case-control	Stool	60 / 43	49.7 ± 8.50 / 42.2 ± 9.61	White: 58 (97%) / 43 (100%) Black: 2 (3%) / 0 (0%) Hispanic: 1 (2%) / 0 (0%)	27.2 ± 4.7 / 26.4 ± 6.3	Yes; method not reported.	Fair
Cosorich, 2017, Italy	Case-control	Duodenal mucosa tissue	19 / 17	41 ± 2 / 48 ± 3	NA	NA	Yes; method not reported.	Fair
Swidsinski, 2017, Germany	Intervention, case-control	Stool	10 / 14	NA	NA	NA	NA	Poor / High risk of bias. Taxa quantification method for assessing our study questions is obsolete
Berer, 2017, Germany	Co-twin, case-control	Stool	34 / 34	MS and controls combined: 41.3 ± 10.8 (21–63)	NA	NA	Yes; method not reported.	Fair
Cekanaviciute, 2017, United States	Case-control	Stool	71 / 71	40.7 ± 11.9 (19–64) / 44.6 ± 14.4 (22–71). Missing 9 controls and 4 cases	NA	NA	NA	Fair

Characteristics of the study participants relate to the time of microbiota sample collection (typically stool). Jangi et al. (2016) were missing clinical data for 8 subjects; they did not report which clinical data was missing. Continuous data are expressed as mean ± SD. NA, not available; MS, multiple sclerosis; BMI, body mass index; NIH, National Institutes of Health.

participants while one collected duodenal mucosal samples. Characteristics of the included studies are summarized in Table 1. Study quality was 'fair' for nine articles and 'poor' for one. The study identified as poor used an obsolete method (fluorescence *in situ* hybridization; FISH) for characterizing the gut microbiota, thus making it a high risk of bias in the context of our systematic review-specific questions (Swidsinski et al., 2017).

Across the nine studies which reported participant demographics, females predominated. Overall the number (%) of females/males were: 182/104 (64%/36%) for cases and 181/111 (61%/38%) for controls (sex was missing for four controls in one study (Cekanaviciute et al., 2017)). The average ages of cases and controls ranged from 12–54 and 13–54 years, respectively across studies (age was missing for nine controls and four cases in one study (Cekanaviciute et al., 2017)). Across the four studies that reported race and/or ethnicity, the majority of participants were Caucasian, totaling 87/101 (86%) of MS cases and 75/84 (89%) of controls. Six studies recruited participants from the United States, two from Germany, one from Italy, and one from Japan. The most commonly reported lifestyle factors were diet (five studies) and BMI (five studies), both diet and BMI were available in three studies (Table 1). Diet was collated via a validated food frequency questionnaire in two US-based studies; three did not specify. Both diet metrics and BMI (based on descriptive group means) were similar when cases were compared to controls, except for one study, which, for MS cases, observed a higher intake of specific dietary elements (i.e., carotenoids) (Cree et al., 2016).

The majority (267/286; 93%) of MS cases had a relapsing-remitting disease course at the time of sample collection. Five studies used the McDonald 2010 criteria for MS diagnosis, one used the Poser criteria and four did not specify (Poser et al., 1983; Thompson et al., 2018). Five studies reported the disease duration of the MS cases, which ranged, on average from 0.9–15.3 years (Table 2).

Medication exposure status was reported in nine of ten studies; all focused on IMDs or immunosuppressants (IMS) used to treat MS. Overall, 118 (43%) of MS cases ($n = 276$) were exposed to an IMD or IMS near the time of sample collection; 64 (23%) to β-interferon, 35 (13%) glatiramer acetate, 18 (7%) other IMDs, and 1 (<1%) IMS (azathioprine). IMD or IMS exposure was typically defined as exposed in the two or three months prior to the time of microbiota sample collection. The pediatric study included IMD naïve MS cases, explicitly defined as never exposed to an IMD (Tremlett et al., 2016). Of the seven studies that reported use of systemic corticosteroids, 11/229 (5%) of MS cases were exposed to systemic corticosteroids near the time of sample collection (Table 2).

3.2. Sample handling and sequencing procedures

Eight of the ten studies mentioned at least some aspects of the methods related to sample collection. A US-based group sampled participant's microbiota using culture swabs, another US-based group collected stool directly into a dry container, and the Italy-based group collected mucosal biopsies into a culturing solution containing antibiotics (Chen et al., 2016; Cosorich et al., 2017; Cekanaviciute et al., 2017). Four studies shipped stool samples overnight (shipped with ice with the exception of one study) while four groups stored or processed the sample the same day of the collection (Cantarel et al., 2015; Cree et al., 2016; Tremlett et al., 2016; Jangi et al., 2016). Samples not sequenced the same day of collection were stored at −70 °C or −80 °C prior to DNA extraction (Cantarel et al., 2015; Miyake et al., 2015; Chen et al., 2016; Tremlett et al., 2016; Jangi et al., 2016; Berer et al., 2017; Cekanaviciute et al., 2017).

Seven studies sequenced various regions of the 16S rRNA gene using a Next-Generation Sequencing (NGS) platform (either Illumina MiSeq or Roche 454). One study used both Roche 454 and Illumina MiSeq (Table 3) (Berer et al., 2017). Two studies used a DNA microarray platform only (Affymetrix PhyloChip G3) and one used FISH to probe and profile the 16S rRNA genes (Cantarel et al., 2015; Cree et al., 2016; Swidsinski et al., 2017). The 16S rRNA variable-region gene sequenced varied between studies. The method of generating 'Operational Taxonomic Units' (OTUs) also varied. OTUs are clusters of similar sequences—typically >97%—that are assumed to represent a single taxon; for 16S rRNA, OTUs typically have genus-to-species level of taxonomic specificity. The different methods of generating OTUs among the studies that used an NGS platform include: *de novo* OTU clustering (four studies) and closed reference OTUs (three studies). Despite these differences, all studies that used an NGS platform clustered the 16S rRNA sequences into OTUs based on a 97% similarity threshold (Table 3).

The twin cohort study also sequenced the gut metagenome (the collective genomes of all the gut microbes in a stool sample), although authors reported that none of the results reached significance, and no data was provided (Berer et al., 2017).

3.3. Gut microbiota diversity

Eight of ten studies assessed the gut microbiota diversity (alpha- or beta-diversity), Table 4. None of the alpha-diversity metrics, e.g., the number of different species within a sample, differed significantly between cases and controls. Out of seven studies that calculated beta-diversity (the number of microbial species that are not the same in two different environments), two studies reported a difference in a beta-diversity metric, between cases and controls (Table 4) (Miyake et al., 2015; Chen et al., 2016). No studies reported whether potential confounders, such as IMD exposure, were relevant to the significant alpha- or beta-diversity findings.

IMD exposure and gut microbiota diversity were assessed in two US-based studies (Cantarel et al., 2015; Tremlett et al., 2016). Alpha-diversity did not differ between IMD exposed and unexposed MS cases in either study, although beta-diversity did differ in the pediatric MS study (Table 4) (Tremlett et al., 2016).

3.4. Taxa-level findings

Taxa-level differences between cases and controls were reported as follows: phylum level (seven studies), genus (seven studies), and species/OTU level (eight studies). In total, two genera and four species (none of which reached significance at the phylum level) similarly differed in their relative abundances between MS cases compared to controls in a consistent direction for two or more studies (Table 5). Specifically, at the genus level, lower relative abundances of *Prevotella* were observed in three studies and a higher relative abundance of the Archaea *Methanobrevibacter* was observed in two studies. At the species level, there was a higher relative abundance of *Akkermansia (muciniphila)* and *Faecalibacterium prausnitzii*, which was observed in four studies each. Also, a lower relative abundance of *Bacteroides coprophilus* was reported in three studies and a lower *Bacteroides fragilis* abundance was reported in two studies (Table 5). Different statistical tests were used to assess differences in OTU abundance, including: models based on the negative binomial distribution (four studies), t-tests (Welch or Student, three studies), Wilcoxon rank sum test (two studies) and ANOVA (one study, Table 6).

Three studies compared gut microbiota taxa- or OTU-level findings according to IMD exposure status via a sub-group analysis (Cantarel et al., 2015; Tremlett et al., 2016; Jangi et al., 2016; Cosorich et al., 2017; Berer et al., 2017). When IMD unexposed MS cases were compared to controls (Table 5), a higher relative abundance of the following taxa was found *Methanobrevibacter* (two studies, with a combined total of 37/60 cases/controls) (Tremlett et al., 2016; Jangi et al., 2016) and *A. muciniphila* (four studies, with 125/148 cases/controls) (Tremlett et al., 2016; Jangi et al., 2016; Berer et al., 2017; Cekanaviciute et al., 2017). Lower relative abundances were observed for *Prevotella* (two studies, with 99/114 cases/controls), (Jangi et al.,

Table 2
Clinical characteristics of the participants with MS in the included studies.

First Author, Year of Publication	Age Onset of MS (Years)	MS Disease Duration (Years)	Diagnostic Criteria	EDSS Near Time of Sample Collection	MS Disease Course	IMD/IMS Exposed Near Time of Sample Collection	Number of Participants Exposed to a Specific IMD/IMS drug						Systemic Corticosteroids
				0[c]			Interferon-β	Glatiramer Acetate	Natalizumab	Rituximab	Fingolimod	Azathioprine	
Cantarel, 2015	NA	NA	McDonald	3.0 or less	RR 7 (100%)	5 (71%)	0	5 (71%)	0%	0	0	0	0[a]
Miyake, 2015	NA	8.8 ± 6.0	McDonald	NA	RR 20 (100%)	9 (45%)	9 (45%)	0	0%	0	0	0	5 (25%)
Chen, 2016	35.4 ± 10.4	NA	McDonald	< 6, *n* = 22; > 6, *n* = 2	RR 31 (100%)	20 (65%)	14 (45%)	1 (3%)	5 (16%)	0	0	0	NA
Cree, 2016	NA	15.3 ± 8.6	NA	NA	RR 9 (56.3%), SP 4 (25%), PP 3 (18.8%)	5 (31%)	0	0	0	5 (31%)	0	0	NA
Tremlett, 2016	12.1 ± 4.7	0.88 ± 0.54	McDonald	(range 0–4.0) < 2.0, n=7; 2.0 to < 3.0, n=8; >3.0, n=3	RR 18 (100%)	9 (50%)[b]	3 (17%)	5 (28%)	1 (6%)	0	0	0	6 (33%)[b]
Jangi, 2016	NA	12.8 ± 8.3	NA	1.2 (1.0 SD)	RR 60 (100%)	32 (53%)[c]	18 (30%)	14 (23%)	0	0	0	0	0[a]
Cosorich, 2017	NA	NA	Poser	(range 0–5.5) 0.0 to < 2.0, n=8; 2.0 to < 3.0, n=4; 3.0 to < 6.0, n=7	RR/NEDA 9 (47%), RR/EDA 10 (53%)	19 (100%)	7 (37%)		9 (47%)	0	0	3 (16%)	0[c]
Swidsinski, 2017	NA	NA	NA	NA	RR 10 (100%)	NA	NA	NA	NA	NA	NA	NA	NA
Berer, 2017	28.0 ± 9.0	13.2 ± 9.6	McDonald	NA	CIS 3 (9%), RR 22 (65%), SP 7 (21%), PP 2 (6%) PP 2 (6%)	19 (56%)[c]	13 (38%)	1 (3%)	4 (12%)	0	0	1	0[c]
Cekanaviciute, 2017	NA	NA	NA	NA	RR 71 (100%)	0 (0%)[c]	0	0	0	0	0	0	0[c]
Total						**118 (43%)**	**64 (23%)**	**35 (13%)**	**10 (4%)**	**5 (2%)**	**3 (1%)**	**1**	**11 (5%)**

Unless otherwise stated, characteristics shown relate to the time of microbiota sample collection. The frequency of exposures to an immunomodulatory drug, immunosuppressant and systemic corticosteroids at the time of microbiota sample collection were typically within 2 or 3 months prior to sample collection. One study did not report treatment status. Systemic corticosteroids are not included in the number of MS cases exposed to IMD/IMS (column 7). All percentages are rounded to the nearest whole numbers. Continuous data are expressed as mean ± SD. NA, not available; MS, multiple sclerosis; RR, relapse-remitting multiple sclerosis; SP, secondary progressive multiple sclerosis; PP, primary progressive multiple sclerosis; CIS, clinically isolated syndrome; RR-EDA, evidence of disease activity in patients with relapse-remitting multiple sclerosis; NA, not available; RR-NEDA, no evidence of disease activity in patients with relapse-remitting multiple sclerosis; F, female; M, male; EDSS, Expanded Disability Status Scale. IMD, Immunomodulatory drug; IMS, immunosuppressant; [a]Exposed within 1 month; [b]Exposed within 2 months; [c]Exposed within 3 months.

Table 3
Technical and computational methods used to process and quantify the microbiota.

First Author, Date of Publication	Microbiota Quantifying Instrument, 16S rRNA Region	Method Generating OTUs	Normalization of Sample Sequence Depth
Cantarel, 2015	DNA microarray (Affymetrix PhyloChip)	The array contains representative sequences from OTUs, which were clustered at 95% similarity	NA
Miyake, 2015	Roche 454, V1-V2	Individual taxa analysis: Closed reference OTUs at 97% similarity. Diversity analysis: *De novo* OTU clustering at 96% similarity	Rarefied to 3000 sequence per sample
Chen, 2016	Illumina MiSeq,V3-V5	*De novo* OTU clustering at 97% similarity using IM-TORNADO	Rarefied to 10,000 sequence per sample
Cree, 2016	DNA microarray (Affymetrix PhyloChip)	NA	NA
Tremlett, 2016	Illumina MiSeq, V4	*De novo* OTU clustering at 97% similarity using QIIME	Rarefied to 201,546 sequence per sample
Jangi, 2016	Roche 454, V3-V5;Illumina MiSeq, V4	*De novo* OTU clustering at 97% similarity using Mothur	NA
Cosorich, 2017	Roche 454, V3-V5	*De novo* OTU clustering generaed using QIIME	At least 3000 sequence per sample
Swidsinski, 2017	Fluorescence *in situ*hybridization (FISH)	NA	NA
Berer, 2017	Roche 454, V3–V5	Closed reference OTUs with 97% similarity using QIIME	Diversity analysis: Rarefied to 10,975 sequences per sample.Individual taxa analysis: variance-stabilizing transformation
Cekanaviciute, 2017	Illumina MiSeq, V4	Closed reference OTUs with 97% similarity using QIIME	Diversity analysis: Rarefied to 10,000 sequence per sampleIndividual taxa analysis: variance-stabilizing transformation

Closed-reference OTU assignment assigns query sequences to OTUs generated from an external reference database, whereas sequences in *de novo OTU clustering* are clustered against one another. The two common methods for normalizing read counts were *rarefying* and *scaling.* Rarefying refers to randomly discarding reads in each sample until each sample has an equal number of sequences. Scaling often includes transforming, e.g., variance stabilizing transformation. OTU, operational taxonomic units; NA, not available; V, variable region of 16S rRNA gene; IM-TORNADO, Illinois Mayo Taxon Organization from RNA Dataset Operations; Qiime, Quantitative Insights Into Microbial Ecology.

2016; Cekanaviciute et al., 2017) *B. coprophilus* and *B. fragilis* (two studies, with 80/87 cases/controls) (Tremlett et al., 2016; Cekanaviciute et al., 2017). Findings for *F. prausnitzii* were mixed (Tremlett et al., 2016; Cekanaviciute et al., 2017).

4. Discussion

We reviewed the recent literature (2008–2018) on the MS gut microbiota composition. Of the ten studies comparing the gut microbiota between MS participants and controls, the majority found no major differences in the overall composition of the gut microbiota in children or adults with MS relative to controls, as judged by alpha- or beta-diversity measures. Instead, subtle differences in the gut microbial communities were generally observed. At least two or more studies reported a higher relative abundance of *Akkermansia* and *Methanobrevibacter* and a lower relative abundance of *Prevotella, Bacteroides (coprophilus* and *fragilis)* and *Faecalibacterium prausnitzii* for MS cases relative to controls. Studies were generally too modest in size to adequately assess potential effect modifiers (confounders) such as drug exposure relevant to diversity or taxa-level findings.

4.1. Microbiota diversity

No study observed a significant difference in alpha-diversity between MS cases and controls and the majority found no differences in beta-diversity. However, the metrics and methods used varied across studies. While this makes comparisons challenging, the use of different diversity measures was not unexpected, as no single index perfectly summarizes local diversity (Morris et al., 2014). Beta-diversity specifically quantifies the variation in the taxonomic composition between samples; two of seven studies reported a significant difference between MS cases and controls (Miyake et al., 2015; Chen et al., 2016). However, it remains possible that findings were affected by IMD exposure; differences in beta-diversity were found in one study when IMD exposure was examined (Tremlett et al., 2016).

4.2. Individual taxa

We identified several taxa that differed in their relative abundance between MS cases and controls across two or more studies, though their role in MS are largely unknown. *Methanobrevibacter,* an archaeal anaerobe and methanogen, was enriched in MS cases relative to controls (Gaci et al., 2014). Consistent with this are the higher methane breath test results observed in MS patients compared to controls (Jangi et al., 2016). However, enrichment of methanogens has also been associated with constipation (Gaci et al., 2014; Falony et al., 2016; Vandeputte et al., 2016), a condition which is common in MS. *A. muciniphila* was also enriched in MS cases relative to controls. A similar relationship was reported in Parkinson's disease (Bedarf et al., 2017; Collado et al., 2007; Heintz-Buschart et al., 2018; Hill-Burns et al., 2017). *A. muciniphila* has been shown to elicit a pro-inflammatory T lymphocyte response *in vitro*; however, *in vivo* studies using mouse models of MS have so far failed to elicit a similar reponse (Cekanaviciute et al., 2017). Intriguingly, *A. muciniphila* may be beneficial in the setting of obesity or metabolic disorders, by supporting metabolic health and improving the intestinal barrier (Greer et al., 2016; Plovier et al., 2017; Schneeberger et al., 2015). These context-specific observations highlight the complexity of the gut microbiome, and a need to understand the underlying biology.

The remaining taxa identified—all of which are also common commensal bacteria of the human gastrointestinal tract—were all lower in relative abundance for MS cases relative to controls. *Faecalibacterium prausnitzii* is known for mitigating inflammation and may be depleted in the gut of individuals with other diseases, such as inflammatory bowel disease and irritable bowel syndrome (Liu et al., 2017; Lopez-Siles et al., 2017; Prosberg et al., 2016; Wright et al., 2015).

Two studies reported conflicting relative abundances, within each study, for several OTUs classified as *F. prausnitzii* (Table 5) (Tremlett et al., 2016; Cekanaviciute et al., 2017). These conflicting OTUs may actually be two different recently discovered phylogroups within the species *F. prausnitzii* which have recently been identified (Lopez-Siles et al., 2017). The two phylogroups share 97% 16S rRNA gene sequence similarity but have different metabolic properties.

Table 4
Gut Microbiota Diversity: MS cases versus controls.

First Author, Date of Publication	Diversity Metric	MS vs Controls (Main Analyses)		IMD-Related Subgroup Analyses	
		Statistical Test Used	Main Findings (R and P-values)	IMD Exposed MS vs IMD Unexposed MS	IMD Unexposed MS vs Control
Cantarel, 2015	β-diversity (weighted UniFrac)	PERMANOVA	$P = 0.74$	$P = 0.66$	NA
Miyake, 2015	α-diversity (richness: Choa1)	Welch's test	$P > 0.05$	NA	NA
	α-diversity (diversity: Shannon index)	Welch's test	$P > 0.05$	NA	NA
	β-diversity (weighted UniFrac)	ANOSIM	$\mathbf{R = 0.24, P \leq 0.0009}$	NA	NA
	β-diversity (unweighted UniFrac)	ANOSIM	$\mathbf{R = 0.21, P \leq 0.002}$	NA	NA
Chen, 2016	α-diversity (richness: observed OTUs)	NA	$P = 0.73$	NA	NA
	α-diversity (diversity: Shannon index)	NA	$P > 0.05$	NA	NA
	β-diversity (Bray-Curtis)	PERMANOVA	$\mathbf{P < 0.001}$	NA	NA
Cree, 2016	NA	NA	NA	NA	NA
Tremlett, 2016	α-diversity (evenness)	Mann-Whitney	$P > 0.2$	NA	$P > 0.05$
	α-diversity (richness)	Mann-Whitney	$P > 0.2$	NA	$P > 0.05$
	α-diversity (Faith's phylogenic diversity)	Mann-Whitney	$P > 0.2$	NA	$P > 0.05$
	β-diversity (Canberra)	PERMANOVA	$P > 0.05$	$\mathbf{P = 0.016}$	NA
Jangi, 2016	α-diversity (diversity: Shannon index)	Wilcoxon rank-sum test	$P > 0.05$	NA	NA
	β-diversity (weighted UniFrac)	AMOVA	$P > 0.05$	NA	NA
	β-diversity (unweighted UniFrac)	AMOVA	$P > 0.05$	NA	NA
	β-diversity (Bray-curtis)	AMOVA	$P > 0.05$	NA	NA
Cosorich, 2017	α-diversity (richness: observed OTUs)	Student's *t*-test	$P > 0.05$	NA	NA
Swidsinski, 2017	NA	NA	NA	NA	NA
Berer, 2017	α-diversity (Faith's phylogenetic diversity)	NA	$P > 0.05$	NA	NA
	β-diversity (weighted UniFrac)	NA	$P > 0.05$	NA	NA
Cekanaviciute, 2017	α-diversity (richness: Choa1)	NA	NA	NA	$P > 0.05$
	β-diversity (unweighted UniFrac)	NA	NA	NA	$P > 0.05$

Diversity tests that were statistically significant ($P < 0.05$) are in bold. Blank cells indicate a diversity test result was not reported. Not all diversity metrics were reported for every alpha-diversity measure. IMD Exposed: MS cases exposed to an immunomodulatory drug within 3 months of sample collection. IMD, Immunomodulatory drug; ANOSIM, The ANalysis Of SIMilarity; PERMANOVA, PERmutational Multivariate ANalysis Of VAriance; AMOVA, Analysis of MOlecular Variance; NA, not available.

Table 5
Key findings from taxa-level relative abundances: MS cases versus controls *(all MS cases* versus *controls are initially shown, regardless of immunomodulatory drug (IMD) exposure. In addition, findings from IMD unexposed MS cases relative to controls are also depicted*).

		First Author, Year of Publication					
Genus	Comparison	Miyake, 2015	Tremlett, 2016	Jangi, 2016	Swidsinski, 2017	Berer, 2017	Cekanavic-iute, 2017
Methanobrevibacter	MS vs Control					NS	
	IMD Unexposed MS vs Control						
Prevotella	MS vs Control			NS		NS	
	IMD Unexposed MS vs Control						
Species	Comparison						
Akkermansia muciniphila	MS vs Control					NS	
	IMD Unexposed MS vs Control						
Bacteroides coprophilus	MS vs Control					NS	
	IMD Unexposed MS vs Control						
Bacteroides fragilis	MS vs Control					NS	
	IMD Unexposed MS vs Control						
Faecalibacterium prausnitzii	MS vs Control					NS	
	IMD Unexposed MS vs Control						

Taxa ↓ in MS vs control; Taxa ↑ in MS vs control; Mixed findings; NS Not significant; Not reported

Taxa listed here are differentially significant across 2 or more studies. Red and green cells indicate a lower and higher relative abundance, respectively. Empty cells indicates that the respected study did not report the differential abundance of the respected taxa between the two groups. 'MS vs Control' refers to all of the MS participants included in the study, regardless of IMD exposure, compared to the control participants. 'IMD unexposed MS vs Control' refers to the MS cases unexposed to an IMD at the time of microbiota sample collection, compared to the controls. Genus *Akkermansia*is represented as *Akkermansia muciniphila*. The 4 studies that compared taxa-level findings between IMD unexposed MS cases vs. controls are (1st author, num. MS cases vs. controls): Tremlett, $n = 9$ vs. $n = 17$; Jangi, $n = 28$ vs $n = 43$; Berer, $n = 17$ vs. $n = 17$; Cekanaviciute, $n = 71$ vs. $n = 71$. If a study reported a higher and lower relative abundances with the same taxonomy assignment, the cell was split into two colors, as was the case for *Faecalibacterium prausnitzii*. If a study reported a taxonomy assignment confidence score, only scores of 95% or better were included in the table. All taxa are statistically significant ($P < 0.05$) after FDR adjustment. NS, not significant; IMD, immunomodulatory drug; untr, IMD untreated; tr, IMD treated; MS, multiple sclerosis; NS, not significant.

Future studies planning to assign taxonomy may find it helpful to further classify *F. prausnitzii* into phylogroups when possible to better resolve the mapping contradiction and serve as a better discriminating biomarker (Lopez-Siles et al., 2017).

The effects of *Prevotella* may differ by species. *P. histicola* has been shown to suppress or prevent disease activity in a mouse model of MS (Mangalam et al., 2017). Interestingly, the relative abundance of *Prevotella* was lower in relapsing-remitting MS patients with 'evidence of

Table 6
Operational taxonomic unit (OTU)-level findings: MS cases versus controls.

First Author, Date of Publication	Differential Abundance Statistical Method	Total OTUs Generated	Number of OTUs or Species Differentialy Abundant After Multiple-Testing Adjustment
Cantarel, 2015	Wilcoxon rank sum test, with Bonferroni correction	NA	Total 359 OTUs
Miyake, 2015	Welch's *t*-test, with Benjamini-Hochberg correction.	130 OTUs	Total 21 OTUs (2 OTUs enriched, 19 OTUs reduced)
Chen, 2016	Wilcoxon rank-sum test, with Benjamini-Hochberg correction	NA	NA
Cree, 2016	ANOVA test, with Bonferroni correction	2621 OTUs	0 OTUs (277 OTUs before adjustment)
Tremlett, 2016	Negative binomial regression, with Bonferroni correction	25,134 OTUs	Total 323 OTUs (160 OTUs enriched and 163 taxa OTUs reduced MS).
Jangi, 2016	DESeq2, with Benjamini and Hochberg correction	Roche 454: 4317 OTUs. 426 OTUs (after filtering). MiSeq: 10,620 OTUs. 1191 OTUs (after filtering)	NA
Cosorich, 2017	t-Student test	NA	None statistically significant
Swidsinski, 2017	t-Student test	NA	NA
Berer, 2017	DESeq2, with Benjamini and Hochberg correction	NA	None statistically significant
Cekanaviciute (2017)	DESeq2, with Benjamini and Hochberg correction	1462 OTUs (after filtering)	247 OTUs (161 reduced in MS and 86 enriched in MS)

Different statistical tests were used to assess differences in OTU abundance. DESeq2 is a differential gene expression analysis based on the negative binomial distribution. Filtering is the process in which undesired (e.g., unreliable) OTUs are removed. Often when filtering is used, OTUs present in less than 5% of samples are discarded. In some cases, it was not possible to determine if the total OTU count was filtered or not before reporting. ANOVA, Analysis of variance; NA, not available.

disease activity' relative to those with 'no evidence of disease activity' in one study (Cosorich et al., 2017). *Prevotella* species were also present in the oral microbiota of new-onset rheumatoid arthritis participants, but not in controls, suggesting a possible role in this autoimmune inflammatory condition (Scher et al., 2012).

B. fragilis is thought to benefit human health by, for example, breaking down dietary fibers to produce short-chain fatty acids and anti-inflammatory polysaccharides (Lukiw, 2016; Ochoa-Repáraz et al., 2010). *B. fragilis* is also considered a pathobiont, having an inflammatory pathogenic potential via its production of endotoxins (Lukiw, 2016). While there is limited relevant literature for *Bacteroides coprophilus,* this species merits further investigation for its potential role in MS.

Experimental studies allude to a pro-inflammatory MS gut microbiota. Neurological symptoms were exacerbated in animal models of MS when stool from individuals with MS was transplanted into the gut of mice with spontaneous or induced autoimmune encephalomyelitis, further supporting the association of the gut microbiota with MS (Berer et al., 2017; Cekanaviciute et al., 2017; Procaccini et al., 2015).

4.3. Potential confounders

Of the few confounding factors assessed to date in MS, exposure to disease-modifying drugs appears to be a likely candidate. However, most studies were too modest in size to formally assess the effect of confounders, hence much remains unknown. For example, at least 20 samples per group have been suggested in order to detect differences in taxonomic relative abundances (Weiss et al., 2017). Exposure to several medications as well as stool consistency (a reflection of gut transit time) are considered important factors in explaining microbiome variation and are related to MS itself (Falony et al., 2016; Vandeputte et al., 2016; He et al., 2018; Maier et al., 2018). A cross-sectional study assessing the differences between the gut microbiota of MS cases treated with either dimethyl fumarate (DMF; $n = 33$ cases) or glatiramer acetate (GA, $n = 60$) relative to IMD naïve cases ($n = 75$) was published in 2018, and although did not fulfill our inclusion criteria (due to absence of controls without MS), it is worthy of comment (Katz Sand et al., 2019). Authors reported that MS cases exposed to either DMF or GA had lower relative abundances of the *Lachnospiraceae* and *Veillonellaceae* families compared to IMD naïve cases (Katz Sand et al., 2019).

Constipation (reflecting a slow gut transit time) is common in MS, may influence the gut microbiota composition and contribute to a pro-inflammatory local environment. Microbiota from chronically constipated individuals was demonstrated to damage the intestinal barrier and further contribute to constipation (Cao et al., 2017). An enrichment of *A. muciniphila* and *Methanobrevibacter* spp. could be related to a slow gut transit and constipation, as shown in other conditions, including Parkinson disease (Gaci et al., 2014; Falony et al., 2016; Vandeputte et al., 2016; Cao et al., 2017; Chia et al., 1995; Stocchi and Torti, 2017). Methanogens may thrive in a gut with reduced motility, and may contribute to a slow colonic transit by augmenting methane production which acts as a neuromuscular transmitter, and has been shown to slow bowel movement (Gaci et al., 2014; Vandeputte et al., 2016; Pimentel et al., 2006). Understanding how these factors relate to MS and the gut microbiota's composition and function may clarify a possible causal role of the gut microbiota in MS. Alternatively, findings might point towards an opportunity to modify the gut microbiota to improve outcomes in MS. Future studies could assess stool consistency using proxy markers, such as the Bristol Stool Scale (Koh et al., 2010). Polypharmacy is common in MS and ideally, all recent drug exposure should be captured (Falony et al., 2016; Maier et al., 2018). The relationship between the IMDs used to treat MS and the gut microbiota is particularly intriguing and could provide additional mechanistic insights. Sufficiently powered, prospective longitudinal studies are needed to better understand the complex and likely dynamic relationship between MS, the gut microbiome, comorbidities, medication exposure, diet, and other lifestyle factors.

4.4. Heterogeneity of study design

Heterogeneity in the microbiota composition across studies may relate to differences in the sourcing of cases and controls and their characteristics, including: the broad age range of participants (from children to adults); (Falony et al., 2016) MS course (2 studies included participants with primary or secondary progressive MS (Cree et al., 2016; Berer et al., 2017)) and disease duration (which ranged from a few months to decades); host geographic location (although all studies were from largely westernized populations) and ethnicity (He et al., 2018; Deschasaux et al., 2018; Gaulke and Sharpton, 2018; McDonald et al., 2018). The degree to which these differences contribute to variation in the gut microbiota are complex, context specific and is not fully understood (He et al., 2018). For example, although it is possible that individuals with a progressive disease course might differ in terms of their gut microbiota composition from those with a relapsing disease course, insufficient data and cases were available. Further, teasing apart the effects of age, disease duration, accrual of comorbidities and medication exposures from the underlying disease course would likely require access to a sizable cohort of individuals with progressive and relapsing MS.

Technical methods for quantifying and analyzing the gut microbiota also differed, including the choice of: quantification instrument, 16S rRNA sequences regions(s), and gastrointestinal tract site sampled. Computational methods also differed, including: the bioinformatics pipeline used to generate OTUs, OTU abundance normalization, and statistical tests employed. While the method of generating OTUs varied, all studies using an NGS platform generated OTUs at the same taxonomic resolution by clustering the 16S rRNA sequences based on a 97% similarity threshold.

4.5. Strengths and limitations

The strengths of this review include its systematic, reproducible approach, and pre-registered protocol. Our systematic review also provides insights into the heterogeneity in microbiome study design including an overview of the differences in the computational pipelines. However, all studies included were relatively modest in size, with the total number of available data pertaining to 286 MS cases and 296 controls from a limited number of regions in the world. It remains possible that associations have been missed, particularly with the lower abundant taxa. For simplicity, we only reported findings on taxa that were similarly observed across two or more studies. Further, all studies were considered together, including one which sampled duodenal mucosal tissue. It remains possible that different physiological niches in the gastrointestinal tract will harbor distinct microbiota communities of relevance in MS. Interrogation of the gut microbiota in MS was primarily conducted using 16S rRNA sequencing which is typically unable to assign taxonomy below species level and is incomplete at low taxonomic ranks. It was not possible to match and compare individual OTUs identified across studies. We found no published study investigating the virome or mycobiome (fungi microbiome) in MS.

4.6. Concluding remarks

To our knowledge, this article is the first to systematically review the scientific literature investigation the link between the gut microbiome and MS. Despite the modest cohort sizes, diversity in the geographical location of participants and sample processing and bioinformatics pipelines used, consistent patterns are emerging: several taxa were similarly identified as being over or underrepresented in MS versus controls. A better understanding of a possible causal role of the microbiota in either facilitating the onset of MS, or outcomes in MS, including perpetuating comorbidities will facilitate our ability to

harness the microbiome to affect positive change in MS.

5. Addendum

5.1. Summary of articles published between June 20th 2018 and August 24th, 2019

The systematic literature search was updated on August 24th, 2019 by one reviewer (AM). Of 122 new articles identified, three fulfilled the inclusion criteria, one from the US, one from Canada, and one from China (Cekanaviciute et al., 2018; Forbes et al., 2018; Zeng et al., 2019).

Briefly, the US-based study included stool samples from 25 RRMS cases, all unexposed to IMDs or corticosteroids 3 months prior to stool collection (80% were women, mean age = 44.0 years), and 24 controls (12.5% women, mean age = 49.3 years). All bacteria as well as the spore-forming bacterial fractions alone were sequenced at the 16S rRNA V4 region, using the Illumina NextSeq platform and clustered into closed-reference OTUs (97% sequence similarity). No differences were observed in alpha-diversity (Chao1) or beta-diversity (unweighted UniFrac) between cases and controls for either the spore-forming bacteria or total bacteria. Differences between MS cases and controls in the relative abundance of OTUs of spore-forming bacteria fractions including: lower *Ruminococcus gnavus, Ruminococcus bromii, Veillonella dispar,* and a higher relative abundance of *Propionibacterium acnes, Staphylococcus epidermidis, Clostridium perfringens, and Clostridium citroniae* were reported (all $P < 0.05$). Taxa-level differences between cases and controls among total bacteria and the effects of possible confounders were not reported (Cekanaviciute et al., 2018).

The Canada-based study included stool samples from 19 MS cases (average age = 47.3 years; 14 were women; MS course was not reported) and 23 healthy controls (average age = 32.4 years; 12 were women). In addition, the authors included 20 Crohn's disease cases, 19 ulcerative colitis cases, and 21 rheumatoid arthritis cases (not reported here). DNA extracts were sequenced at the 16S rRNA V4 region using the Illumina MiSeq platform, clustered into *de novo* OTUs (97% sequence similarity). Because the dataset had an unusually low abundance of Gram-negative bacteria, the differential taxa abundance testing included only OTUs from Gram-positives phyla (i.e., OTUs within the phyla *Firmicutes, Actinobacteria*, and *Tenericutes*). The author's main goal was to compare across all the immune-mediated diseases relative to controls. Here, we report only the comparisons between the MS cases and controls. Alpha-diversity (i.e., Chao1, ACE, Shannon index, and Simpson diversity index, based on all phyla) did not differ between MS cases and controls. Beta-diversity was not directly compared between MS cases and controls. Significant differences between MS cases and healthy controls in the relative abundance of gram-positive bacteria at the genera level included: Lower *Butyricicoccus, Dialister, Faecalibacterium* (consistent with a previous finding (Miyake et al., 2015)), *Fusicatenibacter, Gemmiger, Lachnospira, Sporobacter*, and *Subdoligranulum* in MS cases relative to controls and higher *Actinomyces, Eggerthella, Anaerofustis, Clostridium* group III, *Clostridium* group XlVa, *Clostridium sensu stricto, Faecalicoccus, Streptococcus* and *Turicibacter* in MS cases relative to healthy controls ($P < 0.05$, Kruskal–Wallis test and Dunn's post hoc tests for multiple comparisons, with FDR correction) (Forbes et al., 2018).

The third study included stool samples from 34 RRMS cases (21 unexposed and 13 exposed to an immunosuppressant [e.g., azathioprine, methotrexate, or mycophenolate]) and 34 healthy controls from China (in addition to 34 individuals with neuromyelitis optica spectrum disorder). DNA extracts were sequenced at the 16S rRNA V3-V4 region using the Illumina MiSeq platform and clustered into *de novo* OTUs (97% sequence similarity). Alpha-diversity (i.e., Chao1, Shannon index, and Simpson diversity index) did not differ between all MS cases and healthy controls. Although beta-diversity did differ for both exposed MS cases and unexposed MS cases compared to healthy controls, (using a non-phylogenetic gain distance metric, $P < 0.01$ and $P < 0.05$, respectively; Mann−Whitney U test), the authors used an unconventional approach, by comparing distance values computed from the first principal coordinate of a principal coordinate analysis. The relative abundance of *Prevotella* was lower for the MS cases compared to controls, consistent with findings from prior studies in MS (exposed MS cases vs. controls, $P < 0.05$ and unexposed MS cases vs. controls, $P < 0.0001$; Mann−Whitney U test). As in the prior Canadian study (Forbes et al., 2018), the relative abundance of *Streptococcus* was higher for the MS cases compared to controls, (exposed MS cases vs. controls, $P < 0.001$ and unexposed MS cases vs. controls, $P < 0.0001$) (Zeng et al., 2019).

5.2. Summary comments

The new articles support the conclusion that there is no major differences in the overall composition of the gut microbiota in individuals with MS relative to controls, as assessed using alpha- or beta-diversity measures. Two studies consistently identified two taxa that differed in their relative abundance between MS cases and controls: lower *Faecalibacterium* and higher *Streptococcus* in MS cases relative to controls (Forbes et al., 2018; Zeng et al., 2019). The former observation concurs with a finding from a study in our systematic review, the latter represents a 'new' observation.

Author contributions

AM and HT contributed to conception and design of the study, acquisition, analysis and interpretation of data, and drafted the first version of the manuscript, tables and figures. JF, FZ, CB, GV, MG, and EW contributed to acquisition and interpretation of data and provided critical review of the manuscript.

Potential conflict of interest

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CB, GV, MG, and EW have no conflict of interest to report.

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Appendix 1. Information retrieved and extracted from each included study

1. *Publication information*: name of author(s) and year of publication,
2. *Study information*: study design, geographic location of recruits (e.g., National Center of Neurology and Psychiatry Hospital, Tokyo, Japan), MS diagnosis criteria (e.g., McDonald criteria), and eligibility criteria, and enrolment period.
3. *Case-control demographics and characteristics close to the time of microbiota sample collection:* age, sex, race/ethnicity, MS disease course (e.g., relapsing-remitting onset MS or primary progressive), case or control status, MS disease duration, disability level (e.g., the Expanded Disability Status Scale (EDSS) score), lifestyle factors, as available (e.g., BMI, diet-related metrics). Medication use, including drugs used to treat MS, e.g., the immunomodulatory drug (IMDs), captured as therapeutic class or generic name (e.g., interferon-beta, glatiramer acetate) and IMD treatment status [treated/untreated/naïve (as defined by the study authors)]. Systemic corticosteroid around the time of sample collection (yes, no). Other medication use, including antibiotics.
4. *Microbiota sampling and quantification information:* body site sampled (e.g., stool, mucosa biopsy of small intestines, etc.), number of samples collected, number of samples excluded, DNA isolation kit, microbiota quantification instrument, sequence molecule/ region, primer sequence, method of generating OTUs ('Operational Taxonomic Units'; species-like taxonomy), and OTU statistical normalization method.
5. *Microbiota analysis results:* Total OTUs, differential abundance statistical method, taxa-level [phylum, genus, species (or individual OTUs)] differences between cases and control, number OTUs differentially abundant before and after multiple-testing adjustment, potential confounding factors considered in the study design and/or adjusted statistically in the analyses, diversity metric(s) considered, diversity comparisons between groups, e.g., MS vs. controls, and when available, untreated MS vs controls and treated MS vs untreated MS.

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