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Gut dysbiosis and lack of short chain fatty acids in a Chinese cohort of patients with multiple sclerosis

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ABSTRACT

Background: Recent studies, mostly conducted in Western countries, showed that gut microbes are involved in the pathogenesis of multiple sclerosis (MS).
Objective: The aim of this study was to investigate whether gut dysbiosis is relevant to the initiation and progression of MS in a Chinese population.
Methods: Next-generation sequencing (NGS) and gas chromatography (GC) were integrated and used to compare the fecal bacterial communities and the short-chain fatty acid (SCFA) levels among relapsing-remitting MS (RRMS) patients (n = 34), neuromyelitis optica spectrum disorder (NMOSD) patients (n = 34), and healthy controls (HCs) (n = 34). T-cell profile analyses were performed by flow cytometry for MS patients and matched controls (n = 12).
Results: (1) The gut microbiome of MS patients was characterized by an increase of *Streptococcus* and a decrease of *Prevotella_9*; additionally, compared to NMOSD patients, *Prevotella_9* was found to be much more abundant in MS patients. (2) A striking depletion of fecal acetate, propionate, and butyrate was observed in MS patients compared to HCs. (3) The abundance of *Streptococcus* was negatively correlated with the proportion of pTregs ($P < 0.05$) and positively correlated with Th17 cells ($P < 0.05$) in the peripheral blood, while the abundance of *Prevotella_9* was negatively correlated with the Th17 cell frequency ($P < 0.01$), and the fecal SCFA level was positively correlated with pTreg frequency ($P < 0.05$).
Conclusions: Gut dysbiosis and a lack of SCFAs exist in Chinese MS patients, which might be related to an aberrant immune response of MS; this relationship may have a diagnostic and therapeutic value for patients with MS.

1. Introduction

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS), characterized by demyelination, axonal damage, and progressive neurologic disability. Although the etiology of MS remains elusive, clinical and experimental studies, to date, have indicated that a combination of genetic and environmental factors is involved in the pathogenesis of the disease (Weng and Walker, 2013; Wekerle, 2015). In addition, the autoimmune basis of MS was suggested to stem from an imbalance between pathogenic pro-inflammatory Th1 and/or Th17 cells and anti-inflammatory or regulatory mechanisms of immune cells, including Treg cells (Zhou et al., 2011). However, the specific causes of the immune dysfunction remain unknown.

For a long time, viral and bacterial infections were suspected to be the triggers of MS (Buljevac et al., 2002). Recently, the intestinal flora has been reported to be closely linked to various autoimmune diseases,

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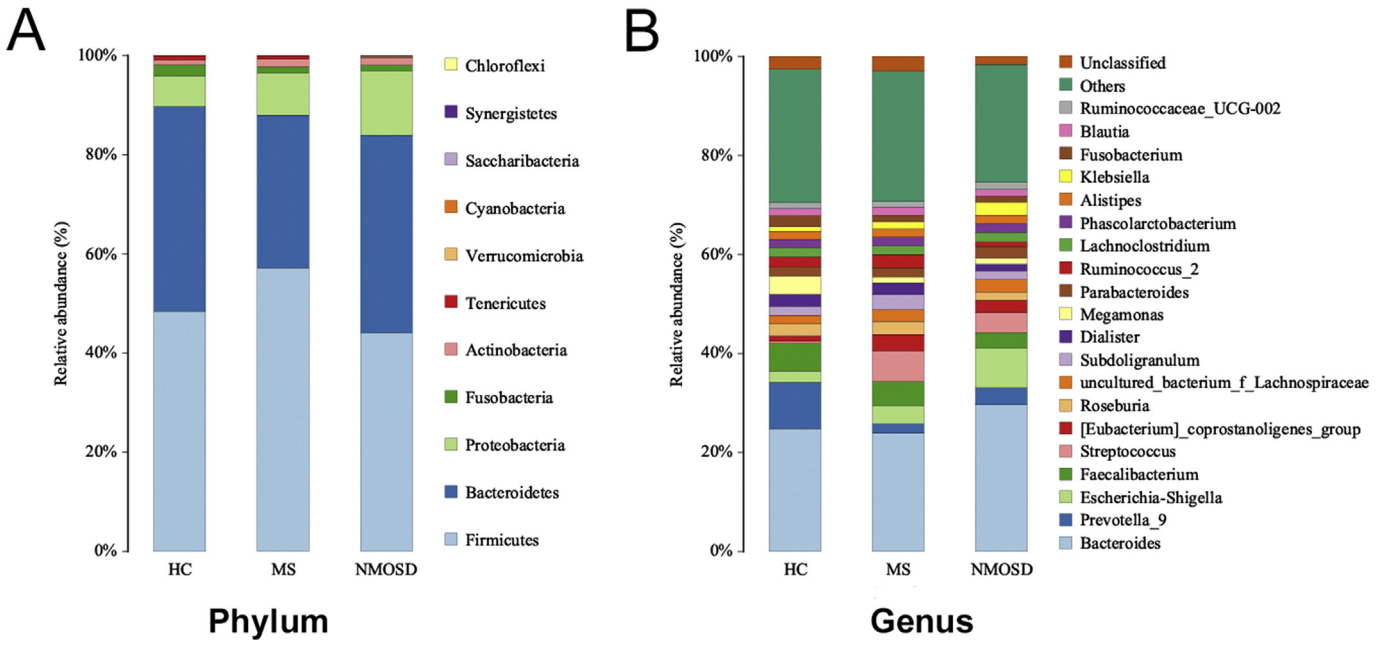

Fig. 1. Overview of community structures in gut microbiota of MS, NMOSD patients and healthy controls (HC) at (a) phylum level and (b) genus level.

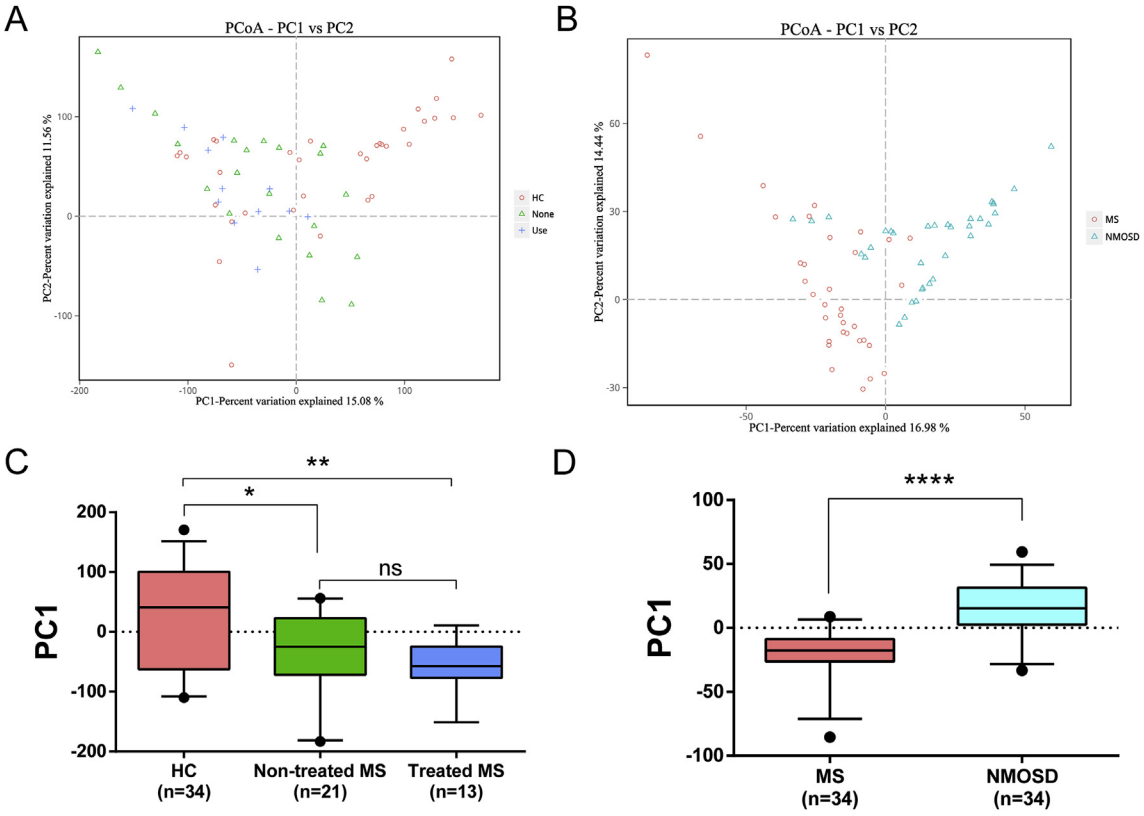

Fig. 2. Cluster analysis among HC, immunosuppressant-treated and non-treated MS patients (a) PCoA analysis between patients with MS and NMOSD(b) . (* denotes $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$ by Mann- Whitney *U* test). PC: principal coordinate.

including MS (Chen et al., 2016; Jangi et al., 2016; Miyake et al., 2015; Rothhammer and Quintana, 2016). Through gut bacterial transplant and *in vitro* immune stimulation, Berer et al. (2017) and Cekanaviciute et al. (2017) confirmed that the intestinal microbiota is able to transfer the phenotype in an MS disease model and contributes to the pathogenesis of MS. Features of the intestinal microbiota in MS patients have been characterized in various reports, including Castillo et al. (2016) from the UK, Jangi et al. (2016) from the USA, and others (Miyake et al., 2015; Rothhammer and Quintana, 2016), which suggested that MS patients exhibit gut microbial dysbiosis. However, studies on the intestinal microflora of MS patients have mainly been conducted in Western countries, which may be due to the high incidence of MS there. In Asia, the only published report on the microflora characteristics of MS patients was that of Miyake et al. (2015), which revealed a striking

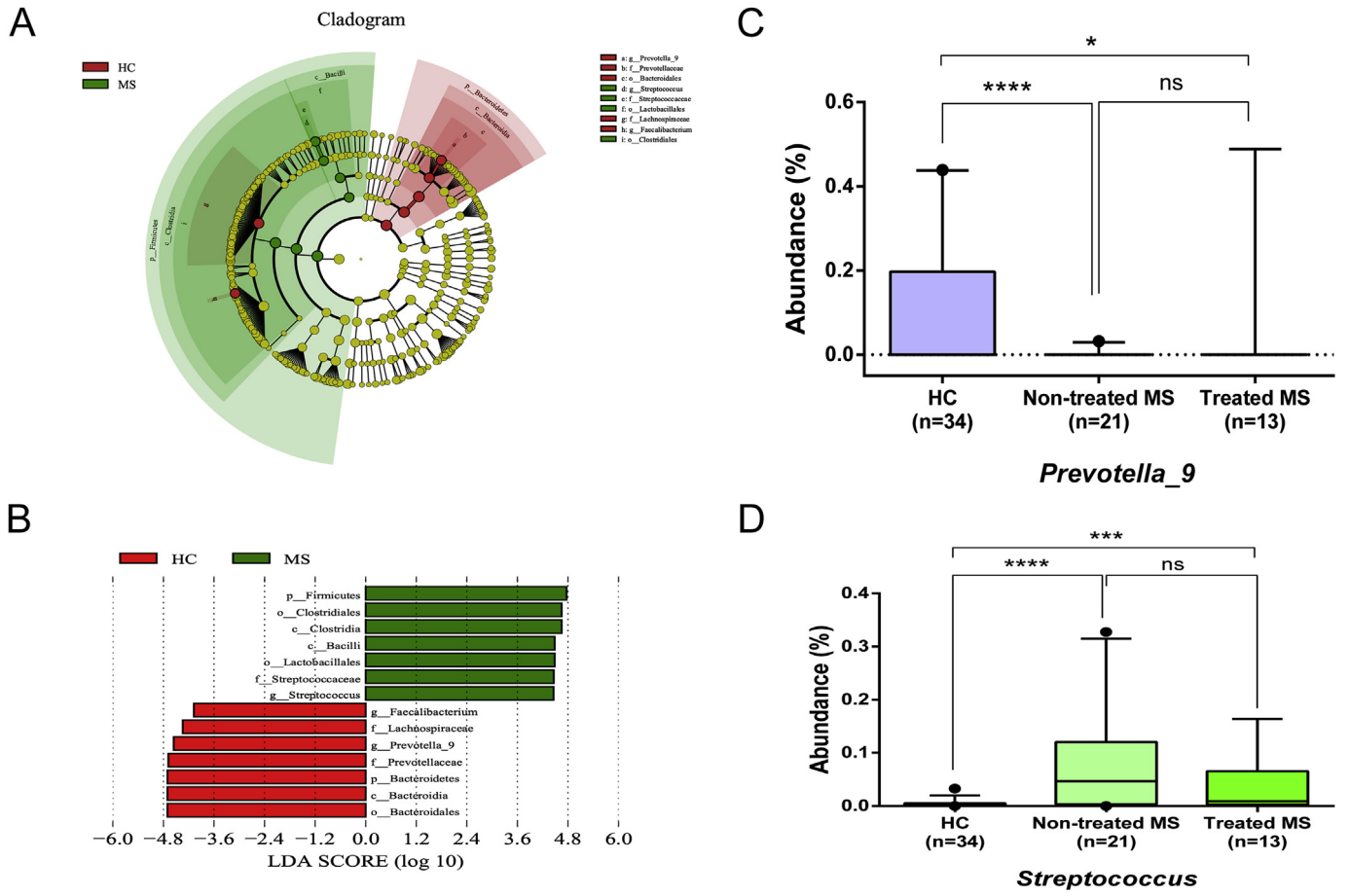

Fig. 3. Relevant features of the intestinal microbiota between MS patients and HCs. (a) Based on the linear discriminant analysis (LDA) and effect size (LEfSe) pineline, cladogram showed the phylogenetic distribution of gut microbiota associated with MS (green) and HC (red); (c) LDA scores revealed the significant bacterial difference between MS and HC; (c,d)comparing the relative abundances of *Prevotella_9 and Streptococcus among* immunosuppressant-treated and non-treated MS patients and control. (* denotes $p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$ by Mann- Whitney *U* test).

depletion of butyrate-producing bacteria in Japanese MS patients. Meanwhile, the association of metabolic products with these bacteria, as well as the relationship between the flora and the immune system, remains to be elucidated. The intestinal microbiome is susceptible to influence from one's geographical region, diet, and genetic background. MS patients in Asia have different manifestations than Western patients, including fewer brain and cerebellar lesions, a lower proportion of progressive disease, and less incidence of positive oligoclonal bands (Chong and Tan, 2008). Therefore, it is worth expanding the scope of this research to Chinese MS patients to get a clearer picture of the intestinal microflora imbalance in MS patients and provide a basis for the study of using microbes as the therapeutic targets.

Moreover, the metabolic products of bacteria, especially short-chain fatty acids (SCFAs), play an important role in immune disorders, including MS (Morris et al., 2017). Haghikia et al. (2016) reported that dietary SCFAs can expand gut Treg cells and ameliorate experimental autoimmune encephalomyelitis (EAE) disease course via long-lasting imprinting on lamina propria-derived Treg cells. However, the fecal SCFA level of MS patients, and its relationship with altered gut microbial composition as well as aberrant immune responses, has not yet been evaluated.

In this study, the intestinal microbial signatures, SCFA levels, as well as T-cell profiles of Chinese MS patients, were determined and analyzed. In addition, neuromyelitis optica spectrum disorder (NMOSD), also an inflammatory demyelinating disease, is frequently misdiagnosed as MS, but prognosis and optimal treatments differ (Yokote and Mizusawa, 2016). It was found that interferon (IFN)-β, widely used and proven effective in treating patients with MS, sometimes exacerbates NMOSD (Papadopoulos et al., 2014). Moreover, NMOSD has a worse outcome than MS, with frequent and early relapses (Lennon et al., 2005). Despite these distinctions, NMOSD shares a similar pathophysiological background and immunoreaction with MS (Yokote and Mizusawa, 2016). So far, quite a few studies have identified the close connection between the gut microbiota and the two diseases. Therefore, to better understand the differences and similarities between these two diseases, it is crucial to perform a comparative analysis on the intestinal microbial signatures and SCFA levels of the two diseases. In sum, our aim was to reveal the unique intestinal flora and SCFA levels of Chinese patients with MS, as well as their correlation with immunity dysfunction.

2. Methods

2.1. Ethics approval and patient consent

This study was approved by the Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University. Informed written consent was provided by all participants.

2.2. Subjects and clinical assessments

Stool samples were collected from a cohort consisting of 34 MS patients [fulfilling the McDonald diagnostic criteria for MS (Thompson et al., 2018)], 34 NMOSD patients [fulfilling the criteria of Wingerchuk,

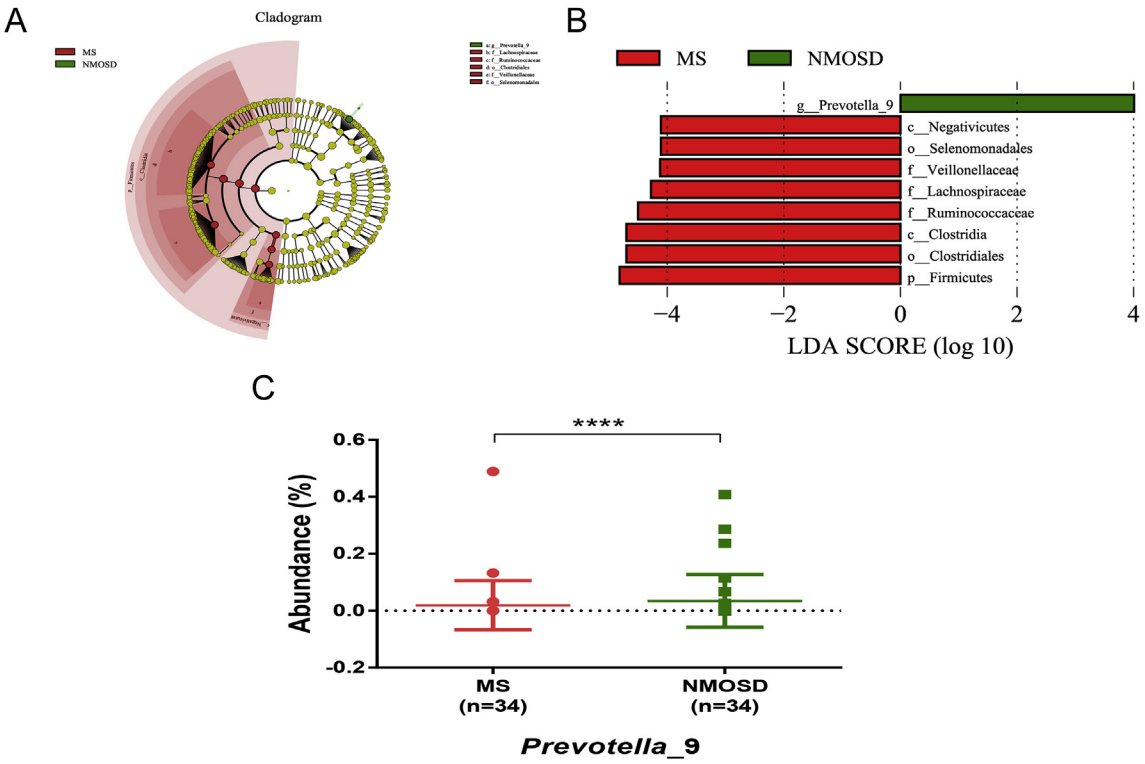

Fig. 4. Gut microbiota differs between MS and NMOSD. (a) The significant taxa were highlighted on the phylogenetic tree and showed the distribution of gut microbiota associated with NMOSD (green) and MS (red); (b) LDA scores-based analysis showed the gut microbiome found to be differentially abundant in MS and NMOSD; (c)comparing the relative abundances of *Prevotella_9* between MS and NMOSD. (**** denotes $p < 0.0001$ by Mann- Whitney *U* test).

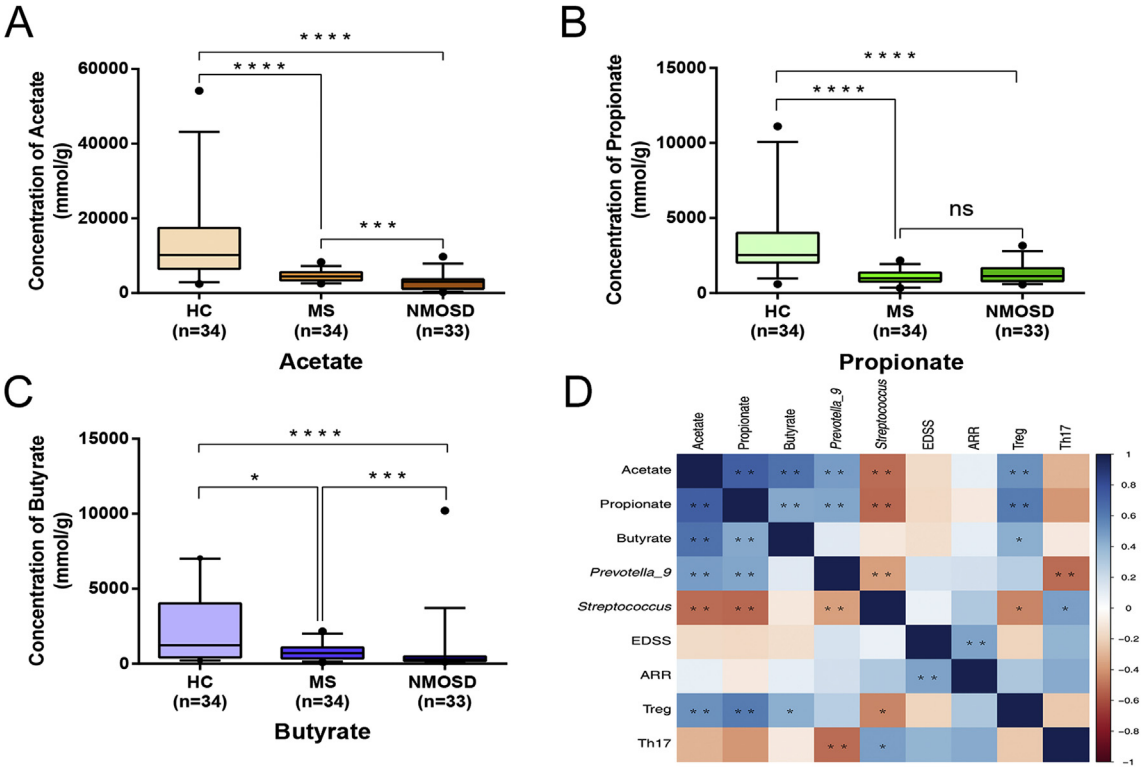

Fig. 5. Concentration of fecal SCFAs among MS, NMOSD and HC, and numerical correlation between differential microbials, SCFAs, and T cells of MS patients. (a) Fecal acetate concentration among MS, NMOSD and HC; (b) Fecal propionate concentration among MS, NMOSD and HC; (c) Fecal butyrate concentration among MS, NMOSD and HC. (d)Spearman's correlation coefficient was calculated among the concentration of SCFAs, the abundance of *Prevotella_9* and *Streptococcus,* the proportions of Treg and Th17 number, EDSS and ARR (* denotes $p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$ by Mann- Whitney *U*- test).

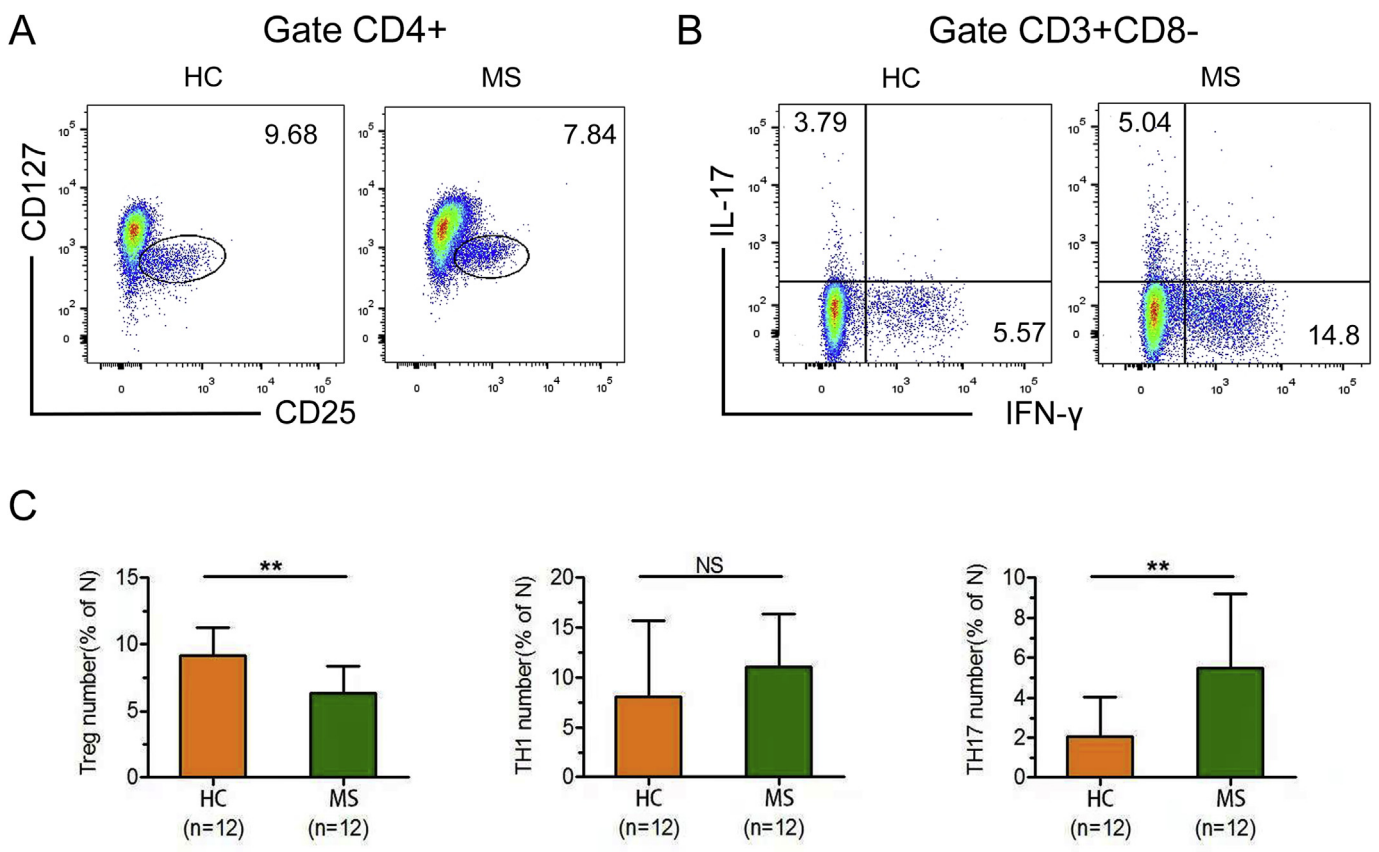

Fig. 6. T-cell profile analyses of peripheral blood in MS patients. (a) We first measured by flow cytometry the proportions of Tregs, stained for $CD4^+CD25^+$ CD127-. (b) For flow cytometry Th profile analyses, cells were stimulated for 5 h with PMA/ionomycin/brefeldin, and then stained for CD3, CD8 and IFN-γ or IL-17. (c) The proportions of $CD25^+$ CD127-(Treg) in the number of $CD4^+$ T cells, and IFN-γ+ (Th1) or IL-17+ (Th17) cells in the number of $CD3^+CD8^-$ T cells in both HC and MS were made comparison statistically. (** denotes $p < 0.01$) (see Table 1).

seropositive for AQP4-IgG (Wingerchuk et al., 2013)], and 34 healthy controls (HC). All the patients, who had different degrees of disability [Expanded Disability Status Scale (EDSS) score from 0 to 6], were enrolled from the Department of Neurology; a control group matched for BMI, age, and gender was recruited from the Health Examination Center of the Third Affiliated Hospital of Sun Yat-sen University. The basic and clinical data of the groups in the study cohort are summarized in Tables 1 and S1. In our cohort, MS patients within a month of a relapse were considered to be in an active disease state, and the rest were defined as being in a remission phase. Some of the MS and NMOSD patients were treated with immunosuppressants, such as azathioprine, methotrexate, or mycophenolate, which are listed in detail in Table S1. Any patients or controls who had taken probiotic supplements or antibiotics within one month before admission were excluded. Other exclusion criteria included gastrointestinal operations and having a known history of autoimmune diseases such as rheumatoid arthritis.

2.3. Sampling, DNA extraction, and PCR processing

Stool samples were collected between December 2016 and December 2017 at one study center and stored at −80 °C immediately. The bacterial DNA was extracted from fecal samples using the QIAamp DNA Stool Mini Kit (Qiagen, Germany), following the manufacturer's instructions. The bacterial DNA was amplified using barcoded primers that amplified the V3–V4 hypervariable region of the 16S rRNA gene (~500 bp long). PCR products were examined on 2% (w/v) agarose gel and further purified using an E.Z.N.A. Gel Extraction Kit (Omega Biotek). Purified amplicons were pooled in equimolar amounts for library preparation. Construction of sequencing libraries and paired-end sequencing (2 × 250 bp) was performed on an Illumina MiSeq platform at Biomarker Technologies Co., Ltd (Beijing, China) according to the standard protocols.

2.4. 16S rRNA gene amplicon sequencing and analysis

Custom Perl and Bash scripts were used to demultiplex the reads and assign barcoded reads to individual samples. Reads were kept only when the sequence included a perfect match to the barcode and the V4 16S rRNA gene primers and were within the length expected for the V3–V4 variable region. The raw data were merged using FLASH (Magoc and Magoc, 2011). Sequences were quality filtered using Trimmomatic (Bolger et al., 2014), and chimera sequences were removed using a UCHIME algorithm (Robert et al., 2011).

2.5. Detection of SCFA concentration

The determination and analysis of fecal SCFAs were performed by the Shenzhen Academy of Metrology and Quality Inspection. Before GC analyses, feces samples were thawed, homogenized (Ultra Turrax T 25, Sweden), centrifuged, and finally filtered. Acetate, propionate, and butyrate standards (analytical quality of at least 99% purity) were purchased from Sigma-Aldrich (Bornem, Belgium). Chromatographic analysis was carried out using an Agilent 7890B GC system equipped with a flame ionization detector (FID) and an Agilent 7693 automatic liquid sampler (Agilent, USA). Relative data were dissected with HP ChemStation Plus software (A.09.01).

Table 1
Clinical and demographic features of MS patients and control.

Data	RRMS			NMOSD	HC
	Active	Remission	Total		
N	26	8	34	34	34
Sex (M/F)	8/16	5/5	13/21	3/31	13/21
Age (SD)	26.66 (10.70)	36.63 (11.16)	29 (10.70)	31.21 (10.93)	35.18 (9.03)
Age Of Onset	22.20 (10.03)	32 (10.33)	24.5 (10.03)	27.59 (12.29)	
BMI	21.12 (3.28)	22.27 (3.40)	21.39 (3.28)	21.11 (2.91)	21.72 (2.77)
ARR					
≤1	18	5	23	11	
> 1	8	3	11	23	
OCB					
+	18	2	20	0	
–	8	6	14	34	
Disease severity					
EDSS					
< 3	9	3	12	14	
3-5	17	6	21	20	
HHcy	8	1	9	4	
Sleep disorders	9	3	12	9	3
Therapy					
Aza	3	2	5	14	
MTX	1	1	2	0	
Else	5	1	6	13	
None	17	4	21	7	
GC	17	1	18	25	

OCB: oligoclonal bands; HHcy: hyperhomocystinemia; Aza: Azathioprine; MTX: Methotrexate; Else: including Tacrolimus, Mycophenolate Mofetil, and Rituximab; None: untreated with immunosuppressor; GC: glucocorticoids.

2.6. Flow cytometry

Peripheral blood mononuclear cells (PBMC) were isolated and extracted from the peripheral blood of the participants. For surface staining, surface markers were stained with fluorescein-conjugated Abs against CD4, CD8, CD3, CD25, and CD127 on the nonmanipulated cells for 20 min. Intracellular staining was performed after stimulation with PMA (0.05 μg/ml) and ionomycin (0.5 μg/ml) for 1 h, and brefeldin A (1:1000 dilution, eBioscience) was added for an additional 4 h. Cells were fixed and permeabilized (Fix/Perm) according to the manufacturer's instructions and stained with fluorescein-conjugated Abs against IL-17 A and IFN-γ as we previously reported. The percentages of cells expressing each molecule in gated T cells were determined using a BD FACSCalibur flow cytometer, and data were analyzed with FlowJo software (Tree Star, Ashland, OR).

2.7. Statistical analyses

The effective sequences were binned into operational taxonomic units (OTUs) using USEARCH software with a cutoff of 97% identity (Edgar, 2010). α-diversity (Chao1 richness estimator, Shannon – Wiener diversity index, Simpson diversity index) was calculated based on the rarefied OTU counts. Differences between individuals and groups could be observed through principal coordinate analysis (PCoA) performed from binary_otu_gain distance, the linear discriminant analysis (LDA) effect size (LEfSe) pipeline (Segata et al., 2011), and Metastats (White et al., 2009). The Boruta algorithm was employed to select the taxa that had predictive power (R package "Boruta"). Predictors of metabolic potential from taxonomic information were obtained from 16S data assessed by the functional analysis (PICRUSt) (Langille et al., 2013). The correlations among the abundance of microbes, fecal SCFA levels, and immune cells were calculated using a Pearson correlation coefficient and visualized by a heatmap in R using the "corrplot" package (Friendly, 2002). Statistical analysis was performed using an

Table 2
Comparative analyses of the fecal microbial communities in MS from different countries.

Country	Subjects (n, M/F)	Changes in abundance between MS vs. HC (G-genus)		Reference
		Increased in MS	Decreased in MS	
USA	RRMS (n = 31,10M/21F) HC (n = 36, 14M/22F)	*Pseudomonas, Mycoplana, Haemophilus, Blautia, Dorea, Pedobacter and Flavobacterium*	*Prevotella, Parabacteroides, Adlercreutzia, Collinsella, Lactobacillus, Coprobacillus and Haemophilus*	Chen et al. (2016)
USA	RRMS(n = 60, 19M/41F) HC (n = 43, 6M/37F)	*Methanobrevibacter, Akkermansia*	*Butyricimonas, Prevotella*	Jangi et al. (2016)
USA	RRMS (n = 7) HC (n = 8) no gender data	*Ruminococcus*	*Fecalibacterium*	Cantarel et al. (2015)
USA	RRMS(n = 71, 27M/44F) HC(n = 71, 39M/32F)	*Akkermansia and Acinetobacter*		Cekanaviciute et al., 2017
Germany	MS twins (n = 34, 8M/26F) HC twins (n = 34, no gender data)	*Akkermansia*		Berer et al. (2017)
UK	RRMS (n = 30) HC (n = 14) no gender data	*Prevotella copri DSM 18205*		Castillo et al. (2016)
Japan	RRMS (n = 20, 6M/14F) HC(n = 40, 20M/20F)	*Bifidobacterium, Streptococcus, thermophilus, Eggerthella lenta*	*Bacteroides (B. stercoris, B. coprocola, and B. coprophilus), Fecalibacterium, Prevotella (P.copri) Anaerostipes, Clostridium, Sutterella (S. wadsworthensis)*	Miyake et al. (2015)
China	RRMS (n = 34, 13M/21F) HC(n = 34, 13M/21F)	*Streptococcus*	*Prevotella_9*	In this study

Table 3
Microbial changes in Central nervous system diseases.

Neuronal diseases	Examples of microbiota changes in humans		Reference
	Increased	Decreased	
NMOSD	*Streptococcus and Shigella*	*Faecalibacterium*	Gong et al. (2018)
ASD	*Erysipelotrichaceae, Clostridium lituseburense, and Terrisporobacter with low tryptophan; Lachnoclostridium bolteae, Lachnoclostridium hathewayi, and Flavonifractor plautii with high serotonin*	*Prevotella*	Strati et al. (2017) and Luna et al., 2017
ALS	*Dorea*	*Oscillibacter, Anaerostipes, and Lachnospiraceae*	Fang et al. (2016)
PD		*Prevotellaceae and Lactobacillaceae*	Scheperjans et al. (2015) and Unger et al. (2016)
AD	*Escherischia/shigella*	*Eubacterium rectale*	Cattaneo et al. (2017)
MSA	*Bacteroides*	*Paraprevotella*	Tan et al., 2018

unpaired two-tailed *t*-test on GraphPad Prism 6.0 software, and the Wilcoxon rank sum test was used to test for significant taxonomic differences among the three groups. A value of $P < 0.05$ was considered statistically significant in the compared groups. The results are expressed as the median and range.

3. Results

3.1. Characteristics of the gut microbiota in patients with MS

Fecal samples from 34 MS patients, 34 NMOSD patients, as well as 34 HCs were analyzed by sequencing of 16S rRNA gene amplicons (Tables 1 and S2). After removing singletons, the sequences were clustered into OTUs based on 97% sequence similarity (Table S3).

Taxonomic classification at the phylum level revealed that the intestinal bacteria detected consisted mainly of *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* (Fig. 1A), and a much more complex gut microbial composition was observed at the genus level. The top 20 genera (mainly *Bacteroides*, *Prevotella_9, Escherichia-Shigella, Faecalibacterium*, and *Dialister*) composed more than 70% of the total microbiota (Fig. 1B). The α-diversity, including species richness (represented by observed OTUs, Chao1) and richness and evenness (represented by the Shannon index), did not differ significantly among MS patients, NMOSD patients, and HCs (Fig. S1). Based on the binary_otu_gain distance of 16S rRNA sequence profiles, PCoA analysis showed that the three groups generally clustered separately, and the diversity of the gut microbiome of MS patients in both immunosuppressant-treated and non-treated states decreased compared to HCs ($P < 0.05$ and $P < 0.01$, respectively; Mann−Whitney *U* test; Fig. 2C) and NMOSD patients ($P < 0.0001$, Fig. 2D). Moreover, no significant difference was observed in the microbial composition between treated and untreated MS patients, indicating a consistent composition of their gut microbiota (Fig. 2A, C).

3.2. Differentially distributed gut microbial taxa and function in MS

LEfSe analysis was performed on differentially distributed taxa between MS patients and HCs. The results showed that two bacterial genera, *Prevotella_9* and *Streptococcus*, were differentially distributed between MS patients and HCs [LDA score ($\log_{10} = 4$)] (Fig. 3A and B). *Prevotella_9* was found to be deficient in the MS group, both with and without immunosuppressant treatment ($P < 0.05$ and $P < 0.0001$, respectively; Mann−Whitney *U* test; Fig. 3C); *Streptococcus* was higher in both treated and untreated MS groups than in the HC group ($P < 0.001$ and $P < 0.0001$, respectively; Fig. 3D). Interestingly, there were no significant differences in *Prevotella_9* and *Streptococcus* between treated and untreated MS groups. A further characterization of the *Streptococcus* strains by quantitative polymerase chain reaction (qPCR) revealed that *S. salivarius* and *S. parasanguinis* were significantly higher in MS patients than in HCs (Table S7, Fig. S3). Beyond that, no statistically significant difference in the abundance of *Prevotella_9* and *Streptococcus* was observed between the MS patients in remission and active states (Fig. S4). Additionally, compared to the gut microbial composition of NMOSD patients, the abundance of *Prevotella_9* was much lower in MS patients ($P < 0.0001$, Fig. 4), indicating that a deficiency in *Prevotella_9* is unique to the intestinal microbiome of MS patients.

Additionally, 30 differentially distributed genera were observed between MS and HC by the Metastats algorithm, of which 16 had an abundance greater than 10^4 (Table S4). Importantly, Boruta algorithm analysis showed that *Streptococcus*, *Atopobium*, *Actinomyces*, and *Rotinia* might be sensitive biomarkers for disease diagnosis. Of them, *Streptococcus* exhibited the highest Z-score (Fig. S5A). Functional analysis by PICRUSt revealed downregulated pathways involved in the metabolism of energy, cofactors, and vitamins in MS ($P < 0.01$ and $P < 0.001$, respectively; Fig. S5B).

3.3. Levels of fecal SCFAs in MS

We used the GC-FID method to analyze three kinds of fecal SCFAs, namely, acetate, propionate, and butyrate (Table S5). Compared to HCs, a striking depletion of fecal acetate and propionate was observed in MS patients ($P < 0.0001$; Fig. 5A and B). Although no complete deficiency was identified, a much lower level of butyrate was also detected in MS ($P < 0.05$; Fig. 5C). However, compared to patients with NMOSD, MS patients had a higher level of acetate and butyrate ($P < 0.001$; Fig. 5A, C). Spearman's correlation revealed a significantly positive correlation between the abundance of *Prevotella_9* and fecal acetate and propionate levels ($P < 0.01$; Fig. 5D). We also collected data on dietary and health habits, e.g., vegetarianism, physical activity, smoking, and alcohol intake (Table S1). After analysis, we did not observe any effects of these factors on the composition of the microbiota or SFCA production in the gut (data not shown).

3.4. Characteristics of peripheral T-cell differentiation in MS

T-lymphocyte cells were isolated from the peripheral blood of MS patients without immunosuppressant treatment and matched controls, and analysis of the T-cell profile was performed by flow cytometry (Table S6). Compared to the HCs, expansion of peripheral regulatory T cells (pTregs, $CD4^+CD25^+CD127^-$; Fig. 6A) was significantly blunted ($P = 0.0012$, Fig. 6C), and the frequency of Th17 cells ($CD3^+CD8^-IL17^+$; Fig. 6B) was increased in MS patients ($P = 0.0104$; Fig. 6C). No statistically significant alterations between MS and HC were observed in the proportions of IFNγ-producing $CD3^+CD8^-$ T cells (Th1; Fig. 6B) ($P = 0.2830$; Fig. 6C). Spearman's correlation revealed that the abundance of *Streptococcus* was significantly negatively correlated with the proportion of pTreg cells and positively correlated with the proportion of Th17 cells ($P < 0.05$; Fig. 5D). The abundance of *Prevotella_9* was significantly negative correlated with Th17 cell frequency ($P < 0.01$; Fig. 5D), and the concentration of fecal SCFAs was positively correlated

with the proportion of pTreg cells ($P < 0.05$ and $P < 0.01$, respectively; Fig. 5D).

4. Discussion

Recently, several studies showed that the intestinal microbiome in patients with MS is characterized by moderate dysbiosis (Chen et al., 2016; Jangi et al., 2016; Miyake et al., 2015), suggesting an association between MS and the gut microbiome. Moreover, replacing some of the bacterial population in the gut could lead to a pro-inflammatory state, indicating a potential microbiota mechanism causing MS in humans (Palm et al., 2015; Branton et al., 2016). A comparison of the dominant and depleted fecal microbes between Chinese and non-Chinese MS patients indicates that at the genus level, patients in China and the USA showed a similar decrease of *Prevotella* (Jangi et al., 2016), while Chinesepatients exhibited a similar increase of *Streptococcus* to Japanese patients (Miyake et al., 2015). Nonetheless, as shown in Table 2 and reviewed in other publications, there were still remarkable differences in most microbial communities among the different countries (Cantarel et al., 2015; Castillo et al., 2016; Chen et al., 2016, 2017; Jangi et al., 2016; Miyake et al., 2015; Zheng et al., 2010). Additionally, *Akkermansia muciniphila*, which has been reported to be increased in Western countries (Jangi et al., 2016), was expressed at low levels in Chinese cohorts, and no differences between patients with MS and healthy controls were found. Overall, Chinese MS patients could be characterized by a remarkable increase of *Streptococcus* and a significant decline of *Prevotella*_9.

Cree et al. (2016) proposed that *Clostridium perfringens* could be a specific pathogen in the promotion of NMOSD through a molecular mimicry hypothesis. However, our study has shown that in Chinese NMOSD patients, the abundance of *Streptococcus* is increased. Furthermore, to our surprise, although there are differences between the pathogenesis and clinical symptoms of MS and NMOSD, the abundance of *Streptococcus* was significantly increased in both of them. *Streptococcus* has been reported to be involved in the pathogenesis of many autoimmune diseases, such as rheumatic heart disease (Fae et al., 2004; Guilherme et al., 2006; Kirvan et al., 2006) and poststreptococcal acute glomerulonephritis (Batsford et al., 2005) through a molecular simulation mechanism or through its innate immune signals. Moreover, *Streptococcus* has been reported to be capable of promoting the differentiation of proinflammatory Th1 and Th17 cells in humans (Engen et al., 2014). For instance, Group B *Streptococcus* (GBS) has been reported to be able to stimulate neonatal neutrophils to promote the generation of Th1 and Th17 cells (Lin et al., 2018). In addition, *Streptococcus pyogenes* has been found to not only reduce the frequency of Tregs in tumor-associated exudate fluids but also inhibit their function via IL-12 produced by antigen-presenting cells (APCs) (Guilherme et al., 2001). In our study, the decreased frequency of pTregs and increased frequency of peripheral Th17 cells were found to be significantly associated with the abundance of *Streptococcus* in MS patients; the imbalance between these cell populations is associated with the pathogenesis and development of autoimmune and inflammatory disease (Horwitz et al., 2008). Therefore, we speculated that an anomalous immune response to *Streptococcus* infections might be a key factor in the development of MS.

Emerging studies have reported that the abundance of specific *Prevotella* strains is associated with inflammatory diseases mediated by Th17-related immune responses (Marietta et al., 2016). In our study, *Prevotella*_9 was found to be significantly depleted in MS compared to both HC and NMOSD. Furthermore, after comprehensive analysis of the genus members of *Prevotella*, a significant difference was found between MS and HC (data not shown), consistent with the results reported by Chen et al. (2016) and Jangi et al. (2016). Some studies on human MS have indicated that individual or combined strains of *Prevotella* may have anti-inflammatory and neuroprotective effects. Mangalam et al. (2017) showed that *P. histicola* can suppress disease in EAE by inducing CD4 + FoxP3 + regulatory T cells, tolerogenic dendritic cells, and suppressive macrophages. Researchers have discovered that *Prevotella* can produce SCFAs from starch/fiber (Kovatcheva et al., 2015) and increase the production of phytoestrogens (Schogor et al., 2014). SCFAs are known to induce Treg cells (Smith et al., 2013) and suppress inflammatory disease in animal models of MS (Haghikia et al., 2016); meanwhile, estrogen has been suggested to possess immunomodulatory properties and can reduce the severity of EAE (Lang, 2004). Unexpectedly, with regard to the existence of certain potential factors affecting Tregs, no statistical correlation was found between the abundance of *Prevotella*_9 and Tregs frequency in this study; however, the increased proportion of Th17 cells in peripheral blood in MS patients was inversely associated with a reduced relative abundance of *Prevotella*_9, which is consistent with the previous report on Italian MS patients (Cosorich et al., 2017). Hence, *Prevotella*_9 might play an important anti-inflammatory role in the pathogenesis of MS.

In addition, our findings showed a significantly decreased amount of fecal SCFAs in MS. The potential role of SCFAs as key microbial mediators in the microbiota–gut–brain axis has been receiving increased attention. A multitude of studies have shown the beneficial effects of butyrate in the brain, such as facilitating neuronal plasticity and long-term memory formation (Lattal et al., 2007) and restoring cognitive function (Govindarajan et al., 2011). Moreover, Hoyles et al. (2018) found that propionate had protective effects against lipopolysaccharides (LPS) -induced blood–brain barrier disruption and oxidative stress. Furthermore, Haghikia et al. (2016) demonstrated that SCFAs, in general, promote T-cell differentiation toward regulatory subtypes (Treg cells), which ameliorate the autoimmune process in the brain. *In vitro* studies and animal experiments have suggested that butyrate and propionate promote peripheral regulatory T-cell generation dependent upon intronic enhancer CNS1 (Arpaia et al., 2013; Josefowicz et al., 2012; Zheng et al., 2010). Moreover, we previously reported that sodium butyrate (NaB), one of the short-chain fatty acids, significantly increased the number of T-regulatory (Treg) cells in both draining lymph nodes and spleens of experimental autoimmune uveitis (EAU) mice (Chen et al., 2017). In this study, fecal SCFAs were also found to be significantly positively correlated with the proportions of pTregs. Taken together, these observations provide evidence supporting the potential role of anti-inflammatory metabolites in MS pathogenesis.

Last but not least, since intestinal dysbiosis has also been found in other neurological diseases, including autism spectrum disorder, amyotrophic lateral sclerosis, and Parkinson's disease, it is indispensable to conduct a brief bibliographic comparison between the gut microbiome in MS and these diseases, as shown in Table 3. Through comparative analysis, it is not difficult to discover that the intestinal microbiome of MS patients is unique.

To sum up, we observed a significantly distinctive fecal microbiome and SCFA depletion in Chinese MS patients compared to NMOSD patients and HCs, which might be involved in the aberrant immune response of MS. Understanding the distinction between the microbial structures in MS and NMOSD can facilitate our comprehension of disease pathogenesis, including the radiological and immunological aspects and clinical manifestations of the two diseases. In clinical practice, it can help to diagnose and choose an appropriate treatment for the disease. However, the sample size in our study was not large, and experimental models should be conducted to explore a possible cause-and-effect relationship of MS with an altered microbiome, the SCFA differences, and the generation or promotion of MS. Further research is needed to clarify the role of the gut microbiota and its metabolites on immune dysregulation in MS, with whole metagenomic and metabolic analysis, animal model, and *in vitro* cellular experiments.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuint.2019.104468.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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