frontiers | Research Topics

The role of gut microbes and their metabolites in immune-related diseases

Edited by
Chuanxing Xiao, Zhe-Sheng Chen, Xiangtian Yu, Yufang Wang and Qinglong Wu

Published in
Frontiers in Cellular and Infection Microbiology

ISSN 1664-8714
ISBN 978-2-8325-3538-7
DOI 10.3389/978-2-8325-3538-7

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

The role of gut microbes and their metabolites in immune-related diseases

Topic editors

Chuanxing Xiao — Xiamen University, China
Zhe-Sheng Chen — St. John's University, United States
Xiangtian Yu — Shanghai Jiao Tong University, China
Yufang Wang — Sichuan University, China
Qinglong Wu — International Flavors and Fragrances, United States

Citation

Xiao, C., Chen, Z.-S., Yu, X., Wang, Y., Wu, Q., eds. (2023). *The role of gut microbes and their metabolites in immune-related diseases*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-3538-7

Table of contents

05 Washed microbiota transplantation improves patients with metabolic syndrome in South China
Lei Wu, Xin-Jian Lu, De-Jiang Lin, Wen-Jia Chen, Xing-Ying Xue, Tao Liu, Jia-Ting Xu, Ya-Ting Xie, Man-Qing Li, Wen-Ying Lin, Qing Zhang, Qing-Ping Wu and Xing-Xiang He

23 Antibiotic ampicillin induces immune tolerance in renal transplantation by regulating the proportion of intestinal flora in mice
Xiaoqiang Wu, Guanghui Cao, Zhiwei Wang, Xuan Wu, Xiangyong Tian, Yue Gu, Fengmin Shao and Tianzhong Yan

35 Alterations of the gut microbiota and short chain fatty acids in necrotizing enterocolitis and food protein-induced allergic protocolitis infants: A prospective cohort study
Jing Xiong, Xing-Sheng Liao, Tong Yin, Xiao-Chen Liu, Lei Bao and Lu-Quan Li

50 Fecal microbiota transplantation treatment of autoimmune-mediated type 1 diabetes: A systematic review
Shuo Zhang, Feiying Deng, Jingxian Chen, Fengwu Chen, Zezhen Wu, Liping Li and Kaijian Hou

63 Fecal microbiota transplantation reverses insulin resistance in type 2 diabetes: A randomized, controlled, prospective study
Zezhen Wu, Bangzhou Zhang, Fengwu Chen, Rongmu Xia, Dan Zhu, Baolong Chen, Aiqiang Lin, Chuyan Zheng, Ducheng Hou, Xiaoyu Li, Shuo Zhang, Yongsong Chen and Kaijian Hou

77 Specific fungi associated with response to capsulized fecal microbiota transplantation in patients with active ulcerative colitis
Qiongyun Chen, Yanyun Fan, Bangzhou Zhang, Changsheng Yan, Zhangran Chen, Lin Wang, Yiqun Hu, Qingwen Huang, Jingling Su, Jianlin Ren and Hongzhi Xu

91 Combined microbiome and metabolome analysis of gut microbiota and metabolite interactions in chronic spontaneous urticaria
Zhen Luo, Zhangsi Jin, Xiaoran Tao, Ting Wang, Panling Wei, Caihong Zhu and Zaixing Wang

103 Oxymatrine ameliorates experimental autoimmune encephalomyelitis by rebalancing the homeostasis of gut microbiota and reducing blood-brain barrier disruption
Ming-Liang Zhang, Wei-Xia Li, Xiao-Yan Wang, Ya-Li Wu, Xiao-Fei Chen, Hui Zhang, Liu-Qing Yang, Cheng-Zhao Wu, Shu-Qi Zhang, Yu-Long Chen, Ke-Ran Feng, Bin Wang, Lu Niu, De-Xin Kong and Jin-Fa Tang

120 Gut-joint axis: Gut dysbiosis can contribute to the onset of rheumatoid arthritis *via* multiple pathways
María del Socorro Romero-Figueroa, Ninfa Ramírez-Durán, Alvaro José Montiel-Jarquín and Gabriel Horta-Baas

137 Shifts and importance of viable bacteria in treatment of DSS-induced ulcerative colitis mice with FMT
Jinglong Liu, Hao Lin, Man Cao, Tan Lin, Aiqiang Lin, Wei Xu, Han Wang, Jianquan He, Yuantao Li, Hailing Tang and Bangzhou Zhang

148 Research progress of gut microbiota and obesity caused by high-fat diet
Shuyi Fan, Suyun Chen and Lin Lin

158 *Lactobacillus rhamnosus* GG ameliorates noise-induced cognitive deficits and systemic inflammation in rats by modulating the gut-brain axis
Xiaofang Li, Pengfang Zheng, Wa Cao, Yang Cao, Xiaojun She, Honglian Yang, Kefeng Ma, Fangshan Wu, Xiujie Gao, Yu Fu, Jiayi Yin, Fei Wei, Shoufang Jiang and Bo Cui

frontiers | Frontiers in Cellular and Infection Microbiology

TYPE Original Research
PUBLISHED 15 November 2022
DOI 10.3389/fcimb.2022.1044957

OPEN ACCESS

EDITED BY
Chuanxing Xiao,
Xiamen University, China

REVIEWED BY
Rongmu Xia,
Xiamen University, China
Kumar Prafull Chandra,
healthcity Vistaar Multispeciality Hospital, India
Lubia Velázquez López,
Instituto Mexicano del Seguro Social, Mexico

*CORRESPONDENCE
Xing-Xiang He
hexingxiang@gdpu.edu.cn
Qing-Ping Wu
wuqp203@163.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Intestinal Microbiome, a section of the journal Frontiers in Cellular and Infection Microbiology

RECEIVED 15 September 2022
ACCEPTED 27 October 2022
PUBLISHED 15 November 2022

CITATION
Wu L, Lu X-J, Lin D-J, Chen W-J, Xue X-Y, Liu T, Xu J-T, Xie Y-T, Li M-Q, Lin W-Y, Zhang Q, Wu Q-P and He X-X (2022) Washed microbiota transplantation improves patients with metabolic syndrome in South China. *Front. Cell. Infect. Microbiol.* 12:1044957.
doi: 10.3389/fcimb.2022.1044957

Washed microbiota transplantation improves patients with metabolic syndrome in South China

Lei Wu[1,2,3†], Xin-Jian Lu[1†], De-Jiang Lin[1†], Wen-Jia Chen[1†], Xing-Ying Xue[4], Tao Liu[1], Jia-Ting Xu[1], Ya-Ting Xie[1], Man-Qing Li[1], Wen-Ying Lin[1], Qing Zhang[1], Qing-Ping Wu[2*] and Xing-Xiang He[1*]

[1]Department of Gastroenterology, Research Center for Engineering Techniques of Microbiota-Targeted Therapies of Guangdong Province, The First Affiliated Hospital of Guangdong Pharmaceutical University, Guangzhou, China, [2]Guangdong Provincial Key Laboratory of Microbial Safety and Health, State Key Laboratory of Applied Microbiology Southern China, Institute of Microbiology, Guangdong Academy of Sciences, Guangzhou, China, [3]School of Biology and Biological Engineering, South China University of Technology, Guangzhou, China, [4]Xiamen Treatgut Biotechnology Co., Ltd., Xiamen, China

Background: Metabolic syndrome (MS) is a growing public health problem worldwide. The clinical impact of fecal microbiota transplantation (FMT) from healthy donors in MS patients is unclear, especially in southern Chinese populations. This study aimed to investigate the effect of washed microbiota transplantation (WMT) in MS patients in southern China.

Methods: The clinical data of patients with different indications receiving 1-3 courses of WMT were retrospectively collected. The changes of BMI, blood glucose, blood lipids, blood pressure and other indicators before and after WMT were compared, such as fasting blood glucose (FBG), glycated hemoglobin (HbA1c), total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-c)), high-density lipoprotein cholesterol (HDL-c), non-high-density lipoprotein (non-HDL-c), systolic blood pressure (SBP), diastolic blood pressure (DBP), etc. At the same time, comprehensive efficacy evaluation and atherosclerotic cardiovascular disease (ASCVD) grade assessment were performed on MS patients. Finally, 16S rRNA gene amplicon sequencing was performed on fecal samples of MS patients before and after transplantation.

Results: A total of 237 patients were included, including 42 in the MS group and 195 in the non-MS group. For MS patients, WMT significantly improved the comprehensive efficacy of MS in short term 40.48% ($p<0.001$), medium term 36.00% ($p=0.003$), and long term 46.15% ($p=0.020$). Short-term significantly reduced FBG ($p=0.023$), TG ($p=0.030$), SBP ($p=0.026$) and BMI ($p=0.031$), and increased HDL-c ($p=0.036$). The medium term had a significant reduction in FBG ($p=0.048$), TC ($p=0.022$), LDL-c ($p=0.043$), non-HDL-c ($p=0.024$) and BMI

(p=0.048). WMT had a significant short term (p=0.029) and medium term (p=0.011) ASCVD downgrading effect in the high-risk group of MS patients. WMT improved gut microbiota in MS patients.

Conclusion: WMT had a significant improvement effect on MS patients and a significant downgrade effect on ASCVD risk in the high-risk group of patients with MS. WMT could restore gut microbiota homeostasis in MS patients. Therefore, the regulation of gut microbiota by WMT may provide a new clinical approach for the treatment of MS.

KEYWORDS

fecal microbiota transplantation (FMT), washed microbiota transplantation (WMT), metabolic syndrome, comprehensive efficacy, atherosclerotic cardiovascular disease (ASCVD)

Introduction

Metabolic syndrome (MS) has become one of the major public health challenges worldwide. It is a group of risk factors combined with obesity, hyperglycemia (diabetes or impaired glucose regulation), dyslipidemia (hyperglycemia and/or low HDL-c hyperemia), and hypertension of clinical syndromes (O'Neill and O'Driscoll, 2015; Rossi et al., 2022). These conditions co-occur in individuals more frequently than expected by chance. When these factors are combined, they directly contribute to the development of ASCVD and also increase the risk of developing type 2 diabetes (Grundy, 2016; Guembe et al., 2022; Tang et al., 2022). There has been a dramatic increase in the number of people living with MS worldwide, and this increase has been associated with a global epidemic of obesity and diabetes (Grundy, 2008; Simmons et al., 2010; Gurka et al., 2017). With the increased risk of diabetes and cardiovascular disease associated with MS (Gami et al., 2007; DeBoer et al., 2015), strategies to prevent emerging global epidemics are urgently needed.

MS combines multiple symptoms, such as obesity, dyslipidemia, hyperglycemia, and hypertension, which significantly increase the risk, progression rate, and harm of diabetes and cardiovascular disease. Therefore, a scientific and reasonable treatment strategy for MS should be comprehensive, including measures such as blood glucose, blood lipid, blood pressure, weight control, and lifestyle improvement. These treatment strategies are mainly lifestyle intervention and drug therapy. Previous studies have confirmed that exercise has both alleviating and therapeutic effects in improving abnormal glucose and lipid metabolism. For people who are sedentary, current recommendations are to gradually increase aerobic exercise, and moderate physical activity can reduce the risk of metabolic diseases (Donnelly et al., 2009). A meta-analysis of 48 studies reported significant improvements in lipid metabolism in 2990 subjects with MS who underwent moderate-intensity aerobic exercise training (40 to 60% of heart rate reserve or maximal oxygen uptake) (Wood et al., 2022). Various drugs are used to treat MS, such as hypoglycemic agents: metformin (Ludvik et al., 2021), alpha-glucosidase inhibitors (Krentz et al., 2008), targeting the glucagon-like peptide GLP -1 receptor agonists, etc. (Rosenstock et al., 2021; Sattar et al., 2021). Lipid-lowering drugs: statins (Briguori et al., 2009; Di Sciascio et al., 2009; Ray et al., 2021), ezetimibe, etc. (Kim et al., 2022). Antihypertensive drugs: diuretic antihypertensives (Bakris et al., 2012), sympathetic inhibitor (Azizi et al., 2015), calcium antagonists (Carey et al., 2018), renin-angiotensin system inhibitor et al. (Bobrie et al., 2012). Diet pills: orlistat (Dombrowski et al., 2014), topiramate (Yanovski and Yanovski, 2014), liraglutide (Bray et al., 2016), lorcaserin (Gadde et al., 2018). However, long term use of drug therapy can have significant side effects. Therefore, it is of great significance to comprehensively analyze the related factors of MS and find a treatment method with less side effects.

The human gut microbiota has an average of 10-100 trillion microorganisms, more than ten times the estimated number of human cells (Turnbaugh et al., 2007). The impact of a healthy and diverse gut microbiota on metabolic system, immune system, and gut homeostasis is increasingly evident (Jung et al., 2015). The gut microbiome is increasingly recognized as playing an important role in human physiology and health. Gut dysbiosis, defined as a decrease in bacterial diversity or a change in bacterial species compared to healthy controls, is associated with the development of multiple diseases (Machiels et al., 2014; Cani, 2017). Modulation of the gut microbiota to restore a balanced and diverse microbiota may be of great value for the treatment or prevention of microbiome-related diseases. Altering the composition of the gut microbiota has received

attention as a novel therapeutic modality to improve insulin sensitivity (Khan et al., 2014). Fecal microbiota transplantation (FMT) is a novel therapeutic approach that uses healthy microbial profiles to replace the patient's own disturbed microbiota (Bafeta et al., 2017). FMT has a tendency not only to improve the function of commensal host bacteria, but also to completely reshape the entire host microbiome. FMT does this by altering the actual composition and proportions of the resident symbiotic species present in the host. FMT is now included in guidelines recommending FMT as the standard of care in the setting of recurrent *Clostridium difficile* infection (Varier et al., 2015). FMT is of increasing interest (de Groot et al., 2020). FMT is now being tested in clinical trials for other diseases, such as inflammatory bowel disease (IBD) (Moayyedi et al., 2015; Paramsothy et al., 2017; Qazi et al., 2017), Crohn's disease (Paramsothy et al., 2017), obesity (Allegretti et al., 2020), and functional gastrointestinal disorders (Pamer, 2014), which are also associated with marked dysbiosis. Whether FMT can improve MS is a topic to be explored in clinical medicine.

Washed microbiota transplantation (WMT) is a microbiota transplantation method that is similar to traditional FMT but adds the safety measure of washed microbiota. The biggest difference between WMT and FMT is that the bacterial solution of WMT is prepared by an intelligent microorganism separation system (GenFMTer), which has gone through a multi-level filtration system, and finally the washed bacterial solution of WMT is obtained after several washed. It has better safety, quality control for bacterial flora disorders and effectiveness (Shi, 2020; Zhang et al., 2020). We tried to investigate whether WMT could improve patients with MS in patients with functional bowel disease and other diseases who received WMT in the Department of Gastroenterology, The First Affiliated Hospital of Guangdong Pharmaceutical University. We hypothesized that WMT could safely and consistently affect patients across various indications, improve MS without side effects. Therefore, we conducted a retrospective trial to collect medical data from patients with MS treated with WMT.

Materials and methods

Patients and experimental design

This study included patients who received WMT for functional bowel disease and other diseases in our hospital from December 2016 to May 2022 and completed 1-3 courses of treatment. Inclusion criteria: patients older than 18 years who volunteered to receive WMT. Exclusion criteria were: pregnant women, patients taking probiotics during WMT treatment. In the end, a total of 237 people met the requirements. This study was approved by the Ethics Committee of the First Affiliated Hospital of Guangdong Pharmaceutical University, Guangzhou, China according to the Declaration of Helsinki (no. 2017-98). Written informed consent was obtained and reviewed from all patients.

The diagnostic criteria for metabolic syndrome in this study refer to the Chinese Guidelines for the Prevention and Treatment of Type 2 Diabetes (2020 Edition) (Chinese Diabetes S, 2021) and the diagnostic criteria for metabolic syndrome of the Diabetes Society of the Chinese Medical Association (Metabolic Syndrome Research Collaboration Group of Diabetes Branch of ChineseMedical Association, 2018). Metabolic syndrome group (MS group) can be diagnosed with 3 or more of the following: (1) BMI ≥ 25kg/m^2. (2) Hyperglycemia: fasting blood glucose ≥ 6.1 mmol/L or 2-hour blood glucose after glucose load ≥ 7.8 mmol/L and (or) those who have been diagnosed with diabetes and treated. (3) Hypertension: blood pressure ≥130/85 mmHg (1 mmHg=0.133 kPa) and (or) confirmed hypertension and treated. (4) Fasting triglyceride (TG) ≥ 1.70mmol/L. (5) Fasting HDL-c < 1.04mmol/L. Those who do not meet the above conditions are the non-metabolic syndrome group (non-MS group). Finally, 42 people in the MS group and 195 people in the non-MS group were included. With reference to the Chinese Guidelines for the Prevention of Cardiovascular Diseases (2017 edition) ("Chinese Guidelines for the Prevention of Cardiovascular Diseases (2017)," 2018), the ASCVD risk was assessed according to baseline and blood lipid status.

Preparation of washed Microbiota and WMT procedure

The procedure of WMT complies with the Nanjing Consensus on the Methodology of Washed Microbiota Transplantation (Shi, 2020). All healthy fecal donors between the ages of 18 and 25 undergo rigorous consultation, psychological and physical examination, biochemical testing and infectious disease screening. To prepare the washed microbiota, each 100 g of feces and 500 mL of 0.9% saline was used to prepare a homogeneous fecal suspension. Then, the washed bacteria solution was prepared by an intelligent microorganism separation system (GenFMTer) (one-hour FMT protocol with relatively low oxygen environment) (Zhang et al., 2020). According to each patient's physical condition and wishes, the washed bacteria solution is injected into the patient's body through the upper gastrointestinal tract (nasojejunal tube) or the lower gastrointestinal tract (endoscopic intestinal tube). The center implements the standard of "three three courses of treatment" of WMT. That was, do a WMT course every month for the first three months, and then do a WMT course three months apart after the third month. Among them, a WMT course of 3 days, once a day, once a 120mL of the washed bacteria solution was used to patients (Mullish et al., 2018). The results of blood tests and other tests before the first

course of treatment were the baseline values, and relevant indicators were obtained before each subsequent course of treatment. Time could be divided into short term: about 1 month after the first WMT course; medium term: about 2 months after the first WMT course; long term: about 6 months after the first WMT course. All patients underwent at least 2 WMT procedures and completed follow-up.

Clinical data collection

Baseline values, short, medium, and long term outcomes of patients before treatment were collected. Data included age (year), sex n (%), BMI (kg/m^2), disease or indication for WMT, laboratory test results. Mainly include blood glucose indicators: fasting blood glucose (FBG, mmol/L), glycosylated hemoglobin (HbA1c, %). Insulin index: fasting insulin (FI, μU/mL), and calculate the insulin resistance value (HOMA-IR, insulin resistance value=fasting blood glucose*fasting insulin/22.5). Blood lipid indexes: total cholesterol (TC, mmol/L), triglyceride (TG, mmol/L), low density lipoprotein cholesterol (LDL-c, mmol/L), high density lipoprotein cholesterol (HDL-c, mmol/L), Apolipoprotein B (ApoB, g/L), non-high density lipoprotein (non-HDL-c, mmol/L), lipoprotein a (LIP, mmol/L). Blood pressure indicators: systolic blood pressure (SBP, mmHg) on admission, diastolic blood pressure (DBP, mmHg) on admission. Adverse events (AEs): abdominal pain, diarrhea, nausea and vomiting, dizziness, fatigue, etc. After all patients received WMT treatment and completed follow-up, the results of blood glucose, blood lipids and blood pressure were statistically analyzed and evaluated.

DNA extraction and sequencing

Stool samples from 5 patients with MS, 6 patients with non-MS, and 5 donors were collected for sequencing before and after WMT. All samples were stored at -80°C after collection until DNA extraction. DNA extraction from fecal samples was performed as previously described (Qin et al., 2012). DNA quality and concentration were checked by NanoDrop™ 2000 (Thermo Fisher Scientific, Wilmington, DE, USA). A bacterial 16S rRNA gene fragment (V3-V4) was amplified from the extracted DNA by PCR using primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). Polymerase chain reaction (PCR) conditions: 30 s at 95°C, 30 s at 55°C, and 45 s at 72°C for a total of 25 cycles. PCR products were subjected to agarose gel electrophoresis to determine the size of the amplicon. The constructed library was quantified by Qubit and Q-PCR; after the library was qualified, the NovaSeq6000 (Illumina, San Diego, CA, USA) sequencing platform was used for on-board sequencing.

Amplicon data processing and analysis

The sample data was split from the off-machine data, and the barcode and primer sequences were truncated. FLASH (V1.2.11, http://ccb.jhu.edu/software/FLASH/) (Magoc and Salzberg, 2011) software was used to splicing the reads of the sample to obtain Raw Tags. Then, using fastp(0.19.6) (Chen et al., 2018) software to conduct quality control on the obtained Raw Tags to obtain high-quality Clean Tags. Finally, the Clean Tags are compared with the database to detect and remove chimeras (Haas et al., 2011), so as to obtain the final effective data, namely Effective Tags. Using the DADA2 module (Callahan et al., 2016) in the QIIME2 (version 2020.2) (Bolyen et al., 2019) software, and filtering out sequences with an abundance of less than 5, the final ASVs (Amplicon Sequence Variants) and features sheet were obtained. Subsequently, the obtained ASVs were compared with the database using the classify-sklearn module in the QIIME2 software to obtain the species information of each ASV.

Data analysis

Statistical analysis was performed using SPSS 22.0 (IBM Corp., Armonk, NY, USA) and Prism 8 (GraphPad, San Diego, CA, USA). Results were expressed as frequencies and percentages for categorical variables, mean and standard deviation for continuous variables with a normal distribution. Categorical variables were analyzed using chi-square or Fisher's exact test. For comparison of continuous variables between two independent groups, an unpaired Student's-t test (normally distributed variables) could be used. Paired data were compared using paired Student's-t test (normally distributed variables). Two-tailed p-values < 0.05 were considered statistically significant.

Results

Clinical characteristics of patients undergoing WMT

WMT was completed in the First Affiliated Hospital of Guangdong Pharmaceutical University from December 2016 to May 2022. A total of 237 patients (42 in the MS group and 195 in the non-MS group) met the inclusion criteria. Among them, 121 (51.05%) were male, 116 (48.95%) were female, and the median age was 55 (41-63) years old. The analysis process is shown in Figure 1. Table 1 shows the first six disease characteristics of patients undergoing WMT, which are functional bowel disease (n=128, 54.01%, including irritable bowel syndrome, functional constipation), ulcerative colitis

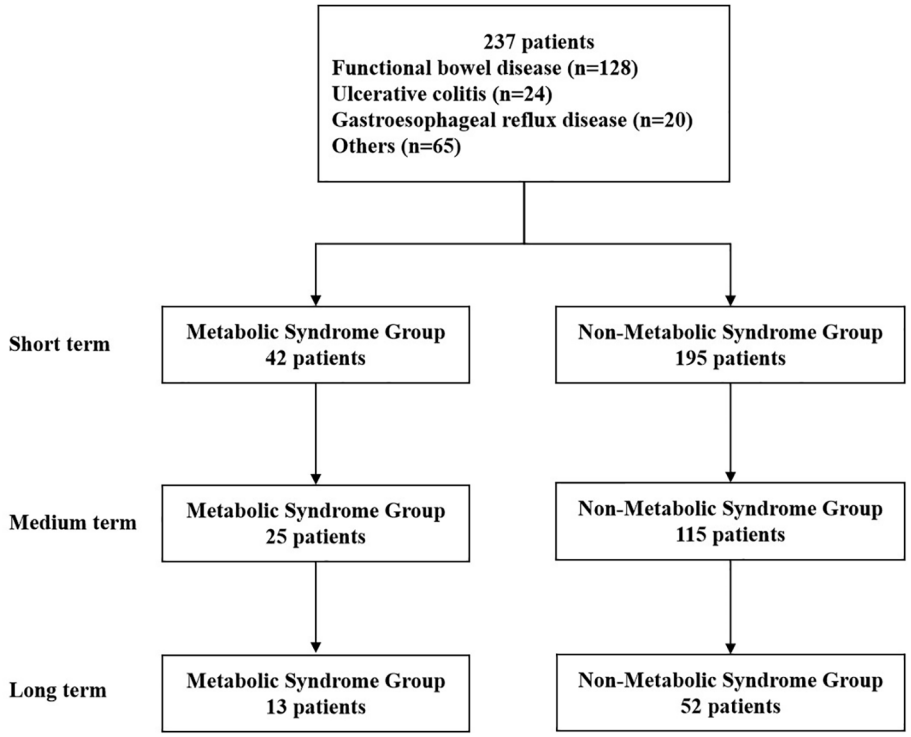


FIGURE 1
Flow chart of this study. Short term: about 1 month; medium term: about 2 months; long term: about 6 months.

(n=24, 10.13%), gastroesophageal reflux disease (n=20, 8.44%), non-alcoholic fatty liver disease (n=11, 4.64%), gouty arthritis (n=7, 2.95%), chemotherapy-related diarrhea (n=7, 2.95%). Due to the different compliance of patients, WMT treatment may not be completed on schedule. In this study, the time interval of WMT in the enrolled patients was counted, and the number of days was expressed as the median (25%-75%). The test results of the patient before the first course of treatment are the baseline value. The second course of treatment was separated by 35 days from baseline (32-42, short term). The third course of treatment was 77 days away from baseline (67-98.75, medium term). The fourth course of treatment was 185 days away from baseline (147.50-210, long term).

The demographic and clinical characteristics of the patients in the MS group and the non-MS group are compared in Table 2. Due to different compliance, not all patients have complete data, so the number of patients in each group is different for each index. There was no significant difference in age and gender ratio between the MS group and the non-MS group, indicating that the basic conditions of the study population were not significantly different, which reduced the confounding factors of this study. BMI (27.56 ± 4.69 vs 21.62 ± 3.41 kg/m^2, p<0.001), FBG (6.00 ± 1.97 vs 4.80 ± 1.07 mmol/L, p<0.001), HbA1c (6.80 ± 1.22 vs 5.84 ± 0.88%, p=0.007), FI (12.67 ± 8.22 vs 8.27 ± 5.57 μU/mL, p=0.011), HOMA-IR (3.44 ± 2.43 vs 1.86 ± 1.68, p=0.003), TC (5.43 ± 2.04 vs 4.69 ± 1.07 mmol/L, p=0.027), TG (3.85 ± 4.72 vs 1.06 ± 0.54 mmol/L, p<0.001), HDL-c (1.00 ± 0.27 vs 1.36 ± 0.31 mmol/L, p<0.001), ApoB (1.07 ± 0.31 vs 0.89 ± 0.24 g/L, p<0.001), non-HDL-c (4.41 ± 2.09 vs 3.33 ± 1.01 mmol/L, p=0.002), SBP (132.57 ± 11.66 vs 120.85 ± 13.94 mmHg, p<0.001), DBP (82.62 ± 10.72 vs 76.50 ± 9.59 mmHg, p<0.001), the above indexes in the MS group were significantly higher than those in the non-MS group except for HDL-c (mmol/L).

Evaluation of clinical comprehensive curative effect of WMT on metabolic syndrome

All enrolled patients were divided into MS group and non-MS group according to the evaluation criteria of MS. Patients were regrouped according to changes in MS symptoms after WMT treatment (Table 3). The comprehensive curative effect of the patients in the MS group changed significantly during the treatment. Short term recovery was 40.48% (p < 0.001), medium term recovery was 36.00% (p = 0.003), and long term recovery was 46.15% (p = 0.020). Our data suggest that WMT has short, medium, and long term significant metabolic syndrome-improving efficacy in patients with MS; however, the efficacy of WMT remains to be explored. There were very few patients in

10.3389/fcimb.2022.1044957

TABLE 1 The main diagnoses of patients receiving washed microbiota transplantation.

Primary cause of WMT	Number (n)	Percentage (%)
Functional bowel disease	128	54.01%
Ulcerative colitis	24	10.13%
Gastroesophageal reflux disease	20	8.44%
Nonalcoholic fatty liver	11	4.64%
Gouty arthritis	7	2.95%
Chemotherapy-Associated Diarrhea	7	2.95%
Atopic dermatitis	6	2.53%
Hyperlipidemia	5	2.11%
Post-hepatitis cirrhosis	5	2.11%
Radiation enteritis	5	2.11%
Crohn's disease	4	1.69%
Hyperlipidemic pancreatitis	2	0.84%
Senile tremor	1	0.42%
Duodenal stasis	1	0.42%
Chronic urticaria	1	0.42%
Parkinson's syndrome	1	0.42%
Bipolar disorder	1	0.42%
Psoriasis vulgaris	1	0.42%
Hyperuricemia	1	0.42%
Perianal eczema	1	0.42%
Autoimmune hepatitis	1	0.42%
Pustular psoriasis	1	0.42%
Neuromyelitis optica	1	0.42%
Eepression	1	0.42%
Functional dysphagia	1	0.42%
total	237	100.00%

TABLE 2 Demographics and clinical characteristics of patients at baseline.

	Metabolic syndrome group (42)	Non-metabolic syndrome group (195)	Donors (5)
Age (year)	54.98 ± 14.53 (n=42)	52.02 ± 15.81 (n=195)	22.80 ± 0.84 (n=5)
Male n (%)	54.76	50.26	80.00
BMI (kg/m^2)	27.56 ± 4.69 (n=38)	21.62 ± 3.41 (n=190)	21.26 ± 1.23 (n=5)
FBG (mmol/L)	6.00 ± 1.97 (n=42)	4.80 ± 1.07 (n=193)	4.60 ± 0.24 (n=5)
HbA1c (%)	6.80 ± 1.22 (n=19)	5.84 ± 0.88 (n=21)	/
FI (μU/mL)	12.67 ± 8.22 (n=28)	8.27 ± 5.57 (n=105)	/
HOMA-IR	3.44 ± 2.43 (n=28)	1.86 ± 1.68 (n=102)	/
TC (mmol/L)	5.43 ± 2.04 (n=42)	4.69 ± 1.07 (n=167)	0.79 ± 0.36 (n=5)
TG (mmol/L)	3.85 ± 4.72 (n=42)	1.06 ± 0.54 (n=167)	2.32 ± 1.21 (n=5)
LDL-c (mmol/L)	2.77 ± 0.98 (n=42)	2.85 ± 0.96 (n=167)	1.25 ± 0.36 (n=5)
HDL-c (mmol/L)	1.00 ± 0.27 (n=42)	1.36 ± 0.31 (n=167)	0.79 ± 0.36 (n=5)
ApoB (g/L)	1.07 ± 0.31 (n=42)	0.89 ± 0.24 (n=167)	/
non-HDL-c (mmol/L)	4.41 ± 2.09 (n=42)	3.33 ± 1.01 (n=167)	2.90 ± 0.29 (n=5)
LIP (mmol/L)	118.16 ± 158.69 (n=19)	139.13 ± 138.18 (n=50)	/
SBP (mmHg)	132.57 ± 11.66 (n=42)	120.85 ± 13.94 (n=195)	122.40 ± 10.64 (n=5)
DBP (mmHg)	82.62 ± 10.72 (n=42)	76.50 ± 9.59 (n=195)	74.00 ± 7.97 (n=5)

Data presented as mean ± standard deviation, or n (%).
BMI (kg/m^2), Body mass index; FBG (mmol/L), Fasting blood glucose; HbA1c (%), Glycated hemoglobin; FI (μU/mL), Fasting insulin; HOMA-IR, Homeostasis model assessment of insulin resistance; TC (mmol/L), Total cholesterol; TG (mmol/L), Triglyceride; LDL-c (mmol/L), Low-density lipoprotein cholesterol; HDL-c (mmol/L), High-density lipoprotein cholesterol; ApoB (g/L), Apolipoprotein B; non-HDL-c (mmol/L), Non-HDL cholesterol; LIP (mmol/L), Lipoprotein; SBP (mmHg), Systolic blood pressure; DBP (mmHg), Diastolic blood pressure.

TABLE 3 Comprehensive clinical efficacy of short, medium and long term treatment on MS.

Data periods	Before therapy (n)	Therapeutic effect base on diagnostic level of MS			
		Unchangedgroup (n)	Changedgroup (n, %)	X^2	p-Value
MS group					
Short term	42	25	17 (40.48%)	21.313	<0.001
Medium term	25	16	9 (36.00%)	8.672	0.003
Long term	13	7	6 (46.15%)	5.417	0.020
Non-MS group					
Short term	195	191	4 (2.05%)	2.273	0.132
Medium term	115	110	5 (4.35%)	3.271	0.071
Long term	52	50	2 (3.85%)	0.510	0.475

The definition of unchanged and changed of MS group were still MS group and changed to Non-MS group.
The definition of unchanged and changed of Non-MS group were still Non-MS and changed to MS group.

the non-MS group who increased during WMT treatment, but there is no statistical difference, which may be caused by changes in living habits and other factors during the treatment process for as short as one month or as long as six months. Our data show that WMT has a significant overall improvement in MS.

Efficacy evaluation of WMT in the treatment of ASCVD risk

According to ASCVD risk stratification, patients were divided into very high risk group, high risk group, intermediate risk group and low risk group. After WMT treatment, patients were regrouped into risk-modified and risk-modified groups (Table 4). Acute coronary syndrome, stable coronary heart disease, ischemic cardiomyopathy, ischemic stroke, transient ischemic attack, and peripheral atherosclerosis were included in the very high-risk group. This group of patients was not reassigned after WMT treatment and is not listed in Table 4.

In terms of ASCVD rating, in the high-risk group of patients with MS, WMT in the short term and medium term had a significant effect, with 25.0% (p=0.029) in the short term and 41.2% (p=0.011) in the medium-risk group. There was no statistically significant effect of short term, medium term and long term WMT on ASCVD rating in the medium-risk group. In conclusion, in terms of ASCVD rating, WMT has a significant short and medium term ASCVD downgrading effect in the high-risk group of patients with MS.

Comparative analysis of each index after WMT treatment and baseline

Previously, we observed that WMT had a significant improvement effect on MS as a whole, and then we further analyzed the specific indicators. Table 5 and Figure 2 showed the effects of WMT on BMI, blood glucose, blood lipids, and blood pressure in patients with MS. The results showed that in the MS group, WMT had a significantly lower effect ($p < 0.05$) on BMI in the short term (from 27.56 ± 4.69 to 26.95 ± 4.47kg/m^2, $p = 0.031$) and in the medium term (from 27.32 ± 3.56 to 26.46 ± 3.70kg/m^2, $p = 0.048$)). And in the long term (from 28.19 ± 3.62 to 27.70 ± 4.24kg/m^2), it also showed a reducing effect, but because the

TABLE 4 Effects of short, medium, and long term treatment on atherosclerotic cardiovascular disease risk classification of patients with MS.

Data periods	Before therapy (n)	Therapeutic effect base on ASCVD risk stratification			
		Unchangedgroup (n)	Risk descended (n, %)	X^2	p-Value
High-risk group					
Short term	24	18	6 (25.0%)	4.762	0.029
Medium term	17	10	7 (41.2%)	6.476	0.011
Long term	8	4	4 (50.0%)	3.000	0.083
Medium-risk group					
Short term	10	6	4 (40.0%)	2.813	0.094
Medium term	7	3	4 (57.1%)	3.150	0.076
Long term	4	3	1 (25.0%)	0.000	1.000

The definition of Risk descended of high- and medium-risk groups were medium-, and low-risk after WMT procedures, respectively.

TABLE 5 The comparison values of each index in high MS group and non-MS group in the short term, medium term and long term with baseline during the treatment of washed microbiota transplantation.

Items	Baseline	Short term	p-Value	Baseline	Medium term	p-Value	Baseline	Long term	p-Value
MS group									
BMI (kg/m^2)	27.56 ± 4.69 (n=38)	26.95 ± 4.47 (n=38)	0.031	27.32 ± 3.56 (n=21)	26.46 ± 3.70 (n=21)	0.048	28.19 ± 3.62 (n=10)	27.70 ± 4.24 (n=10)	0.767
FBG (mmol/L)	6.03 ± 1.98 (n=38)	5.49 ± 1.34 (n=38)	0.023	6.21 ± 1.96 (n=22)	5.66 ± 1.28 (n=22)	0.048	6.24 ± 2.25 (n=8)	5.95 ± 1.89 (n=8)	0.163
HbA1c (%)	7.06 ± 0.78 (n=8)	7.05 ± 0.93 (n=8)	0.949	7.92 ± 0.97 (n=5)	7.50 ± 0.54 (n=5)	0.184	8.55 ± 1.34 (n=2)	7.65 ± 0.64 (n=2)	0.323
FI (μU/mL)	13.83 ± 7.73 (n=20)	14.14 ± 6.37 (n=20)	0.863	12.51 ± 6.40 (n=10)	13.55 ± 6.89 (n=10)	0.662	13.94 ± 6.41 (n=8)	15.28 ± 6.42 (n=8)	0.529
HOMA-IR	3.72 ± 2.35 (n=20)	3.70 ± 2.11 (n=20)	0.971	3.74 ± 1.81 (n=10)	4.08 ± 2.64 (n=10)	0.729	4.00 ± 2.01 (n=7)	4.06 ± 2.01 (n=7)	0.931
TC (mmol/L)	5.42 ± 2.08 (n=40)	4.91 ± 1.26 (n=40)	0.125	5.52 ± 1.59 (n=24)	4.71 ± 1.02 (n=24)	0.022	5.77 ± 1.62 (n=11)	5.47 ± 1.67 (n=11)	0.235
TG (mmol/L)	3.90 ± 4.84 (n=40)	2.62 ± 2.19 (n=40)	0.030	4.10 ± 4.34 (n=24)	3.35 ± 3.11 (n=24)	0.410	3.89 ± 3.15 (n=11)	4.18 ± 5.32 (n=11)	0.820
LDL-c (mmol/L)	2.75 ± 1.00 (n=40)	2.71 ± 1.09 (n=40)	0.836	2.85 ± 1.04 (n=24)	2.36 ± 0.98 (n=24)	0.043	3.01 ± 1.11 (n=11)	2.63 ± 0.94 (n=11)	0.328
HDL-c (mmol/L)	0.99 ± 0.27 (n=40)	1.07 ± 0.29 (n=40)	0.036	1.00 ± 0.33 (n=24)	1.03 ± 0.20 (n=24)	0.608	1.08 ± 0.29 (n=11)	1.05 ± 0.24 (n=11)	0.253
ApoB (g/L)	1.07 ± 0.31 (n=40)	1.02 ± 0.26 (n=40)	0.369	1.13 ± 0.31 (n=24)	0.99 ± 0.25 (n=24)	0.060	1.19 ± 0.35 (n=11)	1.15 ± 0.20 (n=11)	0.627
non-HDL-c (mmol/L)	4.40 ± 2.15 (n=40)	3.84 ± 1.18 (n=40)	0.085	4.46 ± 1.63 (n=24)	3.68 ± 1.05 (n=24)	0.024	4.58 ± 1.50 (n=11)	4.43 ± 1.80 (n=11)	0.591
LIP (mmol/L)	139.67 ± 178.64 (n=14)	153.49 ± 204.34 (n=14)	0.254	69.02 ± 118.45 (n=6)	71.05 ± 105.31 (n=6)	0.807	/	/	/
SBP (mmHg)	132.57 ± 11.66 (n=42)	127.64 ± 10.20 (n=42)	0.026	131.72 ± 13.51 (n=25)	126.40 ± 9.12 (n=25)	0.070	127.08 ± 9.50 (n=12)	125.17 ± 7.22 (n=12)	0.628
DBP (mmHg)	82.62 ± 10.72 (n=42)	78.43 ± 9.13 (n=42)	0.051	81.76 ± 11.18 (n=25)	79.40 ± 7.44 (n=25)	0.323	81.00 ± 9.20 (n=12)	83.08 ± 8.59 (n=12)	0.536
Non-MS group									
BMI (kg/m^2)	21.62 ± 3.42 (n=187)	21.50 ± 3.32 (n=187)	0.279	21.53 ± 3.47 (n=112)	21.30 ± 3.36 (n=112)	0.206	21.69 ± 3.90 (n=51)	21.76 ± 3.66 (n=51)	0.776
FBG (mmol/L)	4.80 ± 1.10 (n=170)	4.72 ± 1.62 (n=170)	0.405	4.77 ± 1.11 (n=98)	4.64 ± 0.90 (n=98)	0.207	4.63 ± 0.59 (n=47)	4.66 ± 0.79 (n=47)	0.775
HbA1c (%)	5.43 ± 1.37 (n=4)	5.45 ± 1.10 (n=4)	0.895	/	/	/	/	/	/
FI (μU/mL)	8.71 ± 6.33 (n=61)	8.53 ± 5.17 (n=61)	0.728	9.78 ± 7.52 (n=32)	10.39 ± 7.56 (n=32)	0.489	10.66 ± 5.67 (n=16)	9.53 ± 4.74 (n=16)	0.299
HOMA-IR	2.02 ± 2.01 (n=60)	1.95 ± 1.64 (n=60)	0.651	2.27 ± 2.50 (n=32)	2.36 ± 2.14 (n=32)	0.699	2.19 ± 1.33 (n=16)	1.99 ± 1.13 (n=16)	0.377
TC (mmol/L)	4.76 ± 1.12 (n=116)	4.67 ± 1.07 (n=116)	0.225	4.72 ± 1.09 (n=70)	4.60 ± 0.90 (n=70)	0.277	4.92 ± 1.21 (n=31)	4.62 ± 1.07 (n=31)	0.051
TG (mmol/L)	1.15 ± 0.59 (n=116)	1.09 ± 0.54 (n=116)	0.129	1.08 ± 0.49 (n=70)	1.03 ± 0.48 (n=70)	0.405	1.12 ± 0.47 (n=31)	1.00 ± 0.43 (n=31)	0.164
LDL-c (mmol/L)	2.90 ± 0.99 (n=116)	2.84 ± 0.96 (n=116)	0.370	2.84 ± 0.94 (n=70)	2.76 ± 0.79 (n=70)	0.352	3.03 ± 1.04 (n=31)	2.78 ± 0.92 (n=31)	0.080
HDL-c (mmol/L)	1.34 ± 0.30 (n=116)	1.33 ± 0.33 (n=116)	0.623	1.38 ± 0.32 (n=70)	1.37 ± 0.28 (n=70)	0.692	1.38 ± 0.31 (n=31)	1.38 ± 0.29 (n=31)	0.982
ApoB (g/L)	0.90 ± 0.25 (n=116)	0.90 ± 0.26 (n=116)	0.874	0.89 ± 0.23 (n=70)	0.89 ± 0.23 (n=70)	0.946	0.93 ± 0.22 (n=31)	0.93 ± 0.24 (n=31)	0.927
non-HDL-c (mmol/L)	3.42 ± 1.05 (n=116)	3.34 ± 1.03 (n=116)	0.255	3.34 ± 1.00 (n=70)	3.23 ± 0.84 (n=70)	0.271	3.54 ± 1.06 (n=31)	3.24 ± 0.94 (n=31)	0.033
LIP (mmol/L)	114.58 ± 93.83 (n=22)	122.48 ± 102.74 (n=22)	0.243	119.23 ± 88.80 (n=12)	124.18 ± 112.83 (n=12)	0.732	87.13 ± 49.56 (n=3)	75.30 ± 56.66 (n=3)	0.442
SBP (mmHg)	120.85 ± 13.94 (n=195)	119.44 ± 12.2 (n=195)	0.164	120.75 ± 13.42 (n=115)	119.20 ± 12.57 (n=115)	0.306	120.57 ± 12.73 (n=51)	119.47 ± 12.03 (n=51)	0.597
DBP (mmHg)	76.5 ± 9.59 (n=195)	75.97 ± 9.05 (n=195)	0.500	76.21 ± 9.54 (n=115)	74.37 ± 8.26 (n=115)	0.082	75.69 ± 8.79 (n=51)	75.96 ± 7.68 (n=51)	0.841

Data presented as mean ± standard deviation, or n (%).

10.3389/fcimb.2022.1044957

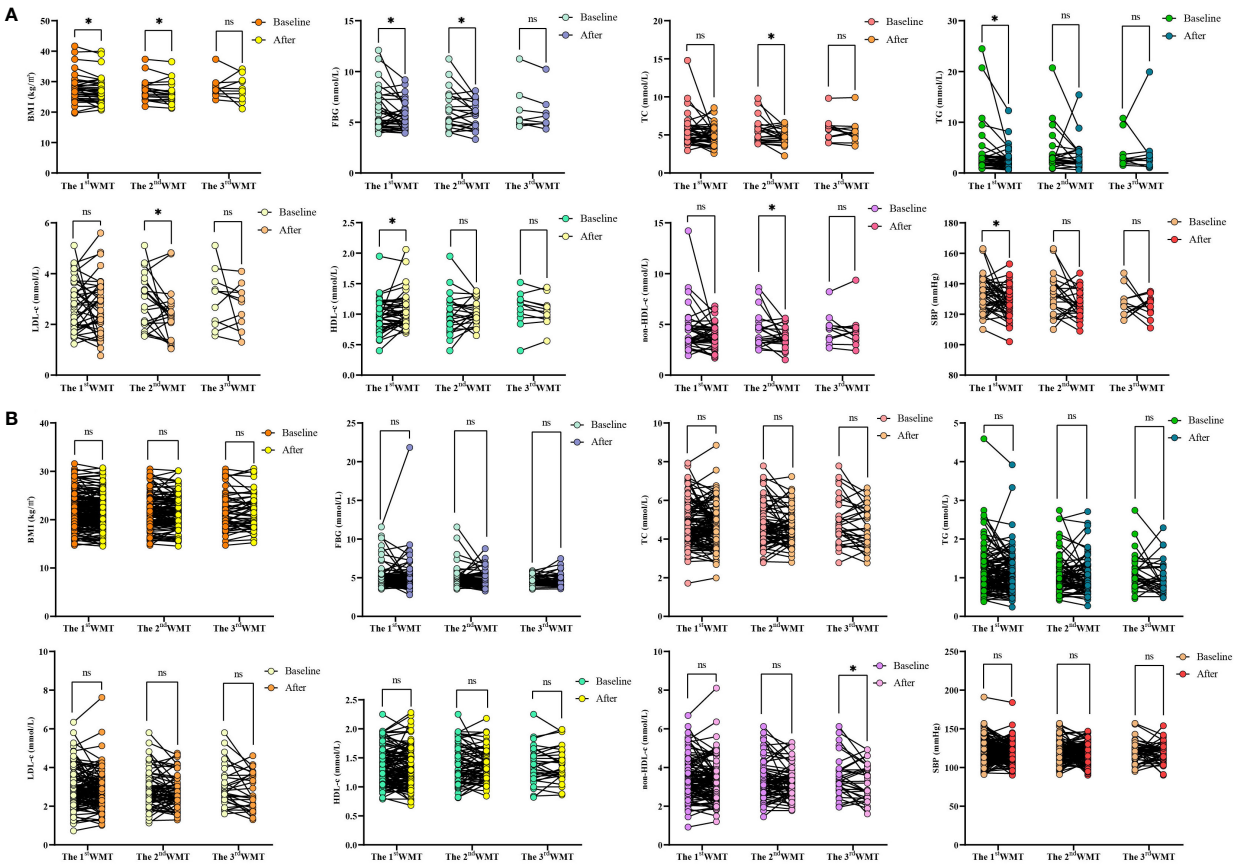


FIGURE 2
Changes of BMI、FBG、TC、TG、LDL-c、HDL-c、non-HDL-c and SBP levels after 1-3 times of WMT. **(A)** Changes of BMI、FBG、TC、TG、LDL-c、HDL-c、non-HDL-c and SBP in MS group; **(B)** Changes of BMI、FBG、TC、TG、LDL-c、HDL-c、non-HDL-c and SBP in non-MS group. BMI, Body mass index; FBG, Fasting blood glucose; TC, Total cholesterol; TG, Triglyceride; LDL-c, Low-density lipoprotein cholesterol; HDL-c, High-density lipoprotein cholesterol; non-HDL-c, Non-HDL cholesterol; SBP, Systolic blood pressure. * indicates $p < 0.05$; ns, not significant. Short term: about 1 month; medium term: about 2 months; long term: about 6 months.

number of people was too small, it was not significant in the long term ($p = 0.767$). This suggests that WMT has a good BMI-improving effect in patients with MS. FBG had a significantly lower effect ($p < 0.05$) in the short term (from 6.03 ± 1.98 to 5.49 ± 1.34 mmol/L, $p = 0.023$) and in the medium term (from 6.21 ± 1.96 to 5.66 ± 1.28 mmol/L, $p = 0.048$). And in the long term (from 6.24 ± 2.25 to 5.95 ± 1.89mmol/L), it also showed a reducing effect, but because the number of people was too small, it was not significant in the long-term ($p = 0.163$). This suggests that WMT has a good blood glucose-improving effect in patients with MS. Meanwhile, in the MS group, WMT significantly reduced TC (from 5.52 ± 1.59 to 4.71 ± 1.02mmol/L, $p = 0.022$) in the medium term ($p < 0.05$). TG (from 3.90 ± 4.84 to 2.62 ± 2.19mmol/L, $p = 0.030$) had a significant short term reduction ($p < 0.05$). LDL-c (from 2.85 ± 1.04 to 2.36 ± 0.98mmol/L, $p = 0.043$) was significantly lower in the medium term ($p < 0.05$). HDL-c (from 0.99 ± 0.27 to 1.07 ± 0.29mmol/L, $p = 0.036$) had a significant increase in the medium term ($p < 0.05$). non-HDL-c (from 4.46 ± 1.63 to 3.68 ± 1.05mmol/L, $p = 0.024$) had a significant lowering effect in the mid-term ($p < 0.05$). Overall, WMT significantly improved blood lipids in the MS group. There was a significant short term reduction ($p < 0.05$) on SBP (from 132.57 ± 11.66 to 127.64 ± 10.20mmHg, $p = 0.026$), indicating that WMT has a significant antihypertensive effect on blood pressure in patients with MS. In the non-MS group, WMT had a significant long term reduction ($p < 0.05$) on non-HDL-c (from 3.54 ± 1.06 to 3.24 ± 0.94mmol/L, $p = 0.033$), however, there was no significant change in FBG, TC, TG, LDL-c, HDL-c in the short term, medium term and long term, that is, WMT had no significant change in the non-MS group.

Correlation analysis of WMT on indicators affecting MS

We previously found that WMT significantly improved BMI, FBG, TC, TG, LDL-c, HDL-c, non-HDL-c and SBP in

the MS group during treatment. In order to find the relevant factors affecting the regulation of WMT on MS, correlation analysis was performed on the above-mentioned indicators with significant regulating effect. As shown in Figure 3, we found that in the MS group, TC was strongly positively correlated with FBG, non-HDL-c, and BMI. FBG was strongly positively correlated with TG and non-HDL-c. There was a strong positive correlation between non-HDL-c and TG and BMI. Our data show that in the process of WMT treatment, while improving BMI, blood glucose and blood lipids are also affected by a good improvement, and BMI, blood glucose, and blood lipids have a strong correlation. This provides us with a good therapeutic idea for the treatment of MS. That is to say, WMT has a significant effect on the treatment MS. It can also play a role in weight loss and lipid lowering while reducing blood glucose, and at the same time, it plays a comprehensive role in these three aspects.

Analysis of the composition of gut microbiota before and after WMT

We analyzed the gut microbiota composition of the MS group, the non-MS group, and donors before and after WMT. The gut microbiota at the phylum level mainly includes *Firmicutes, Bacteroidota, Fusobacteriota, Actinobacteriota and Proteobacteria.* For the MS group, at the phylum level, WMT increased the relative abundance of *Firmicutes* and *Actinobacteriota*; decreased the relative abundance of *Fusobacteriota* and *Proteobacteria* (Figure 4A). At the class level, the relative abundances of

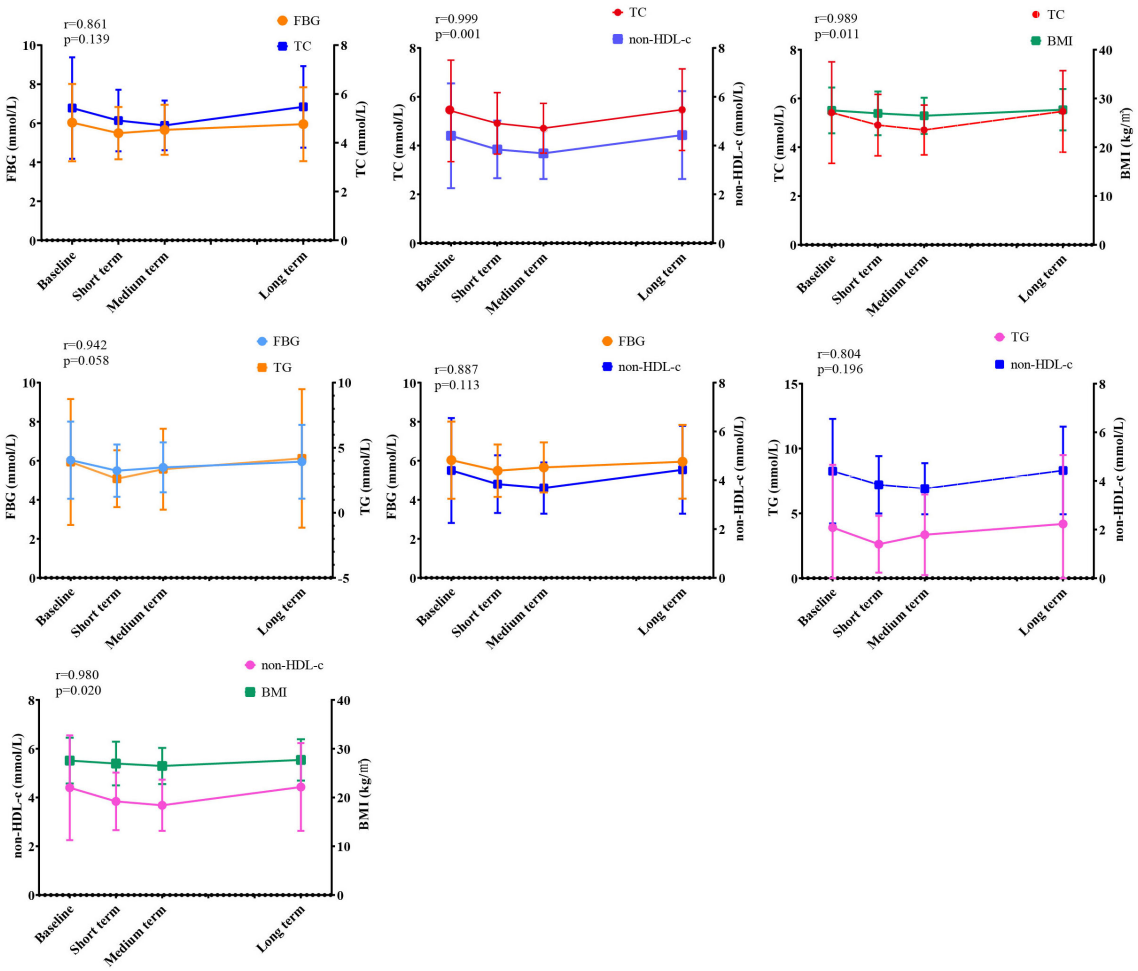


FIGURE 3
Correlation analysis of WMT on MS regulation. BMI, Body mass index; TC, Total cholesterol; FBG, Fasting blood glucose; non-HDL-c, Non-HDL cholesterol; TG, Triglyceride. ($r \leq 0.3$, indicating poor correlation; $0.3 < r \leq 0.6$, indicating moderately strong correlation; $0.6 < r \leq 0.8$, indicating strong correlation; $r > 0.8$, indicating extremely strong correlation). Short term: about 1 month; medium term: about 2 months; long term: about 6 months.

10.3389/fcimb.2022.1044957

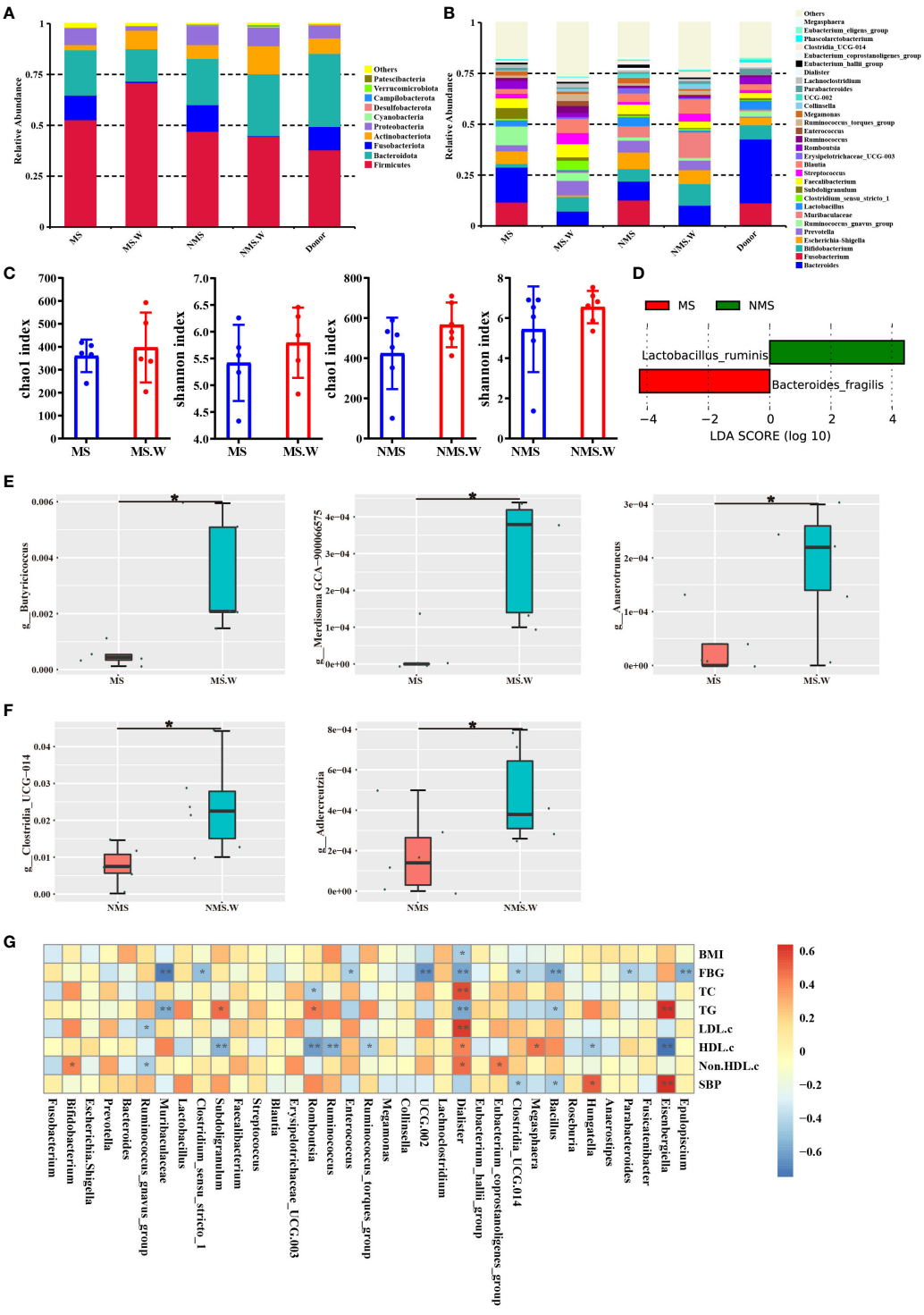


FIGURE 4
The composition of gut microbiota before and after WMT. **(A)** Composition of the top ten gut microbiota at the phylum level. **(B)** Composition of the top 30 gut microbiota at the genus level. **(C)** Chao1 index and Shannon index of alpha diversity analysis. **(D)** LEfSe analysis of MS group and non-MS group. **(E)** T-test analysis of MS group before and after WMT. **(F)** T-test analysis of non-MS group before and after WMT. **(G)** Mutual variation relationship between environmental factors and species. MS: In MS group before WMT. MS.W: In MS group after WMT. NMS: In non-MS group before WMT. NMS.W: In non-MS group after WMT. BMI, Body mass index; FBG, Fasting blood glucose; TC, Total cholesterol; TG, Triglyceride; LDL-c, Low-density lipoprotein cholesterol; HDL-c, High-density lipoprotein cholesterol; non-HDL-c, Non-HDL cholesterol; SBP, Systolic blood pressure. * indicates $p < 0.05$; ** indicates $p < 0.01$. Short term: about 1 month; medium term: about 2 months; long term: about 6 months.

Clostridia, Bacilli, Actinobacteria, and *Coriobacteriia* were increased after WMT; the relative abundances of *Fusobacteriia, Bacteroidia, Gammaproteobacteria*, and *Negativicutes* were decreased after WMT (Supplementary Figure 1A). At the order level, the relative abundances of *Lachnospirales, Bifidobacteriales, Oscillospirales, Lactobacillales, Clostridiales* and *Erysipelotrichales* were increased after WMT; the relative abundances of *Fusobacteriales, Bacteroidales, Enterobacterales* and *Peptostreptococcales Tissierellales* were decreased after WMT (Supplementary Figure 1B). At the family level, the relative abundances of *Lachnospiraceae, Bifidobacteriaceae, Prevotellaceae, Ruminococcaceae* and *Clostridiaceae* were increased after WMT; the relative abundances of *Fusobacteriaceae, Bacteroidaceae, Enterobacteriaceae*, etc. were decreased after WMT (Supplementary Figure 1C). At the genus level, the relative abundances of *Bifidobacterium, Prevotella, Muribaculaceae, Clostridium, Faecalibacterium, Streptococcus*, and *Blautia* were increased after WMT; the relative abundances of *Fusobacterium, Bacteroides, Escherichia-Shigella, Ruminococcus*, etc. were decreased after WMT (Figure 4B). Among them, WMT can not only increase the relative abundance of beneficial bacteria, such as *Bifidobacterium*, *Prevotella*, *Faecalibacterium*, etc. In addition, it can reduce the relative abundance of harmful bacteria, such as poisonous *Fusobacterium*, poisonous *Bacteroides*, *Escherichia-Shigella* and so on. WMT could increase the α-diversity of gut microbiota in both the MS group and the non-MS group, with an increased chao1 index indicating an increase in the total number of community species, and an increased shannon index indicating an increase in community species diversity (Figure 4C). We performed LEfSe analysis on the MS group and the non-MS group in order to find the Biomarker with statistical difference between the two groups. Finally, it was found that the differential species in the MS group was *Bacteroides fragilis*, and the differential species in the non-MS group was *Lactobacillus ruminis* (Figure 4D). Species with significant differences between the MS groups before and after WMT were identified by T-test. We found that WMT significantly increased the relative abundances of *Butyricicoccus*, *Merdisoma*, and *Anaerotruncus* at the genus level of gut microbiota in the MS group compared with baseline (Figure 4E). Meanwhile, WMT significantly increased the relative abundance of *Clostridia* and *Adlercreutzia* at the genus level of the gut microbiota in the non-MS group (Figure 4F). We used Spearman rank correlation to study the mutual change relationship between environmental factors and species, and obtained the correlation and significant P value between the two groups. We found a significant negative correlation between BMI and *Dialister*. FBG was significantly negatively correlated with *Epulopiscium, Parabacteroides, Bacillus, Clostridia, Dialister, Enterococcus, Clostridium* and *Muribaculaceae*. TC was significantly negatively correlated with *Romboutsia*. TG was significantly negatively correlated with *Bacillus*, *Dialister* and *Muribaculaceae*. LDL.c was significantly negatively correlated with *Ruminococcus*. HDL.c was significantly positively correlated with *Megasphaera* and *Eubacterium*. Non.HDL.c was significantly negatively correlated with *Ruminococcus*. SBP was significantly negatively correlated with *Bacillus* and *Clostridia* (Figure 4G).

Prevalence of AEs in WMT patients

We also analyzed the prevalence of AEs in patients receiving WMT. WMT-related AEs were determined on the basis of clinical judgment and all available information (primarily diarrhea, abdominal pain, nausea, vomiting, generalized joint pain, fatigue, convulsions, rash, fever, and dizziness). A total of 679 WMT procedures were analyzed, and the overall incidence of AEs was 2.50%. Diarrhea was the most common AE (7 cases, 1.03%), followed by abdominal pain (2 cases, 0.29%), nausea and vomiting (2 cases, 0.29%), generalized joint pain (1 case, 0.15%), fatigue (1 case, 0.15%), convulsions (1 case, 0.15%), rash (1 case, 0.15%), fever (1 case, 0.15%) and dizziness (1 case, 0.15%). The reality is that these effects quickly go away on their own and pose no greater threat to the patient's health.

Discussion

WMT has a significant improvement effect in patients with MS and a significant downgrade effect on ASCVD risk. In terms of comprehensive efficacy, WMT has significantly improved curative effect in short, medium and long term in patients with MS. In terms of ASCVD rating, WMT has a significant short and medium term ASCVD downgrading effect in the high-risk group of patients with MS. WMT can significantly improve blood glucose, blood lipids, blood pressure and BMI in patients with MS. Our data suggest that modulation of the gut microbiota by WMT may be a novel approach for the treatment of MS.

The gut microbial community is known to be critical for processing dietary polysaccharides, promoting the absorption of monosaccharides in the gut, and inducing hepatic lipogenesis. The gut microbiota also influenced the energy and energy stores that the host obtains from the diet (Turnbaugh and Gordon, 2009). Numerous experiments in animal models or humans showed that FMT played an important role in body weight regulation (Ridaura et al., 2013). In a FMT study of patients with MS, the recipient gut microbiota after transplantation was similar to that of the donor (Li et al., 2016). Donor-specific microorganisms *Roseburia hominis*, *Ruminococcus lactaris* and *A. muciniphila* were able to successfully colonize the recipient (Azad et al., 2018), the latter being associated with improved host glucose tolerance. These results suggested that improving gut dysbiosis with FMT may be an effective treatment for obesity. Specifically, SCFA-producing bacteria, such as *Roseburia gutis*, *Bryantella forexigens*, and *Megamonas hypermegale*, were significantly increased after FMT (Smits et al., 2018), which may help improve insulin sensitivity in patients with MS. Next Lai et al. found that transplantation of fecal microbiota from mice on a normal-fat diet into mice on a high-fat

diet significantly reduced appetitive efficacy, body weight, and metabolic profile in mice on a high-fat diet. The beneficial effects of linking exercise to a normal diet could be transmitted through FMT, and FMT could improve inflammatory status and metabolism in obesity. The transmissible beneficial effects of FMT had been explained by overexpression of *Odoribacter* and oxidative phosphorylation and glycolysis genes (Lai et al., 2018). Consistently, our results showed a similar effect in patients with MS. That was, WMT had a significant short term and medium term improvement effect on BMI in patients with MS. This may be related to the increased abundance of beneficial bacterial species after transplantation. WMT could lead to an increase in the proportion of *Firmicutes* in the recipients as described in the text and in the Figure 4A. It seems reasonable to interpret that the MS group itself contained a high abundance of *Firmicutes*, while receiving *Firmicutes* from donors, resulting in a logical increase in the relative abundance of *Firmicutes*. Similarly, similar results were observed in the non-MS group. Of course, the increased of *Firmicutes* was not a direct addition of numbers. It was possible that the gut microbiota showed up in an interacted form after WMT. *A. muciniphila* was one such species commonly associated with anti-obesity traits. For example, in mouse and a few human studies, *A. muciniphila* significantly improved body composition and nutrient processing in obese subjects. *A. muciniphila* was underrepresented in the microbiome of a mouse model of type 2 diabetes (Okubo et al., 2018). The ratio of *Firmicutes* to *Bacteroidetes* was often used when correlating changes in microbiota composition with obesity phenotypes. *Firmicutes* had been documented to be more abundant than *Bacteroidetes* in obese subjects, whereas lean individuals had more *Bacteroidetes* and less *Firmicutes* (Furet et al., 2010; Louis et al., 2016).

Scientific reports showed that FMT could also improve plasma metabolic parameters in patients with MS. For example, Vrieze et al. found improvements in peripheral and hepatic insulin sensitivity six weeks after infusion of microbiota from lean donors into recipients with MS, and concluded that the gut microbiota could potentially be developed as a therapeutic agent to increase insulin sensitivity in humans (Vrieze et al., 2012). Next, Kootte et al. investigated the effects of lean donor (allogeneic) versus own (autologous) fecal microbiota transplantation in male recipients with MS. Similar to the team of Vrieze et al., at 6 weeks after FMT, the authors observed improved peripheral insulin sensitivity, elevated postprandial plasma triglycerides, and reduced glycated hemoglobin (HbA1c) levels in recipient plasma obtained from allogeneic donors (Kootte et al., 2017). A similarly interesting experiment was performed by Sung et al. The authors administered fecal microbiota to obese mice by oral gavage in their experiments from resveratrol-fed and normally fed groups of donor mice. Their findings showed improved glucose clearance in the group of mice that received fecal suspensions from resveratrol-fed donors compared to the group of normally fed donor mice (Sung et al., 2017). Consistently, our results showed a similar effect in patients with MS. It was shown that WMT had a significant improving effect on blood glucose in patients with MS. WMT had a significant effect on reducing fasting blood glucose in the short and medium term in patients with MS. These data leaded us to conclude that the delivery of beneficial microbiota or metabolites *via* FMT could have ameliorating effects on glycemia in patients with MS.

Hyperlipidemia (HLP) was considered to be an important risk factor for cardiovascular diseases (CVDs) (Roth et al., 2020), atherosclerosis (Panahi et al., 2018; Libby et al., 2019), diabetes (Sun et al., 2022). Evidence suggested that the gut microbiota played an important role in the regulation of energy metabolism and lipid levels in the host (Velagapudi et al., 2010; Mestdagh et al., 2012). A non-human primate model of hyperlipidemia (HLP) was established in cynomolgus monkeys fed a high-fat diet (HFD) for 19 months, according to a recent study. Transplantation of fecal microbiota from HFD-T (high-fat diet-tolerant) monkeys into HFD rats attenuated HLP and hepatic steatosis (Gao et al., 2022). Probiotics could protect the host from intestinal dysbiosis, thereby conferring health benefits to the host. *Lactobacillus* had been reported to have a lipid-lowering effect on hypercholesterolemic and hyperlipidemic rats or mice (Singh et al., 2015; Qian et al., 2019). *A muciniphila* was currently recommended as a new potential complementary therapy for clinical obesity and diabetes (Plovier et al., 2017; Cani and de Vos, 2017). *P. distasonis* had been shown to have metabolic benefits in reducing body weight, hyperglycemia, and hepatic steatosis in genetically obese (ob/ob) and high-fat diet (HFD)-fed mice (Wang et al., 2019). Furthermore, *Faecalibacterium prausnitzii* and its secreted peptides exhibited anti-inflammatory effects against chemically induced colitis in mice (Breyner et al., 2017). Consistently, our results showed a similar effect in patients with MS. It was shown that WMT had a significant improving effect on blood lipids in patients with MS. WMT could significantly reduce triglyceride and increase high-density lipoprotein in patients with MS in the short term. In the medium term, it could significantly reduce total cholesterol, low-density lipoprotein, and non-high-density lipoprotein. Therefore, it is promising to ameliorate such diseases and gut dysbiosis by targeting the modulation of gut microbiota using probiotics or FMT.

Several intervention studies had shown that blood pressure in animal models of hypertension can be altered by altering the gut microbiota. Yang et al. observed a significant decrease in microbial richness, diversity, and uniformity in spontaneously hypertensive rats, and an increase in the ratio of *Firmicutes*/*Bacteroidetes*. These changes were accompanied by a decrease in acetic and butyric acid-producing bacteria (Yang et al., 2015). Furthermore, the microbiota of a small group of human hypertensive patients was found to follow a similar pattern of dysbiosis. Yang et al. discovered a role for the brain-gut-kidney axis in maintaining normal homeostasis and dysregulation of

this axis in chronic kidney disease and hypertension may lead to new therapeutic targets (Yang et al., 2018). In addition, high fiber and SCFA-acetate supplementation had been reported to alter gut microbiota, increase the abundance of acetate-producing bacteria, and prevent hyperactivity in a mouse model of deoxycorticosterone acetate (DOCA)-induced hypertension. Blood pressure (Marques et al., 2017). Another meta-analysis on the antihypertensive effect of probiotics showed that multiple probiotics had a greater effect on blood pressure improvement than a single probiotic (Khalesi et al., 2014). As a multi-species gut microbiota transplant, FMT had significant antihypertensive effects in hypertensive animals (Toral et al., 2019). Consistently, our results showed similar effects in patients with MS. However, our study showed that WMT had a clinically significant short term blood pressure lowering effect, with a trend to lower blood pressure in the medium and long term, but the effect was not significant. Although the significant antihypertensive effect of WMT was short term, it also had a trend of antihypertensive in the medium and long term, and the effect of WMT was generally longer than that of traditional antihypertensive drugs. Further studies are needed to explore how to prolong the antihypertensive effect of WMT. Several clinical studies had found that the gut microbiota of hypertensive patients differs significantly from that of healthy controls, characterized by loss of microbial diversity, loss of beneficial bacteria, and increase in potentially harmful bacteria (Yan et al., 2017; Li et al., 2017). Our 16S rRNA sequencing data suggested that WMT may restore microbial diversity in patients with MS and modulate their microbial composition, similar to that observed in healthy controls. In addition, research by Zhong et al. showed that WMT has antihypertensive effect on hypertensive patients (Zhong et al., 2021). Zhong et al. found that genus-level relative abundance of gut microbiota in hypertensive patients after WMT significantly changed compared with baseline, including increased abundance of *Senegalimassilia* and decreased abundance of *Parasutterella* and *Solobacterium*. Adamberg et al. showed that higher abundance of *Senegalimassilia* was associated with healthy traits. They found that children without obesity had higher levels of *Senegalimassilia anaerobia* compared to overweight children (Adamberg et al., 2018). In addition, diabetic rats treated with formulations that could significantly improve hyperglycemia had also been shown to have a high abundance of *Senegalimassilia* (Gao et al., 2018). In addition, the abundance of *Parasutterella* (increased in hypertensive patients) (Mushtaq et al., 2019) and *Solobacterium* (associated with atherosclerotic cardiovascular disease) (Tierney et al., 2021) were significantly increased after WMT reduce. Consistently, our results showed a similar effect in patients with MS. That was, WMT could significantly improve blood pressure in patients with MS. Therefore, WMT may improve blood pressure in patients with MS by restoring gut microbiota homeostasis.

MS combines multiple symptoms, such as obesity, dyslipidemia, hyperglycemia, and hypertension, which significantly increase the risk, progression rate, and harm of diabetes and cardiovascular disease. Therefore, a scientific and reasonable treatment strategy for MS should be based on the control of blood glucose, blood lipids, blood pressure, and body weight for comprehensive treatment. In our study, WMT significantly improved blood glucose, blood lipids, blood pressure and BMI in patients with MS and had a significant downgrading effect on ASCVD risk. We speculate that the improvement of MS after WMT is due to the improvement of gut microbiota after WMT, which comprehensively regulates blood glucose, blood lipids, blood pressure, and BMI. Although there were several studies on WMT function, such as Liang et al. showed that WMT treatment could alter blood lipids in patients with hyperlipidemia and hypolipidemia without serious adverse events (Liang et al., 2022). Wu et al. showed that WMT can significantly improve blood glucose in patients with high blood glucose (Wu et al., 2022a). Pan et al. showed that WMT significantly improved children with autism spectrum disorder, gastrointestinal symptoms and sleep disturbance, and reduced systemic inflammation (Pan et al., 2022). The mechanism by which FMT contributes to disease remission remains largely unexplained. MS may be alleviated by the synergistic effect of gut commensal microbiota after FMT treatment.

Future research should focus on the bacterial species and functional changes associated with FMT treatment in patients with MS, and how FMT affects the metabolism of other organs in long term improvement. Due to the complexity of the gut microbiota, further studies should explore whether specific microbial community species or communities in FMT are dedicated to the prevention and treatment of MS. This may provide a new perspective and reference. Many factors influenced the outcome of FMT, namely donor selection and preparation, sample handling, mode of administration, and colonization resistance (Leshem et al., 2019). Perhaps the healthy donors from the World Longevity Township in South China can provide a better source of donors for patients in South China (Wu et al., 2021a). FMT-related AEs were a challenge for FMT applications. In most cases, mild gastrointestinal AEs were well tolerated by FMT (Wang et al., 2016). Zhang et al. demonstrated the preparation of washed microflora by repeated centrifugation plus suspension three times on the basis of an automated purification system, which significantly reduced AEs (Zhang et al., 2020). Our WMT project was based on Zhang's standards. No serious AEs were identified during and after WMT. The current understanding of the effects of WMT on the gut microbiota on metabolic diseases is still in its infancy, and data on the effects of WMT on MS are lacking. This is a large-scale retrospective trial of MS in southern China, including a MS group and a non-MS group. The clinical evidence of the effect of WMT on MS has been established, laying a foundation

for the follow-up study of the effects of gut microbiota (Wu et al., 2021b) and metabolic markers (Wu et al., 2022b) on metabolic abnormalities. Taken together, these results suggest that restoring gut microbiota may serve as a promising treatment for MS; however, the mechanism of action requires further investigation.

This study had several limitations. First, this study focused on the analysis of clinical BMI, blood glucose metabolism, lipid metabolism, and blood pressure, as well as gut microbiota amplicons before and after WMT. Given that this was a retrospective study, stool samples from patients with MS were rarely collected. WMT could increase the α-diversity of gut microbiota in both the MS group and the non-MS group, with an increased chao1 index indicating an increase in the total number of community species, and an increased shannon index indicating an increase in community species diversity (Figure 4C). An important reason for the lack of significant differences here was that our sample size was too small. Because our study was a retrospective study, a large number of samples were not collected before, so the current number of samples was too small to have a significant difference. Our situation was similar to that of Zhong et al., (2021). However, the trend of our results fully agreed with the theoretical results on WMT can reshape the intestinal microecology. Gut microbiota metagenomics and metabolomics before and after WMT had not been evaluated. Therefore, the mechanism of action of WMT in improving ms had not been elucidated. Second, the number of patients and the impact of compliance. The overall number of patients with MS was relatively small, and a small number of patients did not receive long term treatment after short term treatment, and returned to the hospital at short or long intervals to evaluate the long term benefit of WMT treatment. Therefore, more samples and data were needed to confirm the long term efficacy of WMT in the treatment of MS. Third, we did not consider potential confounding factors between the main symptoms of WMT treatment and MS. Based on current limitations, although data showed that WMT could improve MS, the findings of WMT-improving MS should be interpreted with caution, and we need large-scale prospective studies to further validate our conclusions. In the future, we plan to conduct a large-sample prospective study to verify the effect of WMT on MS.

Conclusions

WMT had a significant improvement in patients with MS and a significant short and medium term downgrading effect on ASCVD risk in the high-risk group of patients with MS. WMT could restore gut microbiota homeostasis in patients with MS. Therefore, the regulation of gut microbiota by WMT may provide a new clinical approach for the treatment of MS.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, PRJNA881922.

Ethics statement

The studies involving human participants were reviewed and approved by This study was conducted and approved by the Ethics Committee (No. 2017-98) in accordance with the Declaration of Helsinki at the First Affiliated Hospital of Guangdong Pharmaceutical University, Guangzhou, China. The patients/participants provided their written informed consent to participate in this study.

Author contributions

X-XH, Q-PW, and LW designed the concept of the study. X-JL, D-JL, W-JC, X-YX, Y-TX, M-QL, and J-TX collected and analyzed the data. TL and W-YL were the statistics consultant, QZ was the consultant for endocrinology. LW wrote the draft manuscript. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by the Key-Area Research and Development Program of Guangdong Province (No. 2022B1111070006), the Medical Scientific Research Foundation of Guangdong Province (No. B2022209), the Scientific Research Projects of Guangdong Bureau of Traditional Chinese Medicine (No. 20221232), and the Guangdong Innovation Research Team for Higher Education (No. 2021KCXTD025).

Acknowledgments

We sincerely thank all patients in the study and all funding agencies that supported the study.

Conflict of interest

Author X-YX was employed by Xiamen Treatgut Biotechnology Co., Ltd., Xiamen, China.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2022.1044957/full#supplementary-material

SUPPLEMENTARY FIGURE 1
The composition of gut microbiota before and after WMT at the class, order and family level. **(A)** Composition of the top ten gut microbiota at the class level. **(B)** Composition of the top ten gut microbiota at the order level. **(C)** Composition of the top ten gut microbiota at the family level.

References

Adamberg, K., Adamberg, S., Emits, K., Larionova, A., Voor, T., Jaagura, M., et al. (2018). Composition and metabolism of fecal microbiota from normal and overweight children are differentially affected by melibiose, raffinose and raffinose-derived fructans. *Anaerobe* 52, 100–110. doi: 10.1016/j.anaerobe.2018.06.009

Allegretti, J. R., Kassam, Z., Mullish, B. H., Chiang, A., Carrellas, M., Hurtado, J., et al. (2020). Effects of fecal microbiota transplantation with oral capsules in obese patients. *Clin. Gastroenterol. Hepatol.* 18 (4), 855–863. doi: 10.1016/j.cgh.2019.07.006

Azad, M. B., Vehling, L., Chan, D., Klopp, A., Nickel, N. C., McGavock, J. M., et al. (2018). Infant feeding and weight gain: Separating breast milk from breastfeeding and formula from food. *Pediatrics* 142 (4), e20182297. doi: 10.1542/peds.2018-1092

Azizi, M., Sapoval, M., Gosse, P., Monge, M., Bobrie, G., Delsart, P., et al. (2015). Optimum and stepped care standardised antihypertensive treatment with or without renal denervation for resistant hypertension (DENERHTN): a multicentre, open-label, randomised controlled trial. *Lancet* 385 (9981), 1957–1965. doi: 10.1016/S0140-6736(14)61942-5

Bafeta, A., Yavchitz, A., Riveros, C., Batista, R., and Ravaud, P. (2017). Methods and reporting studies assessing fecal microbiota transplantation a systematic review. *Ann. Internal Med.* 167 (1), 34–39. doi: 10.7326/M16-2810

Bakris, G. L., Sica, D., White, W. B., Cushman, W. C., Weber, M. A., Handley, A., et al. (2012). Antihypertensive efficacy of hydrochlorothiazide vs chlorthalidone combined with azilsartan medoxomil. *Am. J. Med.* 125 (12), 1229.e1–1229.e10. doi: 10.1016/j.amjmed.2012.05.023

Bobrie, G., Frank, M., Azizi, M., Peyrard, S., Boutouyrie, P., Chatellier, G., et al. (2012). Sequential nephron blockade versus sequential renin-angiotensin system blockade in resistant hypertension: a prospective, randomized, open blinded endpoint study. *J. Hypertension* 30 (8), 1656–1664. doi: 10.1097/HJH.0b013e3283551e98

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N., Abnet, C. C., Al-Ghalith, G. A., et al. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* 37 (8), 852–857. doi: 10.1038/s41587-019-0209-9

Bray, G. A., Fruhbeck, G., Ryan, D. H., and Wilding, J. P. H. (2016). Management of obesity. *Lancet* 387 (10031), 1947–1956. doi: 10.1016/S0140-6736(16)00271-3

Breyner, N. M., Michon, C., de Sousa, C. S., Boas, P. B. V., Chain, F., Azevedo, V. A., et al. (2017). Microbial anti-inflammatory molecule (MAM) from faecalibacterium prausnitzii shows a protective effect on DNBS and DSS-induced colitis model in mice through inhibition of NF-kappa b pathway. *Front. Microbiol.* 8. doi: 10.3389/fmicb.2017.00114

Briguori, C., Visconti, G., Focaccio, A., Golia, B., Chieffo, A., Castelli, A., et al. (2009). Novel approaches for preventing or limiting events (Naples) II trial impact of a single high loading dose of atorvastatin on periprocedural myocardial infarction. *J. Am. Coll. Cardiol.* 54 (23), 2157–2163. doi: 10.1016/j.jacc.2009.07.005

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. (2016). DADA2: High-resolution sample inference from illumina amplicon data. *Nat. Methods* 13 (7), 581–583. doi: 10.1038/NMETH.3869

Cani, P. D. (2017). Gut microbiota - at the intersection of everything? *Nat. Rev. Gastroenterol. Hepatol.* 14 (6), 321–322. doi: 10.1038/nrgastro.2017.54

Cani, P. D., and de Vos, W. M. (2017). Next-generation beneficial microbes: The case of akkermansia muciniphila. *Front. Microbiol.* 8. doi: 10.3389/fmicb.2017.01765

Carey, R. M., Calhoun, D. A., Bakris, G. L., Brook, R. D., Daugherty, S. L., Dennison-Himmelfarb, C. R., et al. (2018). Resistant hypertension: Detection, evaluation, and management: A scientific statement from the American heart association. *Hypertension* 72 (5), E53–E90. doi: 10.1161/HYP.0000000000000084

Chen, S. F., Zhou, Y. Q., Chen, Y. R., and Gu, J. (2018). Fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* 34 (17), 884–890. doi: 10.1093/bioinformatics/bty560

Chinese Diabetes S (2021). Guideline for the prevention and treatment of type 2 diabetes mellitus in China(2020 edition)(Part 1). *Chin. J. Endocrinol. Metab.* 37 (4), 311–398. doi: 10.19538/j.nk2021080106

Chinese Cardiovascular Disease Prevention Guidelines Writing Group, Editorial Board of ChineseJournal of Cardiovascular Diseases (2018). Chinese Guidelines for the prevention of cardiovascular diseases (2017). *Chin. J. Cardiol.* 46 (1), 10–25. doi: 10.3760/cma.j.issn.0253-3758.2018.01.004

DeBoer, M. D., Gurka, M. J., Woo, J. G., and Morrison, J. A. (2015). Severity of the metabolic syndrome as a predictor of type 2 diabetes between childhood and adulthood: the Princeton lipid research cohort study. *Diabetologia* 58 (12), 2745–2752. doi: 10.1007/s00125-015-3759-5

de Groot, P., Scheithauer, T., Bakker, G. J., Prodan, A., Levin, E., Khan, M. T., et al. (2020). Donor metabolic characteristics drive effects of faecal microbiota transplantation on recipient insulin sensitivity, energy expenditure and intestinal transit time. *Gut* 69 (3), 502–50+. doi: 10.1136/gutjnl-2019-318320

Di Sciascio, G., Patti, G., Pasceri, V., Gaspardone, A., Colonna, G., and Montinaro, A. (2009). Efficacy of atorvastatin reload in patients on chronic statin therapy undergoing percutaneous coronary intervention results of the ARMYDA-RECAPTURE (Atorvastatin for reduction of myocardial damage during angioplasty) randomized trial. *J. Am. Coll. Cardiol.* 54 (6), 558–565. doi: 10.1016/j.jacc.2009.05.028

Dombrowski, S. U., Knittle, K., Avenell, A., Araujo-Soares, V., and Sniehotta, F. F. (2014). Long term maintenance of weight loss with non-surgical interventions in obese adults: systematic review and meta-analyses of randomised controlled trials. *Bmj-British Med. J.* 348, g2646. doi: 10.1136/bmj.g2646

Donnelly, J. E., Blair, S. N., Jakicic, J. M., Manore, M. M., Rankin, J. W., and Smith, B. K. (2009). American College of sports medicine position stand. appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults. *Med. Sci. Sports Exercise* 41 (2), 459–471. doi: 10.1249/MSS.0b013e3181949333

Furet, J. P., Kong, L. C., Tap, J., Poitou, C., Basdevant, A., Bouillot, J. L., et al. (2010). Differential adaptation of human gut microbiota to bariatric surgery-induced weight loss links with metabolic and low-grade inflammation markers. *Diabetes* 59 (12), 3049–3057. doi: 10.2337/db10-0253

Gadde, K. M., Martin, C. K., Berthoud, H. R., and Heymsfield, S. B. (2018). Obesity pathophysiology and management. *J. Am. Coll. Cardiol.* 71 (1), 69–84. doi: 10.1016/j.jacc.2017.11.011

Gami, A. S., Witt, B. J., Howard, D. E., Erwin, P. J., Gami, L. A., Somers, V. K., et al. (2007). Metabolic syndrome and risk of incident cardiovascular events and death - a systematic review and meta-analysis of longitudinal studies. *J. Am. Coll. Cardiol.* 49 (4), 403–414. doi: 10.1016/j.jacc.2006.09.032

Gao, J. M., Rao, J. H., Wei, Z. Y., Xia, S. Y., Huang, L., Tang, M. T., et al. (2022). Transplantation of gut microbiota from high-Fat-Diet-Tolerant cynomolgus monkeys alleviates hyperlipidemia and hepatic steatosis in rats. *Front. Microbiol.* 13. doi: 10.3389/fmicb.2022.876043

Gao, K., Yang, R., Zhang, P., Wang, Z. Y., Jia, C. X., Zhang, F. L., et al. (2018). Effects of qijian mixture on type 2 diabetes assessed by metabonomics, gut microbiota and network pharmacology. *Pharmacol. Res.* 130, 93–109. doi: 10.1016/j.phrs.2018.01.011

Grundy, S. M. (2008). Metabolic syndrome pandemic. *Arterioscler. Thromb. And Vasc. Biol.* 28 (4), 629–636. doi: 10.1161/ATVBAHA.107.151092

Grundy, S. M. (2016). Metabolic syndrome update. *Trends In Cardiovasc. Med.* 26 (4), 364–373. doi: 10.1016/j.tcm.2015.10.004

Guembe, M. J., Fernandez-Lazaro, C. I., Sayon-Orea, C., Toledo, E., and Moreno-Iribas, C. (2022). Risk for cardiovascular disease associated with metabolic syndrome and its components: a 13-year prospective study in the RIVANA cohort. *Cardiovasc. Diabetol.* 19 (1), 195. doi: 10.1186/s12933-020-01166-6

Gurka, M. J., Golden, S. H., Musani, S. K., Sims, M., Vishnu, A., Guo, Y., et al. (2017). Independent associations between a metabolic syndrome severity score and future diabetes by sex and race: the atherosclerosis risk in communities study and Jackson heart study. *Diabetologia* 60 (7), 1261–1270. doi: 10.1007/s00125-017-4267-6

Haas, B. J., Gevers, D., Earl, A. M., Feldgarden, M., Ward, D. V., Giannoukos, G., et al. (2011). Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. *Genome Res.* 21 (3), 494–504. doi: 10.1101/gr.112730.110

Jung, L. W., Lattimer, L. D. N., Stephen, S., Borum, M. L., and Doman, D. B. (2015). Fecal microbiota transplantation: A review of emerging indications beyond relapsing clostridium difficile toxin colitis. *Gastroenterol. Hepatol.* 11 (1), 24–32.

Khalesi, S., Sun, J., Buys, N., and Jayasinghe, R. (2014). Effect of probiotics on blood pressure a systematic review and meta-analysis of randomized, controlled trials. *Hypertension* 64 (4), 897–903. doi: 10.1161/HYPERTENSIONAHA.114.03469

Khan, M. T., Nieuwdorp, M., and Backhed, F. (2014). Microbial modulation of insulin sensitivity. *Cell Metab.* 20 (5), 753–760. doi: 10.1016/j.cmet.2014.07.006

Kim, B. K., Hong, S. J., Lee, Y. J., Hong, S. J., Yun, K. H., Hong, B. K., et al. (2022). Long-term efficacy and safety of moderate-intensity statin with ezetimibe combination therapy versus high-intensity statin monotherapy in patients with atherosclerotic cardiovascular disease (RACING): a randomised, open-label, non-inferiority trial. *Lancet (london england)* 400 (10349), 380–390. doi: 10.1016/S0140-6736(22)00916-3

Kootte, R. S., Levin, E., Salojarvi, J., Smits, L. P., Hartstra, A. V., Udayappan, S. D., et al. (2017). Improvement of insulin sensitivity after lean donor feces in metabolic syndrome is driven by baseline intestinal microbiota composition. *Cell Metab.* 26 (4), 611–619. doi: 10.1016/j.cmet.2017.09.008

Krentz, A. J., Patel, M. B., and Bailey, C. J. (2008). New drugs for type 2 diabetes mellitus what is their place in therapy? *Drugs* 68 (15), 2131–2162. doi: 10.2165/00003495-200868150-00005

Lai, Z. L., Tseng, C. H., Ho, H. J., Cheung, C. K., Lin, J. Y., Chen, Y. J., et al. (2018). Fecal microbiota transplantation confers beneficial metabolic effects of diet and exercise on diet-induced obese mice. *Sci. Rep.* 8 (1), 15625. doi: 10.1038/s41598-018-33893-y

Leshem, A., Horesh, N., and Elinav, E. (2019). Fecal microbial transplantation and its potential application in cardiometabolic syndrome. *Front. Immunol.* 10. doi: 10.3389/fimmu.2019.01341

Liang, F. F., Lu, X. J., Deng, Z. L., Zhong, H. J., Zhang, W., Li, Q., et al. (2022). Effect of washed microbiota transplantation on patients with dyslipidemia in south China. *Front. Endocrinol.* 13. doi: 10.3389/fendo.2022.827107

Libby, P., Buring, J. E., Badimon, L., Hansson, G. K., Deanfield, J., Bittencourt, M. S., et al. (2019). Atherosclerosis. *Nat. Rev. Dis. Primers* 5 (1), 56. doi: 10.1038/s41572-019-0106-z

Li, J., Zhao, F. Q., Wang, Y. D., Chen, J. R., Tao, J. E., Tian, G., et al. (2017). Gut microbiota dysbiosis contributes to the development of hypertension. *Microbiome* 5 (1), 14. doi: 10.1186/s40168-016-0222-x

Li, S. S., Zhu, A., Benes, V., Costea, P. I., Hercog, R., Hildebrand, F., et al. (2016). Durable coexistence of donor and recipient strains after fecal microbiota transplantation. *Science* 352 (6285), 586–589. doi: 10.1126/science.aad8852

Louis, S., Tappu, R. M., Damms-Machado, A., Huson, D. H., and Bischoff, S. C. (2016). Characterization of the gut microbial community of obese patients following a weight-loss intervention using whole metagenome shotgun sequencing. *PloS One* 11 (2), e0149564. doi: 10.1371/journal.pone.0149564

Ludvik, B., Giorgino, F., Jodar, E., Frias, J. P., Lando, L. F., Brown, K., et al. (2021). Once-weekly tirzepatide versus once-daily insulin degludec as add-on to metformin with or without SGLT2 inhibitors in patients with type 2 diabetes (SURPASS-3): a randomised, open-label, parallel-group, phase 3 trial. *Lancet* 398 (10300), 583–598. doi: 10.1016/S0140-6736(21)01443-4

Machiels, K., Joossens, M., Sabino, J., De Preter, V., Arijs, I., Eeckhaut, V., et al. (2014). A decrease of the butyrate-producing species roseburia hominis and faecalibacterium prausnitzii defines dysbiosis in patients with ulcerative colitis. *Gut* 63 (8), 1275–1283. doi: 10.1136/gutjnl-2013-304833

Magoc, T., and Salzberg, S. L. (2011). FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* 27 (21), 2957–2963. doi: 10.1093/bioinformatics/btr507

Marques, F. Z., Nelson, E., Chu, P. Y., Horlock, D., Fiedler, A., Ziemann, M., et al. (2017). High-fiber diet and acetate supplementation change the gut microbiota and prevent the development of hypertension and heart failure in hypertensive mice. *Circulation* 135 (10), 964–977. doi: 10.1161/CIRCULATIONAHA.116.024545

Mestdagh, R., Dumas, M. E., Rezzi, S., Kochhar, S., Holmes, E., Claus, S. P., et al. (2012). Gut microbiota modulate the metabolism of brown adipose tissue in mice. *J. Proteome Res.* 11 (2), 620–630. doi: 10.1021/pr200938v

Metabolic Syndrome Research Collaboration Group of Diabetes Branch of ChineseMedical Association. (2018). Suggestions of Diabetes Branch of Chinese Medical Association onmetabolic syndrome. *Chin. J. Diab* 12 (3), 156–161. doi: 10.3321/j.issn:1006-6187.2004.03.002

Moayyedi, P., Surette, M. G., Kim, P. T., Libertucci, J., Wolfe, M., Onischi, C., et al. (2015). Fecal microbiota transplantation induces remission in patients with active ulcerative colitis in a randomized controlled trial. *Gastroenterology* 149 (1), 102–109. doi: 10.1053/j.gastro.2015.04.001

Mullish, B. H., Quraishi, M. N., Segal, J. P., McCune, V. L., Baxter, M., Marsden, G. L., et al. (2018). The use of faecal microbiota transplant as treatment for recurrent or refractory clostridium difficile infection and other potential indications: joint British society of gastroenterology (BSG) and healthcare infection society (HIS) guidelines. *Gut* 67 (11), 1920–1941. doi: 10.1136/gutjnl-2018-316818

Mushtaq, N., Hussain, S., Zhang, S. R., Yuan, L., Li, H., Ullah, S., et al. (2019). Molecular characterization of alterations in the intestinal microbiota of patients with grade 3 hypertension. *Int. J. Of Mol. Med.* 44 (2), 513–522. doi: 10.3892/ijmm.2019.4235

O'Neill, S., and O'Driscoll, L. (2015). Metabolic syndrome: a closer look at the growing epidemic and its associated pathologies. *Obes. Rev.* 16 (1), 1–12. doi: 10.1111/obr.12229

Okubo, H., Nakatsu, Y., Kushiyama, A., Yamamotoya, T., Matsunaga, Y., Inoue, M. K., et al. (2018). Gut microbiota as a therapeutic target for metabolic disorders. *Curr. Med. Chem.* 25 (9), 984–1001. doi: 10.2174/0929867324666171009121702

Pamer, E. G. (2014). Fecal microbiota transplantation: effectiveness, complexities, and lingering concerns. *Mucosal Immunol.* 7 (2), 210–214. doi: 10.1038/mi.2013.117

Panahi, Y., Ahmadi, Y., Teymouri, M., Johnston, T. P., and Sahebkar, A. (2018). Curcumin as a potential candidate for treating hyperlipidemia: A review of cellular and metabolic mechanisms. *J. Cell. Physiol.* 233 (1), 141–152. doi: 10.1002/jcp.25756

Pan, Z. Y., Zhong, H. J., Huang, D. N., Wu, L. H., and He, X. X. (2022). Beneficial effects of repeated washed microbiota transplantation in children with autism. *Front. Pediatr.* 10. doi: 10.3389/fped.2022.928785

Paramsothy, S., Kamm, M. A., Kaakoush, N. O., Walsh, A. J., van den Bogaerde, J., Samuel, D., et al. (2017). Multidonor intensive faecal microbiota transplantation for active ulcerative colitis: a randomised placebo-controlled trial. *Lancet* 389 (10075), 1218–1228. doi: 10.1016/S0140-6736(17)30182-4

Paramsothy, S., Paramsothy, R., Rubin, D. T., Kamm, M. A., Kaakoush, N. O., Mitchell, H. M., et al. (2017). Faecal microbiota transplantation for inflammatory bowel disease: A systematic review and meta-analysis. *J. Of Crohns Colitis* 11 (10), 1180–1199. doi: 10.1093/ecco-jcc/jjx063

Plovier, H., Everard, A., Druart, C., Depommier, C., Van Hul, M., Geurts, L., et al. (2017). A purified membrane protein from akkermansia muciniphila or the pasteurized bacterium improves metabolism in obese and diabetic mice. *Nat. Med.* 23 (1), 107–113. doi: 10.1038/nm.4236

Qazi, T., Amaratunga, T., Barnes, E. L., Fischer, M., Kassam, Z., and Allegretti, J. R. (2017). The risk of inflammatory bowel disease flares after fecal microbiota transplantation: Systematic review and meta-analysis. *Gut Microbes* 8 (6), 574–588. doi: 10.1080/19490976.2017.1353848

Qian, Y., Li, M. Y., Wang, W., Wang, H. W., Zhang, Y., Hu, Q., et al. (2019). Effects of lactobacillus casei YBJ02 on lipid metabolism in hyperlipidemic mice. *J. Food Sci.* 84 (12), 3793–3803. doi: 10.1111/1750-3841.14787

Qin, J. J., Li, Y. R., Cai, Z. M., Li, S. H., Zhu, J. F., Zhang, F., et al. (2012). A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* 490 (7418), 55–60. doi: 10.1038/nature11450

Ray, K. K., Molemans, B., Schoonen, W. M., Giovas, P., Bray, S., Kiru, G., et al. (2021). EU-Wide cross-sectional observational study of lipid-modifying therapy use in secondary and primary care: the DA VINCI study. *Eur. J. Of Prev. Cardiol.* 28 (11), 1279–1289. doi: 10.1093/eurjpc/zwaa047

Ridaura, V. K., Faith, J. J., Rey, F. E., Cheng, J. Y., Duncan, A. E., Kau, A. L., et al. (2013). Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science* 341 (6150), 1079–U1049. doi: 10.1126/science.1241214

Rosenstock, J., Wysham, C., Frias, J. P., Kaneko, S., Lee, C. J., Lando, L. F., et al. (2021). Efficacy and safety of a novel dual GIP and GLP-1 receptor agonist tirzepatide in patients with type 2 diabetes (SURPASS-1): a double-blind, randomised, phase 3 trial. *Lancet* 398 (10295), 143–155. doi: 10.1016/S0140-6736(21)01324-6

Rossi, J. L. S., Barbalho, S. M., de Araujo, R. R., Bechara, M. D., Sloan, K. P., and Sloan, L. A. (2022). Metabolic syndrome and cardiovascular diseases: Going beyond traditional risk factors. *Diabetes-Metabol. Res. Rev.* 38 (3), e3502. doi: 10.1002/dmrr.3502

Roth, G. A., Mensah, G. A., and Fuster, V. (2020). The global burden of cardiovascular diseases and risks a compass for global action. *J. Am. Coll. Cardiol.* 76 (25), 2980–2981. doi: 10.1016/j.jacc.2020.11.021

Sattar, N., Lee, M. M. Y., Kristensen, S. L., Branch, K. R. H., Del Prato, S., Khurmi, N. S., et al. (2021). Cardiovascular, mortality, and kidney outcomes with GLP-1 receptor agonists in patients with type 2 diabetes: a systematic review and meta-analysis of randomised trials. *Lancet Diabetes Endocrinol.* 9 (10), 653–662. doi: 10.1016/S2213-8587(21)00203-5

Shi, Q. (2020). Nanjing consensus on methodology of washed microbiota transplantation. *Chin. Med. J.* 133 (19), 2330–2332. doi: 10.1097/CM9.0000000000000954

Simmons, R. K., Alberti, K., Gale, E. A. M., Colagiuri, S., Tuomilehto, J., Qiao, Q., et al. (2010). The metabolic syndrome: useful concept or clinical tool? report of a WHO expert consultation. *Diabetologia* 53 (4), 600–605. doi: 10.1007/s00125-009-1620-4

Singh, T. P., Malik, R. K., Katkamwar, S. G., and Kaur, G. (2015). Hypocholesterolemic effects of lactobacillus reuteri LR6 in rats fed on high-cholesterol diet. *Int. J. Food Sci. Nutr.* 66 (1), 71–75. doi: 10.3109/09637486.2014.953450

Smits, L. P., Kootte, R. S., Levin, E., Prodan, A., Fuentes, S., Zoetendal, E. G., et al. (2018). Effect of vegan fecal microbiota transplantation on carnitine- and choline-derived trimethylamine-N-Oxide production and vascular inflammation in patients with metabolic syndrome. *J. Of Am. Heart Assoc.* 7 (7), e008342. doi: 10.1161/JAHA.117.008342

Sung, M. M., Kim, T. T., Denou, E., Soltys, C. L. M., Hamza, S. M., Byrne, N. J., et al. (2017). Improved glucose homeostasis in obese mice treated with resveratrol is associated with alterations in the gut microbiome. *Diabetes* 66 (2), 418–425. doi: 10.2337/db16-0680

Sun, C. Y., Zheng, Z. L., Chen, C. W., Lu, B. W., and Liu, D. (2022). Targeting gut microbiota with natural polysaccharides: Effective interventions against high-fat diet-induced metabolic diseases. *Front. Microbiol.* 13. doi: 10.3389/fmicb.2022.859206

Tang, X. Y., Wu, M. Y., Wu, S. L., and Tian, Y. H. (2022). Continuous metabolic syndrome severity score and the risk of CVD and all-cause mortality. *Eur. J. Clin. Invest.* 52 (9), e13817. doi: 10.1111/eci.13817

Tierney, B. T., Tan, Y. X., Kostic, A. D., and Patel, C. J. (2021). Gene-level metagenomic architectures across diseases yield high-resolution microbiome diagnostic indicators. *Nat. Commun.* 12 (1). doi: 10.1038/s41467-021-23029-8

Toral, M., Robles-Vera, I., de la Visitacion, N., Romero, M., Yang, T., Sanchez, M., et al. (2019). Critical role of the interaction gut microbiota - sympathetic nervous system in the regulation of blood pressure. *Front. Physiol.* 10. doi: 10.3389/fphys.2019.00231

Turnbaugh, P. J., and Gordon, J. I. (2009). The core gut microbiome, energy balance and obesity. *J. Of Physiol.-London* 587 (17), 4153–4158. doi: 10.1113/jphysiol.2009.174136

Turnbaugh, P. J., Ley, R. E., Hamady, M., Fraser-Liggett, C. M., Knight, R., and Gordon, J. I. (2007). The human microbiome project. *Nature* 449 (7164), 804–810. doi: 10.1038/nature06244

Varier, R. U., Biltaji, E., Smith, K. J., Roberts, M. S., Jensen, M. K., LaFleur, J., et al. (2015). Cost-effectiveness analysis of fecal microbiota transplantation for recurrent clostridium difficile infection. *Infect. Control And Hosp. Epidemiol.* 36 (4), 438–444. doi: 10.1017/ice.2014.80

Velagapudi, V. R., Hezaveh, R., Reigstad, C. S., Gopalacharyulu, P., Yetukuri, L., Islam, S., et al. (2010). The gut microbiota modulates host energy and lipid metabolism in mice. *J. Of Lipid Res.* 51 (5), 1101–1112. doi: 10.1194/jlr.M002774

Vrieze, A., Van Nood, E., Holleman, F., Salojarvi, J., Kootte, R. S., Bartelsman, J., et al. (2012). Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* 143 (4), 913–916. doi: 10.1053/j.gastro.2012.06.031

Wang, K., Liao, M. F., Zhou, N., Bao, L., Ma, K., Zheng, Z. Y., et al. (2019). Parabacteroides distasonis alleviates obesity and metabolic dysfunctions via production of succinate and secondary bile acids. *Cell Rep.* 26 (1), 222–235. doi: 10.1016/j.celrep.2018.12.028

Wang, S. A., Xu, M. Q., Wang, W. Q., Cao, X. C., Piao, M. Y., Khan, S., et al. (2016). Systematic review: Adverse events of fecal microbiota transplantation. *PloS One* 11 (8). doi: 10.1371/journal.pone.0161174

Wood, G., Taylor, E., Ng, V., Murrell, A., Patil, A., van der Touw, T., et al. (2022). Determining the effect size of aerobic exercise training on the standard lipid profile in sedentary adults with three or more metabolic syndrome factors: a systematic review and meta-analysis of randomised controlled trials. *Br. J. Sports Med.* 56 (18), 1032–1041. doi: 10.1136/bjsports-2021-103999

Wu, L., Li, M. Q., Xie, Y. T., Zhang, Q., Lu, X. J., Liu, T., et al. (2022a). Washed microbiota transplantation improves patients with high blood glucose in south China. *Front. Endocrinol.* 13. doi: 10.3389/fendo.2022.985636

Wu, L., Xie, X. Q., Liang, T. T., Ma, J., Yang, L. S., Yang, J., et al. (2022b). Integrated multi-omics for novel aging biomarkers and antiaging targets. *Biomolecules* 12 (1), 39. doi: 10.3390/biom12010039

Wu, L., Xie, X. Q., Li, Y., Liang, T. T., Zhong, H. J., Ma, J., et al. (2021b). Metagenomics-based analysis of the age-related cumulative effect of antibiotic resistance genes in gut microbiota. *Antibiotics* 10 (8), 1006. doi: 10.3390/antibiotics10081006

Wu, L., Xie, X. Q., Zhang, J. M., Ding, Y., and Wu, Q. P. (2021a). Bacterial diversity and community in regional water microbiota between different towns in world's longevity township jiaoling, China. *Diversity* 13 (8), 361. doi: 10.3390/d13080361

Yang, T., Richards, E. M., Pepine, C. J., and Raizada, M. K. (2018). The gut microbiota and the brain-gut-kidney axis in hypertension and chronic kidney disease. *Nat. Rev. Nephrol.* 14 (7), 442–456. doi: 10.1038/s41581-018-0018-2

Yang, T., Santisteban, M. M., Rodriguez, V., Li, E., Ahmari, N., Carvajal, J. M., et al. (2015). Gut dysbiosis is linked to hypertension. *Hypertension* 65 (6), 1331–1340. doi: 10.1161/HYPERTENSIONAHA.115.05315

Yan, Q. L., Gu, Y. F., Li, X. C., Yang, W., Jia, L. Q., Chen, C. M., et al. (2017). Alterations of the gut microbiome in hypertension. *Front. Cell. Infect. Microbiol.* 7. doi: 10.3389/fcimb.2017.00381

Yanovski, S. Z., and Yanovski, J. A. (2014). Long-term drug treatment for obesity a systematic and clinical review. *Jama-Journal Of Am. Med. Assoc.* 311 (1), 74–86. doi: 10.1001/jama.2013.281361

Zhang, T., Lu, G. C., Zhao, Z., Liu, Y. F., Shen, Q., Li, P., et al. (2020). Washed microbiota transplantation vs. manual fecal microbiota transplantation: clinical findings, animal studies and in vitro screening. *Protein Cell* 11 (4), 251–266. doi: 10.1007/s13238-019-00684-8

Zhong, H. J., Zeng, H. L., Cai, Y. L., Zhuang, Y. P., Liou, Y. L., Wu, Q. P., et al. (2021). Washed microbiota transplantation lowers blood pressure in patients with hypertension. *Front. Cell. Infect. Microbiol.* 11. doi: 10.3389/fcimb.2021.679624

frontiers | Frontiers in Cellular and Infection Microbiology

TYPE Original Research
PUBLISHED 18 November 2022
DOI 10.3389/fcimb.2022.1048076

OPEN ACCESS

EDITED BY
Chuanxing Xiao,
Xiamen University, China

REVIEWED BY
Stefano Di Bella,
University of Trieste, Italy
Ashraf Kariminik,
Islamic Azad University Kerman, Iran

*CORRESPONDENCE
Fengmin Shao
shaofengmin1818@163.com
Tianzhong Yan
ytz460@hotmail.com

SPECIALTY SECTION
This article was submitted to Intestinal Microbiome, a section of the journal Frontiers in Cellular and Infection Microbiology

RECEIVED 19 September 2022
ACCEPTED 27 October 2022
PUBLISHED 18 November 2022

CITATION
Wu X, Cao G, Wang Z, Wu X, Tian X, Gu Y, Shao F and Yan T (2022) Antibiotic ampicillin induces immune tolerance in renal transplantation by regulating the proportion of intestinal flora in mice.
Front. Cell. Infect. Microbiol. 12:1048076.
doi: 10.3389/fcimb.2022.1048076

Antibiotic ampicillin induces immune tolerance in renal transplantation by regulating the proportion of intestinal flora in mice

Xiaoqiang Wu[1], Guanghui Cao[1], Zhiwei Wang[1], Xuan Wu[1], Xiangyong Tian[1], Yue Gu[2], Fengmin Shao[2]* and Tianzhong Yan[1]*

[1]Department of Urology, Henan Provincial Clinical Research Center for Kidney Disease, Henan Provincial People's Hospital (Zhengzhou University People's Hospital), Zhengzhou, Henan, China, [2]Department of Nephrology, Henan Provincial Clinical Research Center for Kidney Disease, Henan Provincial People's Hospital (Zhengzhou University People's Hospital), Zhengzhou, Henan, China

Objectives: There are significant differences in the composition of intestinal flora in renal transplant recipients before and after an operation, which has a great impact on the prognosis of renal transplantation. The purpose of this project is to study the effect of intestinal flora imbalance on renal transplantation.

Methods: The animal model of renal transplantation was established after intestinal flora imbalance (mice pretreated with compound antibiotics), or the animal model of renal transplantation was established after being pretreated with single antibiotics. HE, PAS, and Masson staining was used to detecting the histopathological changes of transplanted renal. The expression of inflammatory factors and infiltration of inflammatory cells of renal tissue were respectively been detected by ELISA kit and flow cytometry.

Results: Antibiotic pretreatment restored weight loss, and decreased serum creatinine level in mice after renal transplantation. The tissue staining, ELISA assay, and flow cytometry data showed that antibiotic pretreatment alleviated injury of the renal allograft, inhibited the inflammatory factors levels, and reduced inflammatory cell infiltration in mice after renal transplantation. Furthermore, single antibiotic, especially ampicillin pretreatment can also play the same role as compound antibiotics, such as restoring weight loss, decreasing serum creatinine level, alleviating renal allograft injury, inhibiting inflammatory factors levels, and reducing inflammatory cell infiltration in mice after renal transplantation.

Conclusions: Antibiotic ampicillin may inhibit inflammatory cell infiltration after renal transplantation by regulating the proportion of intestinal flora in mice, to reduce renal injury and play a role in renal protection.

KEYWORDS

renal transplant, intestinal flora, antibiotic, 16S rDNA gene sequencing, renal injury

Introduction

The gastrointestinal tract of the human body is rich in hundreds of millions of microbial. The genome encoded by these microbes is 50-100 times that of the human body, and known as the "second genome" of the human body. As a "special organ" of the human body, intestinal flora coexists with the human body and plays an important role in the metabolism of substances and energy, nerve and immune regulation, as well as resisting the invasion of pathogenic microorganisms (Zhou et al., 2020). Numerous studies have shown that dysbacteriosis (changes in the structure of flora) is closely related to the occurrence and development of a variety of diseases, such as cancer (Tilg et al., 2018), infectious diseases (Ghani et al., 2022), cardiovascular diseases (Xu and Yang, 2021), mental diseases (Järbrink-Sehgal and Andreasson, 2020).

Renal transplantation is the first choice for patients with end-stage renal disease. With the use of new immunosuppressants, the short-term prognosis and long-term survival of renal transplant recipients improved (Gioco et al., 2020). However, after kidney transplantation, a large number of inflammatory cells infiltrated the graft. These cells play a role in immune response and inflammatory response after ischemia-reperfusion, resulting in a series of damage to the recipient (Zhao et al., 2018). In previous animal experiments, pretreatment with antibiotics before transplantation can delay the rejection of skin transplantation with major antigen mismatch and heart transplantation with MHC-II molecular mismatch (Lei et al., 2016). Therefore, the composition of intestinal microorganisms may predict the occurrence of rejection. Changing the tissue of intestinal flora by targeting may become one of the strategies to improve the immune tolerance of grafts.

Antibiotics frequently used to treat bacterial infections. Although antibiotics kill bacteria and inhibit their growth, they can also induce drug resistance (Zimmermann and Curtis, 2019). In addition, antibiotic use can temporarily or permanently alter the composition of the intestinal microbiota and promote colonization by intestinal pathogens (Freifeld et al., 2011). In recent years, intestinal flora has become a research hotspot to explore the common complications after renal transplantation from a perspective of intestinal flora. Studies have shown that the composition of intestinal flora in renal transplant recipients is significantly different before and after surgery, which is closely related to the occurrence and development of many complications after renal transplantation and affects the prognosis of recipients (Swarte et al., 2020). Therefore, it is an economic and effective intervention measure to use antibiotic pretreatment to improve the intestinal flora composition and to alleviate the related complications after renal transplantation.

In this study, we first established a renal allograft mouse model after clearing intestinal flora with compound antibiotics, to determine the effect of compound antibiotic pretreatment on renal allograft mice. Then, the model of renal allograft mice was pretreated with a single antibiotic to determine the effect of single antibiotic pretreatment on renal allograft mice. Finally, according to the experimental results of mice, it was determined that the ampicillin played the most significant role, and the 16s RNA gene sequencing technology was used to analyze the effect of ampicillin on the intestinal flora of renal allograft mice. This study aimed to observe the effect of antibiotic pretreatment on inflammatory cell infiltration in mouse renal allografts and to explore the possible mechanisms of antibiotic pretreatment on inflammatory cell infiltration.

Material and methods

Animal experiment

Male Balb/c mice were purchased from Shanghai SLAC Laboratory Animal Co.,Ltd, and maintained in a specific pathogen-free room in the Experimental Animal Center at Zhengzhou University, in cages with free access to water and food and a 12-h/12-h light/dark cycle. The mice were organized into 4 groups (Control group, intestinal flora imbalance group (antibiotics, ABx), renal transplantation group (Transplant), intestinal flora imbalance +renal transplantation group (ABx+ Transplant), every group consists of 6 mice for a total of 30 mice). Mice in ABx group received broad-spectrum compound antibiotic treatment. The antibiotics were added to the drinking water based on the weight of the mice, Amp (100 mg/kg

ampicillin), Van (50 mg/kg vancomycin), Met (100 mg/kg metronidazole), and Neo (100 mg/kg neomycin). The mice received antibiotics for 4 weeks. Mice in ABx+Transplant group were treated with broad-spectrum compound antibiotics (Am, Van, Neo, and Met) for 4 weeks to establish mice with intestinal flora imbalance, and then underwent renal transplantation. The mice were been killed one week after renal transplantation.

Allogeneic renal transplantation in mice

The mouse model of the renal transplant was established as previously described (Liu et al., 2019). In brief, mice underwent a standard midline abdominal incision under anesthesia with inhalation of 2% isoflurane. Then, the donors' left kidneys, aorta, inferior vena cava, and ureter were been removed under a microscope followed by lavage *in situ* with the histidine-tryptophane-ketoglutarate solution. The isolated renal were then implanted below the level of native renal vessels of recipients with left nephrectomy, while the infrarenal aorta and the inferior vena cava were perfectly anastomosed to the recipients. In addition, the ureter was been directly anastomosed to the bladder for urinary tract reconstruction.

Sampling and testing

Blood was collected from mouse orbit and placed at room temperature for 2 hours, then been centrifuged. The supernatant was taken and stored at -20°C. Serum creatinine level was measure by automatic biochemical instrument. Tumor necrosis factor in serum α (TNF-α), interferon-γ (IFN-γ), Interleukin 1β (IL-1β), and Interleukin-6 (IL-6) in renal tissues were determined by enzyme-linked immunosorbent assay (ELISA).

Staining and histopathology

After taking blood, the mice were been killed, and the renal tissue (transplanted renal tissue) was taken out. The floating blood on the surface of renal tissue was been washed with normal saline, dried with filter paper, and fixed in formalin. Histopathological examination: the tissues were been removed from formalin solution, dehydrated, embedded in paraffin, sectioned, stained with conventional HE, PAS, and Masson, and observed and photographed under the optical microscope.

Flow cytometry analysis of cell infiltration in renal tissue

The renal tissues were cut into pieces of approximately 2 mm^2, and digested by tyrosinase plus 0.5% type II collagenase at 37°C with 5% CO_2 for 2 h and meshed through a 200-gauge stainless steel filter. Cells are collected using centrifugation at 1500 rpm for 10 min at 4°C and resuspended in PBS. The single nucleus cell suspension was been collected and analyzed by flow cytometry. The percentage of monocytes ($CD11b^+LY6C^+$), macrophages ($CD11b^+F4/80^+$), and neutrophils ($CD11b^+LY6G^+$) cells in the mouse renal were determined by flow cytometry. Briefly, cell suspension at 106/tube was stained in duplicate with FITC-anti-CD11b, APC-F4/80, and PE-anti-LY6C/LY6G at room temperature for 30 min in the dark. After washed with PBS, the cells were been analyzed by flow cytometry on the FACSCalibur with FACSDiva software.

Sequencing analysis of intestinal flora by Illumina MiSeq

The high-throughput sequencing and analysis of the experimental flora structure were completed by Shanghai CapitalBio Technology Co., Ltd. DNA was extracted from the intestinal mucosa of mice according to the instructions of the qubit dsDNA assay kit (life technologies, q328520). The extracted DNA samples were detected by qubit 2.0 fluorometer (Invitrogen, Carlsbad, CA), and the metavxtm library construction kit was used to construct the sequencing library. Using 30 ~ 50 ng DNA as a template, the V3-V4 variable region on bacterial 16S rDNA was amplified by PCR, 341F: 5'-CCTAYGGGRBGCASCAG-3', 806R: 5'-GGACTACNNGGG TATCTAAT-3' (Jiang et al., 2020). The quality of the library was been detected by Agilent 2100 biological analyzer, and qubit quantification was performed. After the DNA library is mixed, it is been sequenced on the computer, and the sequence information is read by miseq control software. The two terminal sequences are de hybridized, spliced, and chimeric sequences are been removed. Vsearch is used for sequence clustering (the sequence similarity is set to 97%), and the corresponding species information of each OTU is obtained by referring to the silva132 database. RDP classifier software is used for species comparison annotation, and the reserved confidence interval is greater than 0.7. Taxon clustering, OTUs abundance, and α or β diversity analysis were performed on the measured effective data. Lefse analysis was been used to compare the differences in intestinal microflora abundance in renal transplantation mice caused by antibiotics at the level of species classification.

Statistical analysis

Data expressed as the mean ± standard deviation (SD). The comparisons among groups were done by one-way analysis of variance (ANOVA), followed by Tukey *post hoc* testing. The $P< 0.05$ was been considered statistically significant.

Results

ABx pretreatment improves renal function in renal transplantation mice

To study the effect of intestinal flora imbalance on renal transplantation in mice, the mice were divided into 4 groups: Control group, intestinal flora imbalance group (antibiotics, ABx), renal transplantation group (Transplant), intestinal flora imbalance +renal transplantation group (ABx+Transplant). Compared with the control group, the weight of mice in the transplantation group decreased significantly and compared with kidney transplantation mice, ABx pretreatment significantly increased the weight of kidney transplantation mice, while only ABx pretreatment did not affect the body weight of mice (Figure 1A). Creatinine is a product of muscle metabolism in the body, and mainly discharged from the body through the glomerulus. We can judge whether the kidney is healthy by measuring the value of serum creatinine. As shown in Figure 1B, only ABx pretreatment did not affect the serum creatinine level. Compared with the control group, the serum creatinine in the transplant group increased significantly, which decreased in renal transplantation mice that pretreated with ABx. And the (transplanted) renal tissues of mice in each group were stained with H&E and PAS (Figures 1C, D). The results showed that the kidneys of ABx pretreated mice were normal, however, the diffuse inflammatory cells infiltrated the renal parenchyma, necrotic tubules and tubulitis can been observed in the transplanted renal tissues of the mice transplant group. At the same time, ABx pretreatment can effectively alleviate renal inflammation in renal transplantation mice. In addition, we also performed Masson staining on the (transplanted) renal tissues of mice in each group. As shown in Figure 1E, we observed slight collagen fibers in the renal tissues of mice in the transplant group, and renal tissues of mice in the ABx group and ABx+ transplant group showed normal. These results indicated that ABx pretreatment improved renal function in renal transplantation mice.

ABx pretreatment inhibits inflammatory cell infiltration in grafts of renal transplantation mice

To explore the effect of intestinal flora imbalance on inflammatory response in renal transplantation mice, mice grouping was the same as in Figure 1. The expression levels of

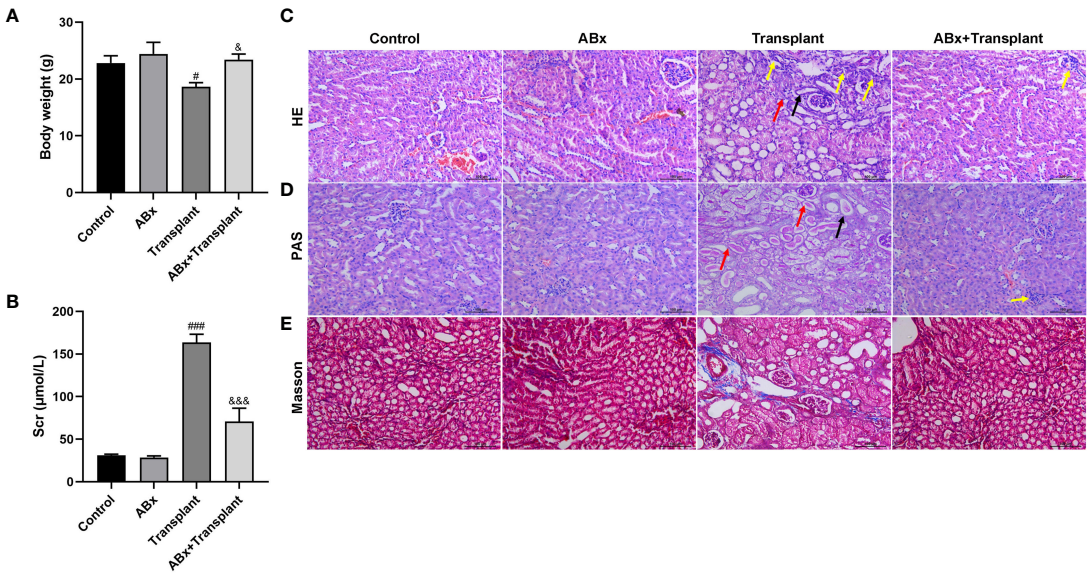


FIGURE 1
Effect of intestinal flora imbalance on renal transplantation in mice. Male BALB/c mice were purchased and randomly divided into 4 groups: Control group, intestinal flora imbalance group (antibiotics, ABx), renal transplantation group (Transplant), intestinal flora imbalance +renal transplantation group (Abx+Transplant). **(A)** The body weights. **(B)** The serum creatinine level. **(C-E)** HE, PAS and Masson staining for pathological examination. The yellow arrow indicates diffuse inflammatory cell infiltration, the black arrow indicates tubular necrosis, and the red arrow indicates tubulitis. $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ vs Control group; $^{\&}p < 0.05$, $^{\&\&\&}p < 0.001$ vs transplant group.

pro-inflammatory and inflammatory (TNF-α、IFN-γ、IL-6, and IL-1β) in (transplanted) renal tissue were detected by ELISA kit. As shown in Figures 2A-D, there are high levels of TNF-α、IFN-γ、IL-6 and IL-1β in the transplanted renal tissue of renal transplantation mice. ABx pretreatment can significantly reduce the inflammatory response of renal transplantation mice. Then, we detected the infiltration of inflammatory cells [monocytes ($CD11b^+LY6C^+$), macrophages ($CD11b^+F4/80^+$) and neutrophils ($CD11b^+LY6G^+$)] in the (transplanted) renal tissues of mice in each group by flow cytometry. The results showed that compared with the control group, ABx pretreatment could reduce the inflammatory cell infiltration in the renal tissue of mice in each group, and at the same time, the inflammatory cell infiltration in the transplanted

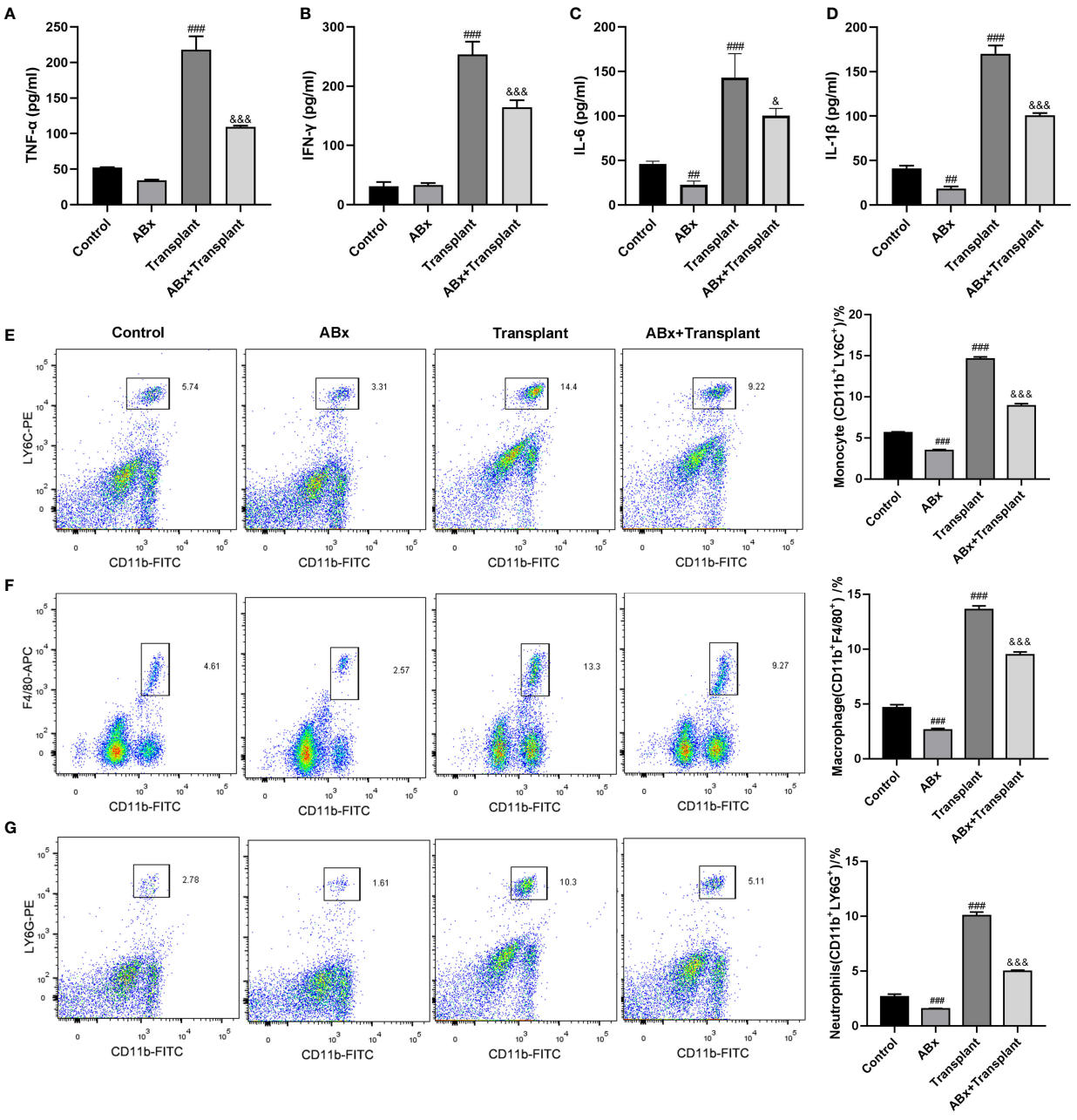


FIGURE 2
Effect of intestinal flora imbalance on inflammatory response in renal transplantation mice. **(A-D)** ELISA was used to detect the expression levels of TNF-α、IFN-γ、IL-6 and IL-1β in renal tissue. **(E-G)** Flow cytometry used to detect the infiltration of inflammatory cells (monocytes, macrophages, neutrophils) in the renal tissue. $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs Control group; $^{\&}p < 0.05$, $^{\&\&\&}p < 0.001$ vs transplant group.

renal tissue of transplantation mice increased significantly. Compared with the renal transplantation group, the inflammatory cell infiltration in the transplanted renal tissue of ABx pretreated renal transplantation mice was significantly reduced (Figures 2E-G). These data suggested that ABx pretreatment inhibits inflammatory cell infiltration in grafts of renal transplantation mice.

Antibiotics ampicillin pretreatment improves renal function in renal transplantation mice

To determine the effect of a single antibiotic on renal transplantation in mice, the mice were divided into 5 groups: Transplant, ampicillin (Amp), vancomycin (Van), neomycin sulfate (Neo), and metronidazole (Met). Except for the simple transplantation group, the other 4 groups of mice were pretreated with corresponding antibodies for 4 weeks before renal transplantation. Compared with the renal transplantation group, the weight of mice in the amp group increased significantly, while the weight of mice in the other 4 antibiotic pretreatment groups did not change significantly (Figure 3A). Compared with the transplantation alone group, the serum creatinine significantly reduced in the Amp and Van pretreatment groups (Figure 3B). As shown in Figures 3C, D, the HE and PAS stain results showed that diffuse inflammatory cells infiltrated the whole renal parenchyma, necrotic tubules and tubulitis were observed in the transplant group. Compared with the transplant group, the renal tissue inflammation in the other 4 antibiotic pretreatment groups were relieved. Among them, the renal tissue of the Amp pretreatment group was the closest to normal renal tissue, and there was no significant difference among Neo, Met and Van pretreatment groups. Masson staining showed that a small number of collagen fibers could be seen in the transplanted renal tissue of mice in the transplant group, and the renal tissue of mice in the other 4 antibiotic pre-treatment was normal (Figure 3E). These results showed that a single antibiotic, especially ampicillin pretreatment, could improve the renal function of renal

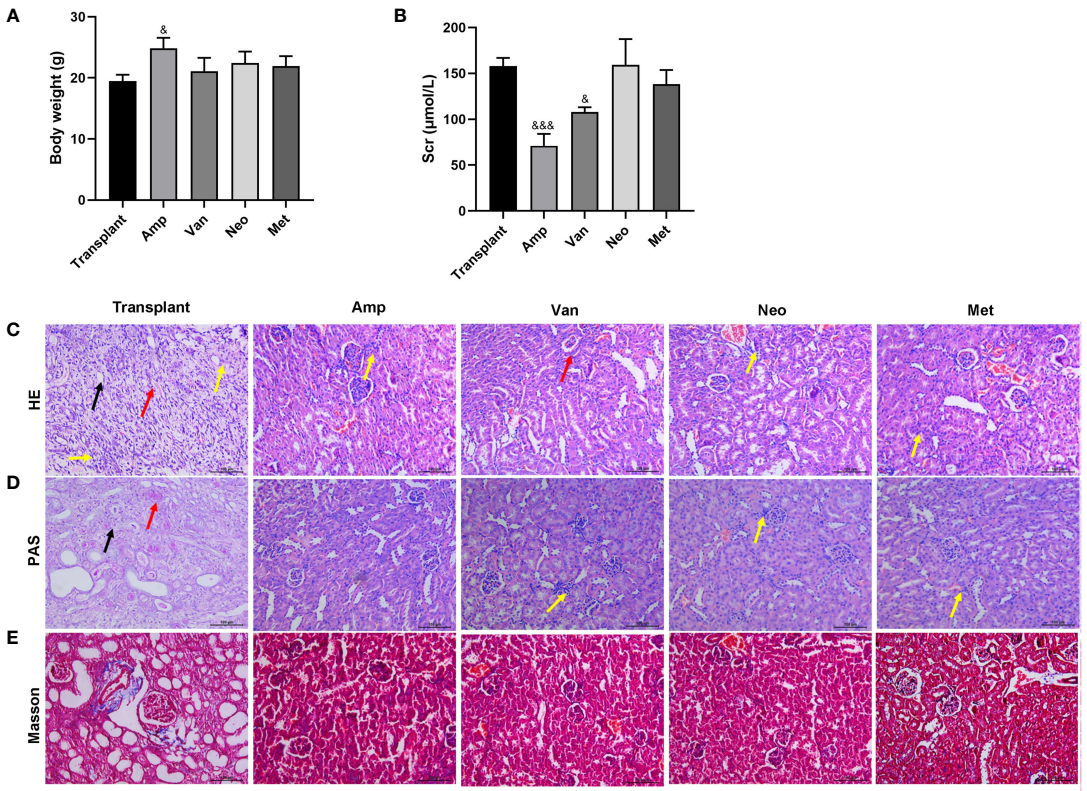


FIGURE 3
Effect of single antibiotic on renal transplantation in mice. Balb/c mice were treated with antibiotics ampicillin (Amp), vancomycin (Van), neomycin sulfate (Neo), and metronidazole (Met), respectively, and then underwent renal transplantation. **(A)** Compare the body weight of mice in each group. **(B)** The serum creatinine level. **(C-E)** HE, PAS and Masson staining were used to pathological examination of the transplanted renal. The yellow arrow indicates diffuse inflammatory cell infiltration, the black arrow indicates tubular necrosis, and the red arrow indicates tubulitis. $^{\&}p < 0.05$, $^{\&\&\&}p < 0.001$ vs transplant group.

transplantation mice. These results suggested that antibiotics ampicillin pretreatment improved renal function in renal transplantation mice.

Antibiotics ampicillin pretreatment inhibits inflammatory cell infiltration in grafts of renal transplantation mice

To further explore the effect of a single antibiotic on inflammatory response in renal transplantation mice, mice grouping was the same as Figure 3. The expression levels of pro-inflammatory and inflammatory factors (TNF-α、IFN-γ、IL-6 and IL-1β) in transplanted renal tissue of ampicillin-pretreated renal transplantation mice were significantly lower than that in the transplant group. Ampicillin pretreatment significantly reduced the TNF-α, IFN-γ, IL-6 and IL-1β levels, vancomycin pretreatment reduced TNF-α, IL-6 and IL-1β levels, neomycin sulfate pretreatment reduced TNF-α and IL-6 level, and metronidazole pretreatment reduced TNF-α and IL-1β level. Only ampicillin pretreatment could inhibit the expression of all pro-inflammatory and inflammatory factors in transplanted kidney tissue of renal transplantation mice (Figures 4A-D). In addition, the flow cytometry results showed that, compared with the renal transplantation group, the inflammatory cell infiltration in the renal tissue of single antibiotic-pretreated renal transplantation mice were all significantly reduced (Figures 4E-G). These data suggested that ampicillin pretreatment inhibits inflammatory cell infiltration in grafts of renal transplantation mice.

Ampicillin pretreatment reduces intestinal microflora diversity in renal transplantation mice

According to the above results, single antibiotic pretreatment, especially ampicillin pretreatment, could improve the renal function of renal transplantation mice, the intestinal mucosa tissues of antibiotic ampicillin pretreated renal transplantation mice (Amp, n=6) and renal transplantation mice (Control, n=6) were collected. Then we used 16S rDNA sequencing method to analyze the intestinal flora in the intestinal mucosa tissues of two groups of mice. In the present study, we first obtained the pairs of reads by sequencing 12 samples using 16S rRNA gene high throughput sequencing, the reads were spliced and filtered to generate clean tags. According to the USEARCH software based on 97% sequence similarity, the tags were clustered into OTUs. As shown in Figure 5A, a total of 500 OTUs were assigned to the simple renal transplantation group and ampicillin pretreated renal transplantation group, 473 and 401 OTUs were found in the renal transplantation group and ampicillin pretreatment group, respectively, and the number of OUTs shared among the two groups was 374. From the comparison of OTU uniform sequence and related diversity index between the two groups, it can be seen that there is no significant difference in Shannon index (p=0.065), Simpson index (p=0.132), Chao I (p=0.132) and ACE index (p=0.8182) between the two groups (Figures 5B-E).

Beta diversity reflects the degree of similarity in species diversity of different sample groups, and the small value of beta diversity indicated that the species of the two groups were similar. When considering the existence of species, the transplantation group and ampicillin pretreatment group had similar microbial species, with unweighted UniFrac distances (R^2 =0.04058; P = 0.8199). When considering species abundance, we find that there were significant changes in the community structure of bacteria of ampicillin pretreatment renal transplantation mice compared to the renal transplantation group, with weighted UniFrac accommodate (R^2 = 0.5793; P =0.0022) (Figures 5F, G). Next, we use a tree branch structure to describe and compare the similarity and differences between multiple samples. The multi-sample clustering tree based on Bray Curtis suggested that the structure of mucosal flora in the two groups was significantly different (Figure 5H).

Ampicillin pretreatment affects the species composition at the phylum and genus levels in renal transplantation mice

The two groups of samples included 20 phyla. The four phyla with the highest abundance in the ampicillin pretreatment group were *verrucomicrobia* (47.57%), *Bacteroidetes* (39.63%), *Proteobacteria* (11.45%) and *Firmicutes* (1.03%). In the control group, *verrucomicrobia* accounted for 15.15%, *Bacteroides* accounted for 20.57%, *proteobacteria* accounted for 9.50% and *Firmicutes* accounted for 43.22% (Figure 6A). Metastatic analysis showed that there were significant differences in three phylum including *Bacteroidetes*, *Firmicutes*, and *verrucomicrobia* between the two groups (Figures 6B-D). Ampicillin pretreatment significantly increased the abundance of *Bacteroides* and *verrucomicrobia* and decreased the abundance of *Firmicutes* at the phylum level.

By comparing with the Silva database, we found 260 genera in 12 samples. Most dominant bacteria in the control group and ampicillin pretreatment group belong to *Firmicutes* and *Proteobacteria* (Figure 6E). We plotted the 20 dominant bacterial genera in the two groups into a heat map (Figure 6F). Ampicillin pretreatment significantly increased the abundance of *akkermansia*, *Klebsiella*, and *Escherichia-Shigella* at the genus level, and decreased the abundance of *Lactobacillus*.

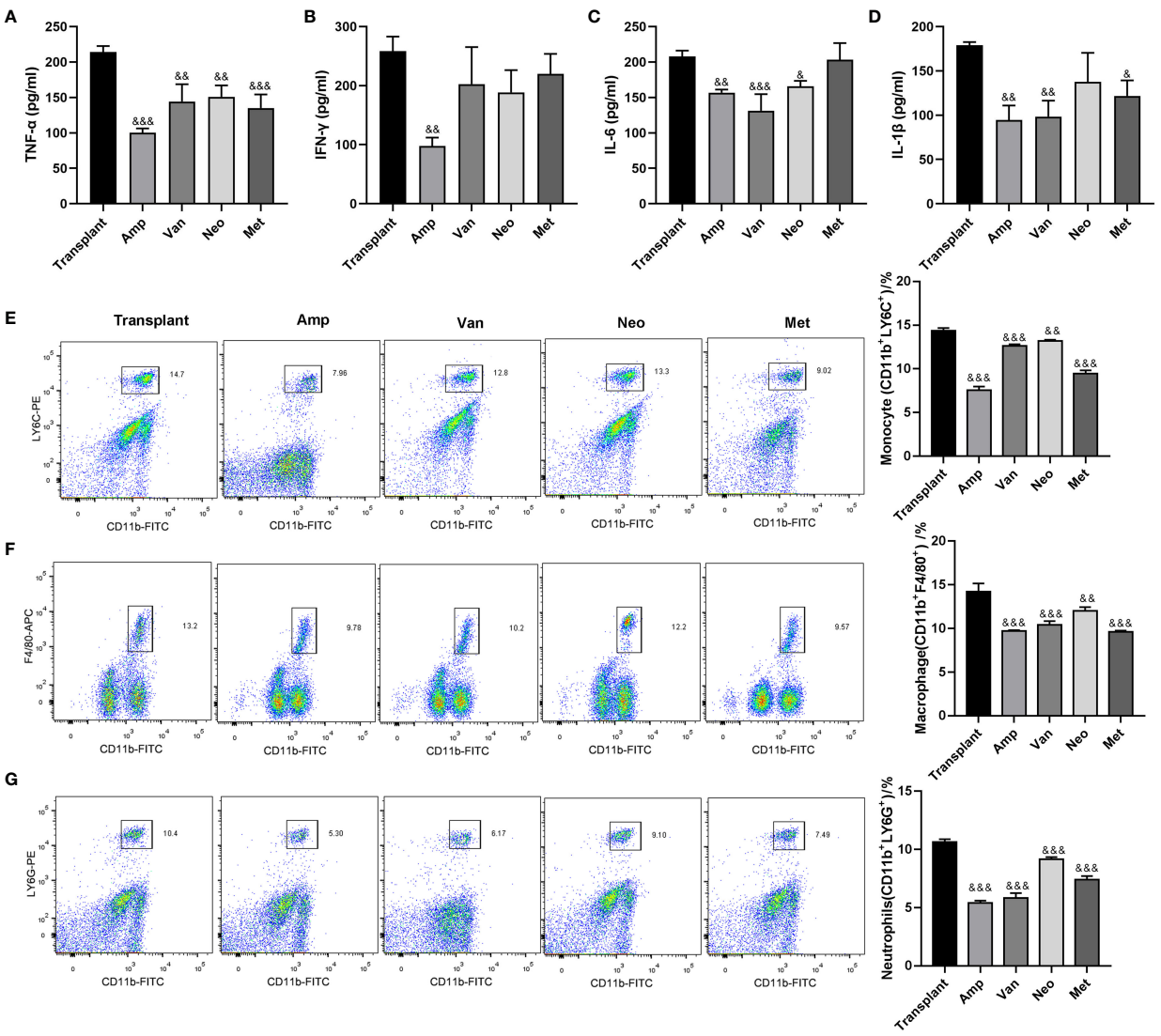


FIGURE 4
Effect of single antibiotic on inflammatory response in renal transplantation mice. **(A-D)** ELISA was used to detect the expression levels of TNF-α、IFN-γ、IL-6 and IL-1β in the transplanted renal. **(E-G)** Flow cytometry used to detect the infiltration of inflammatory cells (monocytes, macrophages, neutrophils) in the transplanted renal. $^{\&}p < 0.05$, $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$ vs transplant group.

Ampicillin pretreatment regulates species abundance in kidney transplantation mice

To identify high-dimensional biomarkers in the gut microbiota in experiment mice, we performed the line discriminant analysis (LDA) effect size (LEfSe) method. The LDA score was set to 3.0, and different species with an LDA score >3 were considered to be important gut microbiota biomarkers. Figure 7A showed the potential microbiota biomarkers at different taxonomic levels in the ampicillin pretreatment group and the most differential biomarkers at the genus level were *Akkermansia*, *Klebsiella*, and *Lactobacillus*. Cladograms of multiple taxonomic level differentiating biomarkers were shown in Figure 7B, illustrating bacterial taxa representation between the transplant group and the ampicillin pretreatment group.

Discussion

Graft injury caused by organ transplantation mainly includes immune rejection and ischemia-reperfusion injury. The injury process is mostly completed by the recipient's

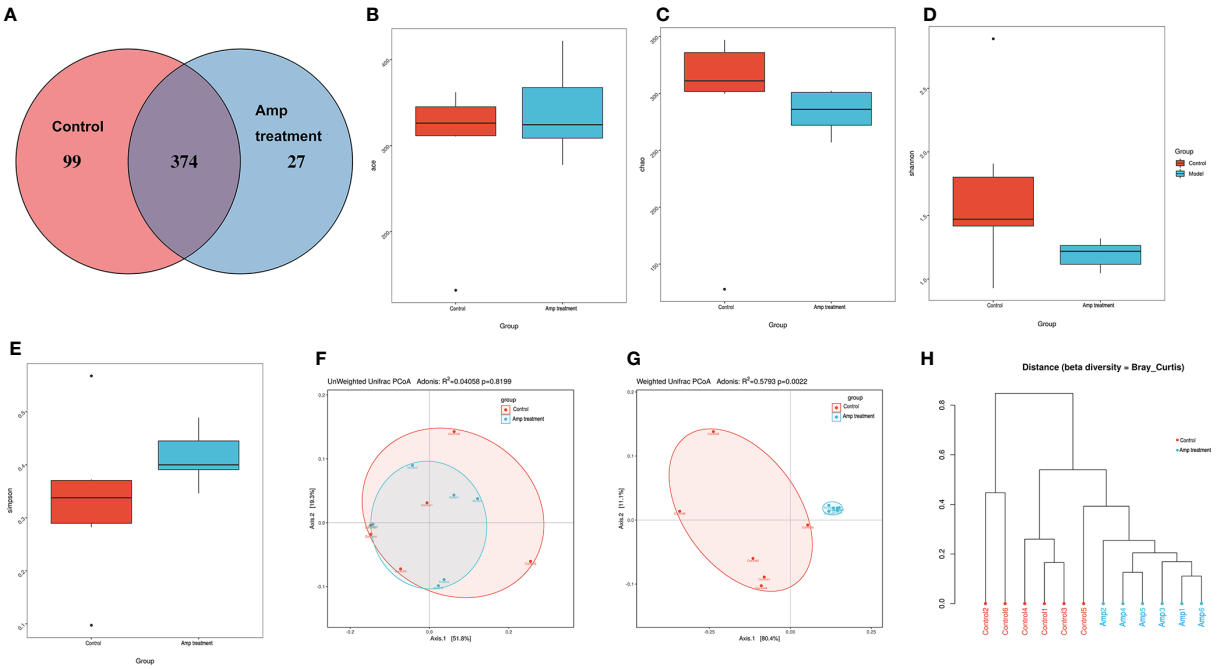


FIGURE 5
Effect of ampicillin on intestinal microflora diversity in renal transplantation mice. **(A)** OTU Venn diagram. Each note represents a group (n=6), and the number of the overlapping part between the color graphs refers to the total number of OTUs between the two groups, while the non-overlapping part refers to the number of OTUs unique to each group. **(B-E)** Difference analysis of alpha diversity index between the two groups. **(B)** ace, P-value: 0.8182. **(C)** chao, P-value: 0.132. **(D)** Shannon, P-value: 0.132. **(E)** simpson, P-value: 0.065. **(F, G)** Difference analysis of beta diversity index between the two groups using principal coordinate analysis (PCoA). PCoA plots using weighted-UniFrac metric and unweighted-UniFrac metric. **(H)** Tree view of similarity of multiple samples.

inflammatory cells infiltrating the graft and secreting inflammatory injury factors (Yu et al., 2013). Our results suggested that ampicillin pretreatment may inhibit inflammatory cell infiltration after renal transplantation by regulating the proportion of intestinal flora in mice, to reduce renal injury and play a role in renal protection. The results showed that after antibiotic pretreatment, the expression of inflammatory factors including TNF-α, IFN-γ, IL-6, and IL-1β in the graft of mice undergoing renal transplantation decreased significantly, and the infiltration of inflammatory cells including monocytes, macrophages and neutrophils in the graft decreased significantly, indicating that antibiotic pretreatment can inhibit the infiltration of inflammatory cells in renal transplantation. Furthermore, we found that ampicillin pretreatment could also reduce the expression of inflammatory factors and inflammatory cell infiltration in the grafts.

The intestinal tract is the reservoir of human bacteria, parasitizing more than 1000 kinds of bacteria. Bacterial ectopia induced by mucosal barrier damage and intestinal flora disorder is an important factor leading to infection and systemic inflammatory response. The disorder of intestinal flora not only disturbs the normal functions of intestinal metabolism and nutrition absorption, but also may lead to infection and disturb the normal immune system (Guo et al., 2017). The human gut microbiome is been influenced by many factors, including the host's genetic factors, dietary habits and drugs. When antibiotics first emerged in the 1940s, it was been called "miracle drugs". Antibiotics have saved countless lives, but almost all antibiotics cause diarrhea through dysfunction in the gastrointestinal tract, including secretion, digestion, absorption and movement. Antibiotics cause dysregulation of normal intestinal flora, including a substantial reduction in physiological bacteria, and an increase in the reproduction of conditional pathogens (Eyler and Shvets, 2019). Penicillins, sulfonamides, carbapenems, cephalosporins, quinolones, macrolides and aminoglycosides could kill pathogens that cause infection. At this cost, symbiotic bacteria in the human body also suffer "heavy casualties". Research shows that the antibiotic-sensitive bacteria in patients receiving broad-spectrum antibiotics for a long time are largely been killed, while the insensitive bacteria take the opportunity to reproduce, causing re-infection. And patients are more likely to suffer from obesity, diabetes, asthma, inflammatory bowel disease and other diseases after heavy antibiotic use (Buffie and Pamer, 2013). Antibiotics have saved countless lives, but with the widespread use of antibiotics, people also pay more attention to the side effects of antibiotics (antibiotic-associated diarrhea). In recent years,

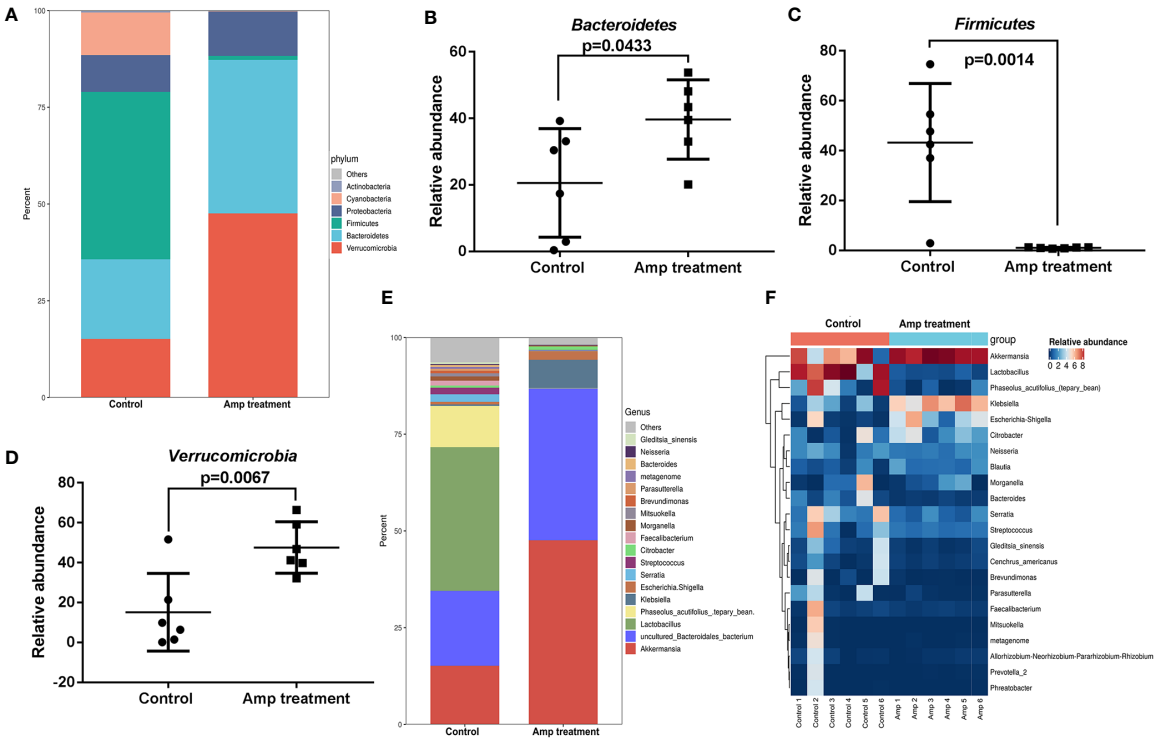


FIGURE 6
Comparison of species composition of intestinal microflora between the two groups at phylum and genus levels. **(A)** Histograms of intestinal mucosal phylum composition in two groups of mice. **(B-D)** Scatter diagram of three phyla in intestinal mucosa of two groups of mice. **(E)** Histograms of intestinal mucosal genus composition in two groups of mice. **(F)** Heat maps of 20 dominant genus in the two groups.

clinical studies have shown that the main treatment for antibiotic-associated diarrhea is probiotics and fecal microbiota transplantation based on conventional antibiotics (such as vancomycin, metronidazole, etc.). Although probiotics therapy and fecal microbiota transplantation have achieved initial results, the mechanism is still unclear, and there are still potential safety hazards (Mekonnen et al., 2020). Therefore, medical researchers will continue to look for effective alternative treatments.

To clarify antibiotics affect the inflammatory response after renal transplantation by regulating the intestinal flora, we performed a 16S rDNA sequencing analysis on the intestinal mucosal tissues of renal transplantation mice and ampicillin pretreated renal transplantation mice. Principal coordinate analysis and similarity clustering tree showed that there were significant differences between the two groups, suggesting that ampicillin pretreatment significantly affected the intestinal flora. Our results showed that *Verrucomicrobia, Bacteroidetes, Proteobacteria and Firmicutes* were the four most abundant bacterial groups in the intestinal mucosal tissue samples of the control group and amp treatment group. As reported in a previous study, species in *Firmicum* and *Bacteroides* accounted for more than 90% of the total intestinal microbiota (Human Microbiome Project C, 2012). Renal transplantation seems to have significantly changed the composition of the body's major intestinal microorganisms. Although the impact of changes in intestinal microbial components on solid organ transplantation is still controversial, studies have shown that the bacterial species in these four phyla can affect the side effects of organ transplantation. For example, some bacteria (such as *Clostridium difficile, Enterococcus faecium*, and *Streptococcus*) belonging to *Firmicutes* can infect recipients of solid organ transplantation and are an important cause of side effects (diarrhea, bloodstream infection, pneumonia) after solid organ transplantation in this population (Mullane et al., 2019; Roca-Oporto et al., 2019; Mercuro et al., 2020). Compared with renal transplant recipients without diarrhea, renal transplant recipients with diarrhea have a lower abundance of *Bacteroides* (Lee et al., 2014). Proteus composed of a variety of Gram-negative bacteria (such as *Klebsiella pneumoniae, Pseudomonas aeruginosa and Escherichia coli*) is a risk factor for increased infection, bacteremia and mortality in solid organ transplant recipients (Bodro et al., 2015; Geladari et al., 2017; Ville et al., 2019; Wu et al., 2020). Further analysis of the samples of the two groups at the genus level showed that compared with the control group, the ampicillin pretreatment group has an increased abundance of *akkermansia, Escherichia-Shigella and Klebsiella* in the intestinal tract, while the abundance of *Lactobacillus* decreased

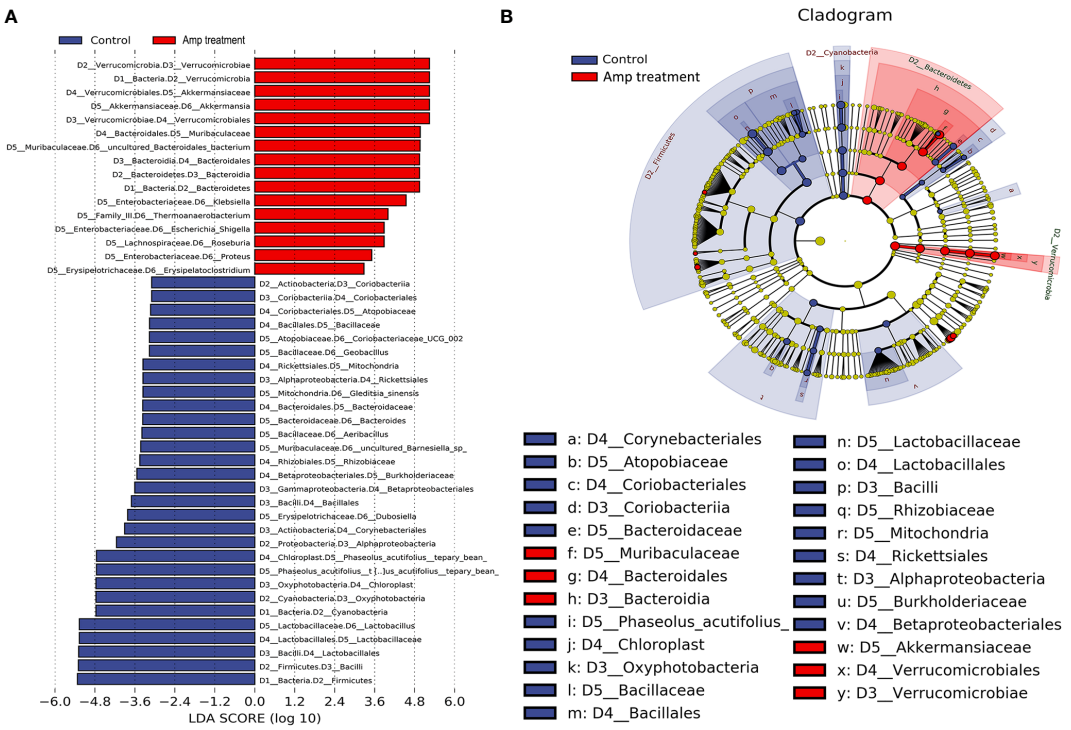


FIGURE 7
Line discriminant analysis (LDA) effect size (LEfSe) histograms and cladograms between two groups. **(A)** The histogram of LDA value distribution (LDA Score >3) shows the genus with significant differences between control and Amp treatment group. **(B)** The LEfSe cladogram shows the evolutionary relationships between genus with significant differences between thecontrol and Amp treatment group.

significantly. Whether these bacteria with significant changes in expression abundance can cause disease, this needs further study.

Although no research on renal transplantation has reported, whether ampicillin or other antibiotics will change the intestinal flora to induce infection and rejection, and whether the application of probiotics can improve the intestinal flora and clinical prognosis of renal transplantation patients deserve further study, which is also the next direction of our research.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/sra/, PRJNA893084.

Ethics statement

The animal study was reviewed and approved by Henan Provincial People's Hospital.

Author contributions

XiW designed the study and wrote the paper. All authors participated in the experiments and analyzed the data. All authors contributed to the article and approved the submitted version.

Funding

This work supported by the Henan Provincial Medical Scientific and Technological Research Project (No. LHGJ20200046); the Key Scientific Research Projects in Universities of Henan Province (No.22A320012).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Bodro, M., Sabe, N., Tubau, F., Llado, L., Baliellas, C., Gonzalez-Costello, J., et al. (2015). Extensively drug-resistant pseudomonas aeruginosa bacteremia in solid organ transplant recipients. *Transplantation* 99, 616–622. doi: 10.1097/TP.0000000000000366

Buffie, C., and Pamer, E. (2013). Microbiota-mediated colonization resistance against intestinal pathogens. *Nat. Rev. Immunol.* 13, 790–801. doi: 10.1038/nri3535

Eyler, R. F., and Shvets, K. (2019). Clinical pharmacology of antibiotics. *Clin. J. Am. Soc. Nephrol.* 14, 1080–1090. doi: 10.2215/CJN.08140718

Freifeld, A. G., Bow, E. J., Sepkowitz, K. A., Boeckh, M. J., Ito, J. I., Mullen, C. A., et al. (2011). Clinical practice guideline for the use of antimicrobial agents in neutropenic patients with cancer: 2010 update by the infectious diseases society of America. *Clin. Infect. Dis.* 52, 427–431. doi: 10.1093/cid/ciq147

Geladari, A., Karampatakis, T., Antachopoulos, C., Iosifidis, E., Tsiatsiou, O., Politi, L., et al. (2017). Epidemiological surveillance of multidrug-resistant gram-negative bacteria in a solid organ transplantation department. *Transpl. Infect. Dis.* 19, e12686. doi: 10.1111/tid.12686

Ghani, R., Mullish, B., Roberts, L., Davies, F., and Marchesi, J. (2022). The potential utility of fecal (or intestinal) microbiota transplantation in controlling infectious diseases. *Gut Microbes* 14, 2038856. doi: 10.1080/19490976.2022.2038856

Gioco, R., Corona, D., Ekser, B., Puzzo, L., Inserra, G., Pinto, F., et al. (2020). Gastrointestinal complications after kidney transplantation. *World J. Gastroenterol.* 26, 5797–5811. doi: 10.3748/wjg.v26.i38.5797

Guo, Y., Yang, X., Qi, Y., Wen, S., Liu, Y., Tang, S., et al. (2017). Long-term use of ceftriaxone sodium induced changes in gut microbiota and immune system. *Sci. Rep.* 7, 43035. doi: 10.1038/srep43035

Human Microbiome Project C (2012). Structure, function and diversity of the healthy human microbiome. *Nature* 486, 207–214. doi: 10.1038/nature11234

Järbrink-Sehgal, E., and Andreasson, A. (2020). The gut microbiota and mental health in adults. *Curr. Opin. Neurobiol.* 62, 102–114. doi: 10.1016/j.conb.2020.01.016

Jiang, Y., Liu, Y., Gao, M., Xue, M., Wang, Z., and Liang, H. (2020). Nicotinamide riboside alleviates alcohol-induced depression-like behaviours in C57BL/6J mice by altering the intestinal microbiota associated with microglial activation and BDNF expression. *Food Funct.* 11, 378–391. doi: 10.1039/C9FO01780A

Lee, J. R., Muthukumar, T., Dadhania, D., Toussaint, N. C., Ling, L., Pamer, E., et al. (2014). Gut microbial community structure and complications after kidney transplantation: A pilot study. *Transplantation* 98, 697–705. doi: 10.1097/TP.0000000000000370

Lei, Y., Chen, L., Wang, Y., Stefka, A., Molinero, L., Theriault, B., et al. (2016). The composition of the microbiota modulates allograft rejection. *J. Clin. Invest.* 126, 2736–2744. doi: 10.1172/JCI85295

Liu, L., Wang, Z., Pang, X., Feng, Y., Wang, J., Xie, H., et al. (2019). Bortezomib ameliorates acute allograft rejection after renal transplant by inhibiting tfh cell proliferation and differentiation *via* miR-15b/IRF4 axis. *Int. Immunopharmacol.* 75, 105758. doi: 10.1016/j.intimp.2019.105758

Mekonnen, S. A., Merenstein, D., Fraser, C. M., and Marco, M. L. (2020). Molecular mechanisms of probiotic prevention of antibiotic-associated diarrhea. *Curr. Opin. Biotechnol.* 61, 226–234. doi: 10.1016/j.copbio.2020.01.005

Mercuro, N. J., Gill, C. M., Kenney, R. M., Alangaden, G. J., and Davis, S. L. (2020). Treatment and outcomes of enterococcus faecium bloodstream infections in solid organ transplant recipients. *Transpl. Infect. Dis.* 22, e13251. doi: 10.1111/tid.13251

Mullane, K. M., Dubberke, E. R., and Practice, A. I. C. O. (2019). Management of clostridioides (formerly clostridium) difficile infection (CDI) in solid organ transplant recipients: Guidelines from the American society of transplantation community of practice. *Clin. Transplant.* 33, e13564. doi: 10.1111/ctr.13564

Roca-Oporto, C., Cebrero-Cangueiro, T., Gil-Marques, M. L., Labrador-Herrera, G., Smani, Y., Gonzalez-Roncero, F. M., et al. (2019). Prevalence and clinical impact of streptococcus pneumoniae nasopharyngeal carriage in solid organ transplant recipients. *BMC Infect. Dis.* 19, 697. doi: 10.1186/s12879-019-4321-8

Swarte, J. C., Douwes, R. M., Hu, S., Vich Vila, A., Eisenga, M. F., van Londen, M., et al. (2020). Characteristics and dysbiosis of the gut microbiome in renal transplant recipients. *J. Clin. Med.* 9, 386. doi: 10.3390/jcm9020386

Tilg, H., Adolph, T. E., Gerner, R. R., and Moschen, A. R. (2018). The intestinal microbiota in colorectal cancer. *Cancer Cell* 33, 954–964. doi: 10.1016/j.ccell.2018.03.004

Ville, S., Ydee, A., Garandeau, C., Canet, E., Tissot, A., Cantarovich, D., et al. (2019). Shiga toxin-producing escherichia coli-associated hemolytic uremic syndrome in solid organ transplant recipients. *Kidney Int.* 96, 1423–1424. doi: 10.1016/j.kint.2019.08.024

Wu, D., Chen, C., Liu, T., and Wan, Q. (2020). Risk factors for acquisition of carbapenem-resistant klebsiella pneumoniae and mortality among abdominal solid organ transplant recipients with k. pneumoniae infections. *Med. Sci. Monit.* 26, e922996. doi: 10.12659/MSM.922996

Xu, J., and Yang, Y. (2021). Gut microbiome and its meta-omics perspectives: profound implications for cardiovascular diseases. *Gut Microbes* 13, 1936379. doi: 10.1080/19490976.2021.1936379

Yu, T., Wen, M., Li, C., Cheng, C., Wu, M., Chen, C., et al. (2013). Expression of hypoxia-inducible factor-1α (HIF-1α) in infiltrating inflammatory cells is associated with chronic allograft dysfunction and predicts long-term graft survival. *Nephrol. Dial. Transplant.* 28, 659–670. doi: 10.1093/ndt/gfs377

Zhao, H., Alam, A., Soo, A. P., George, A. J. T., and Ma, D. (2018). Ischemia-reperfusion injury reduces long term renal graft survival: Mechanism and beyond. *EBioMedicine* 28, 31–42. doi: 10.1016/j.ebiom.2018.01.025

Zhou, B., Yuan, Y., Zhang, S., Guo, C., Li, X., Li, G., et al. (2020). Intestinal flora and disease mutually shape the regional immune system in the intestinal tract. *Front. Immunol.* 11, 575. doi: 10.3389/fimmu.2020.00575

Zimmermann, P., and Curtis, N. (2019). The effect of antibiotics on the composition of the intestinal microbiota - a systematic review. *J. Infect.* 79, 471–489. doi: 10.1016/j.jinf.2019.10.008

frontiers | Frontiers in Cellular and Infection Microbiology

TYPE Original Research
PUBLISHED 21 November 2022
DOI 10.3389/fcimb.2022.1030588

OPEN ACCESS

EDITED BY
Qinglong Wu,
Baylor College of Medicine,
United States

REVIEWED BY
Cecilia Ximenez,
Universidad Nacional Autónoma de México, Mexico
Yina Huang,
Anhui Polytechnic University, China

*CORRESPONDENCE
Lei Bao
1021004676@qq.com
Lu-Quan Li
Liluquan123@163.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Intestinal Microbiome, a section of the journal Frontiers in Cellular and Infection Microbiology

RECEIVED 29 August 2022
ACCEPTED 04 November 2022
PUBLISHED 21 November 2022

CITATION
Xiong J, Liao X-S, Yin T, Liu X-C, Bao L and Li L-Q (2022) Alterations of the gut microbiota and short chain fatty acids in necrotizing enterocolitis and food protein-induced allergic protocolitis infants: A prospective cohort study.
Front. Cell. Infect. Microbiol. 12:1030588.
doi: 10.3389/fcimb.2022.1030588

Alterations of the gut microbiota and short chain fatty acids in necrotizing enterocolitis and food protein-induced allergic protocolitis infants: A prospective cohort study

Jing Xiong[1†], Xing-Sheng Liao[2†], Tong Yin[1], Xiao-Chen Liu[1], Lei Bao[1*] and Lu-Quan Li[1*]

[1]Neonatal Diagnosis and Treatment Center of Children's Hospital of Chongqing Medical University, National Clinical Research Center for Child Health and Disorders, Ministry of Education Key Laboratory of Child Development and Disorders, International Science and Technology Cooperation Base of Child Development and Critical Disorders, Chongqing Key Laboratory of Pediatric, Chongqing, China, [2]Department of Neonatology, The first People's Hospital of Jiulongpo District, Chongqing, China

Background: Even though presenting with similar clinical manifestations, necrotizing enterocolitis (NEC) and food protein-induced allergic protocolitis (FPIAP) have completely different treatments and prognosis. Our study aimed to quantify and evaluate differences in gut microbiota and short chain fatty acids (SCFAs) between infants with NEC and FPIAP to better identify these two diseases in clinical settings.

Methods: A total of 43 infants with NEC or FPIAP in Children's Hospital of Chongqing Medical University, China between December 2020 and December 2021 were enrolled. Stool samples were prospectively collected and froze. Infants defined as NEC were those who presented with clinical courses consistent with NEC and whose radiographs fulfilled criteria for Bell's stage 2 or 3 NEC, while those who were healthy in appearance and had blood in the stool (visible or may be microscopic), had normal bowel sounds in physical examination, were resolved after eliminating the causative food, and/or had recurrence of symptoms after oral food challenge (OFC) were defined as FPIAP. Primers specific for bacterial 16S rRNA genes were used to amplify and pyrosequence fecal DNA from stool samples. Gas chromatography-mass spectrometry (GC-MS) technology was used to determine the concentrations of SCFAs.

Results: Among the 43 infants, 22 were diagnosed with NEC and 21 were diagnosed with FPIAP. The microbial community structure in NEC infant stools differed significantly from those in FPIAP infant stools. NEC infants had significantly higher proportion of Actinobacteria and reduced proportion of Bacteroidetes compared with FPIAP infants, and the proportions of

Halomonas, Acinetobacter, Bifidobacterium, and *Stenotrophomonas* in NEC infants were significantly higher than that of FPIAP infants. In addition, infants with NEC had significantly lower levels of acetic acid, propionic acid, butyric acid, isovaleric acid, and total SCFAs, and higher level of hexanoic acid as compared to the infants of the FPIAP group.

Conclusions: The differences of gut microbiota composition and concentrations of SCFAs might represent suitable biomarker targets for early identification of NEC and FPIAP.

KEYWORDS

necrotizing enterocolitis, food protein-induced allergic protocolitis, gut mictobiota, short chain fatty acids, newborn

Introduction

Necrotizing enterocolitis (NEC) is a destructive gastrointestinal disease that occurs primarily in preterm infants with high morbidity and mortality. It is associated with intestinal inflammation driven by microbiota and is characterized by an exaggerated inflammatory response and necrosis to the intestine resulting in the loss of intestinal barrier integrity and eventually multiple organ failure (Lu et al., 2014; Hackam and Caplan, 2018). A US-nationwide study reported that NEC affects about 8.9% of extremely preterm infants, among them 3.9% infants require surgery (Bell et al., 2022). According to a study by a UK specialist centre, the mortality for surgical NEC is 18.9% (Calvert et al., 2021). Common symptoms of NEC include gastric retention of enteral feedings, abdominal distension, and blood per rectum (Sowden et al., 2022). The onset of the disease is usually fulminant. Antibiotic therapy is usually used and therapeutic strategies for severe cases are limited and often useless.

Food protein-induced allergic proctocolitis (FPIAP) is a non-IgE-mediated gastrointestinal disorder with rising prevalence in food allergy. FPIAP is commonly caused by severe allergic reactions in the digestive system and has some overlapping clinical features with NEC, including hematochezia and diarrhea (Ohtsuka, 2015). The management of FPIAP relies upon avoidance of dietary triggers, with interval challenge to assess for resolution, which usually occurs in the first years of life (Feuille and Nowak-Węgrzyn, 2015). The exact prevalence of FPIAP is not well established. Study from North American revealed that the cumulative incidence of FPIAP was 17% over 3 years (Martin et al., 2020). Conversely, a large study of an Israeli birth cohort reported that the overall prevalence of FPIAP was low, at 0.16% (Elizur et al., 2012). Due to the lack of specific biomarkers, the diagnosis of FPIAP is mainly done by clinical history.

Intestinal dysbiosis has been proposed as one of the possible factors involved in the pathogenesis of NEC (Lindberg et al., 2020; Tarracchini et al., 2021; Thänert et al., 2021). Early life microbiota disruption had also been proven to be related to the development of metabolic disorders and allergies (Savage et al., 2018). Various studies reported that fecal microbiome from infants with NEC had increased relative abundances of Proteobacteria and Klebsiella and deceased relative abundances of Firmicutes and Bacteroidetes (Pammi et al., 2017a; Olm et al., 2019). While study described elevated relative abundances of Firmicutes and Bacteroidetes in FPIAP infants (Berni Canani et al., 2018a). Short chain fatty acids (SCFAs), mainly acetic acid, propionic acid and butyric acid, are the products of bacterial fermentation of carbohydrates in the intestines. Disruption in gut microbiota could subsequently cause the metabolic disorder of SCFAs.

NEC and FPIAP are two major diseases in preterm infants with overlapping clinical features but totally different treatment regimens. However, there is limited data in the literature comparing the gut microbiota and SCFAs between the NEC and FPIAP infants. Therefore, we firstly conducted this study to compare the gut microbiome and SCFAs of infants with NEC or FPIAP at a single tertiary center. We hypothesized that there would be differences in the gut microbial and SCFAs patterns between NEC and FPIAP infants. These differences might represent suitable biomarker targets for early identification of NEC and FPIAP.

Materials and methods

Standard protocol approval, registration, and patient consent

The study was approved by institutional review board for human studies of the Children's Hospital of Chongqing Medical University (project approval No. 2020104) and registered at

Chinese Clinical Trial Registry (Identifier: ChiCTR2000034672) (registration date, July 15, 2020). The study was designed to be prospective protocol, and informed consent was obtained from patient parents.

Patient characteristics and sample collection

This prospective cohort trial was conducted in the tertiary referral NICU of Children's Hospital of Chongqing Medical University between December 2020 and December 2021. A total of 168 neonates who presented clinical features of abdominal distension or hematochezia were recruited and the fecal samples of all the final included neonates were collected. All of the following neonates were excluded from the study: 1. neonates born with congenital intestinal disorders, 2. neonates who received probiotics before recruitment, 3. neonates whose parents refused the treatments, 4. neonates whose parents insist to discharge, 5. spontaneous intestinal perforation without radiographic evidence of NEC, and 6. neonates who lost the follow-up. In the end, 22 of the 168 recruited infants were diagnosed with NEC, while 21 of the 168 recruited infants were diagnosed with FPIAP.

Fecal samples of NEC or FPIAP patients were collected as soon as possible after the diagnosis was made within 24 hours. The samples were collected directly from the diapers by the nursing stuff using a sterile cotton swab, and then were placed into a sterile DNAase-, RNAase-, Eppendorf tube. All samples were frozen and stored at -80°C until processed.

Case definition and clinical management

NEC was diagnosed based on the Vermont Oxford Network criteria (Vermont Oxford Network database,) and staged according to Bell's modified staging criteria (Kliegman and Walsh, 1987). The diagnosis of FPIAP is based on a careful and detailed history (including diet records), clinical manifestation of being healthy in appearance and being the presence of blood in the stool (visible or may be microscopic), physical examination of normal bowel sounds, remission of symptoms after eliminating the causative food, and/or recurrence of symptoms after oral food challenge (OFC) (Burks et al., 2012; Nowak-Węgrzyn et al., 2015; Meyer et al., 2020; Senocak et al., 2022). In addition, it is important to rule out other causes of blood in the stools in infancy such as anal fissures or infectious gastroenteritis (Thompson et al., 1996).

Demographic and clinical variables

Neonatal and maternal medical record data were extracted from the medical record management system. Neonatal factors including: gestational age (GA), birth weight (BW), gender, age at sample collection, age at NEC/FPIAP diagnosis, antibiotic therapy before NEC/FPIAP diagnosis, and dietary information (type of feeding). Maternal factors including: mode of delivery, premature prolonged rupture of membranes (PPROM), meconium-staining amniotic fluid, intrauterine fetal distress, maternal hypertension, maternal diabetes, and use of antenatal corticosteroids.

DNA extraction and 16S rRNA gene sequencing analysis

DNA of each sample was extracted using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) following the manufacturer's instructions. The quality and concentration of DNA were determined by 1.0% agarose gel electrophoresis and a NanoDrop® ND-2000 spectrophotometer (Thermo Scientific Inc., USA) and kept at -80 °C prior to further use. The V3-V4 region of 16S rRNA gene was amplified using primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) by an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA). For each extracted DNA sample, The PCR reaction mixture contains 4 μL 5 × Fast Pfu buffer, 2 μL 2.5 mM dNTPs, 0.8 μL each primer (5 μM), 0.4 μL Fast Pfu polymerase, 10 ng of template DNA, and ddH2O to a final volume of 20 μL. PCR was performed with the following conditions: initial denaturation at 95 °C for 3 min, followed by 27 cycles of denaturing at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 45 s, and single extension at 72 °C for 10 min, and end at 4 °C. All samples were amplified in triplicate.

PCR products purification

The amplification products were separated by 2% agarose gel electrophoresis, purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to manufacturer's instructions, and quantified with a Quantus™ Fluorometer (Promega, USA).

Library preparation and sequencing

DNA library preparation was performed using the TruSeq DNA PCR-Free Sample Preparation Kit (Illumina, San Diego, CA), following the manufacturer's instructions. Sequencing was performed on Novaseq6000 instrument (Illumina, San Diego, CA), following the manufacturer's instructions.

Bioinformatic analysis

Bioinformatic analysis of the gut microbiota was carried out using the Majorbio Cloud platform (https://cloud.majorbio.

com). Sequences were divided into operational taxonomic units (OTUs) using similarity levels with a cutoff of 97% similar. Bacterial OTU representative sequences were assigned to a taxonomic lineage by a Ribosomal Database Project (RDP) classifier version 2.2 against the 16S rRNA gene database (Silva v138). Based on the OTUs information, rarefaction curves and α-diversity indices including Shannon index, Simpson index, Ace index, and Chao1 index were calculated with Mothur v1.30.1 (Schloss et al., 2009). The similarity among the microbial communities in different samples was determined by principal coordinate analysis (PCoA) based on Bray-curtis dissimilarity using Vegan v2.5-3 package. The linear discriminant analysis (LDA) effect size (LEfSe) (http://huttenhower.sph.harvard.edu/LEfSe) was performed to identify the significantly abundant taxa (phylum to genera) of bacteria among the different groups (LDA score≥4, $P < 0.05$) (Segata et al., 2011). Correlation heatmap was conducted to explore the relationship between SCFAs concentrations and the gut microbiota composition based on *Spearman rank correlation* in R.

SCFAs analysis

Fecal SCFAs concentration was determined by using gas chromatography-mass spectrometry (GC-MS) technology (Termo TRACE 1310-ISQ LT, America) as follows: Briefy, fecal pellets were ground twice for three minutes, placed in an ice bath for 30 minutes, held at 4°C for 30 minutes, and centrifuged at 13,000 rpm, at 4 °C for 15 minutes. In addition, ethyl acetate was added to SCFAs (including acetic, propionic, butyric, isovaleric, hexanoic acid, and the total SCFAs) and 2-ethylbutyric acid to obtain standard concentration gradients. Then, a small sample of the supernatant (1 μL) and the standard solution were injected into the column and used for detection by GC-MS. Lastly, Masshunter quantitative software (version10.0.707.0; Palo Alto, USA) was used to automatically identify and integrate target SCFAs. The SCFAs concentrations of each sample were calculated based on standard curves.

Statistical analysis

All data were analyzed using SPSS version 24.0 software (SPSS Inc., USA). Data exhibiting a normal distribution were described as the mean with standard deviation (SD) and were analyzed by means of Student's t-test or one-way analysis of variance (ANOVA). Non-normally distributed measurement data were presented as the median (interquartile range) and were analyzed by means of the Wilcoxon rank-sum test. Categorical data were compared using chi-square tests or Fisher's exact test, when appropriate. Receiver operating characteristic (ROC) curves and all figures were generated with GraphPad Prism (version 9.0; California, USA). Two sided P values < 0.05 were considered statistically significant.

Results

Subjects

A total of 168 infants presenting abdominal distension or hematochezia over one year were enrolled prospectively. 22 (13.1%) of 168 infants developed NEC, while 21 (12.5%) of these infants developed FPIAP (Figure 1). The basic clinical characteristics are shown in Table 1. The median age of onset of NEC was 11.6 days (interquartile range [IQR], 6.8-16.0 days), while that of FPIAP was 15.2 days (IQR, 11.0-22.0 days). In NEC infants, 9.1% of them were exclusively breast-fed, 72.7% were fed with cow's milk (CM)-based formula, and 18.2% were fed by both breast milk and CM-based formula before the onset of symptoms. While the percentages of these three feeding patterns in FPIAP infants were 19.1%, 61.9%, and 19.0%, respectively.

Characterization of gut microbiota

DNA sequences from the 43 fecal samples were analyzed, with a total of 4,438,641 sequences and an average length of 465 base pairs (bps). The rarefaction curves based on sobs index, the Shannone curves based on Shannon index, and the species accumulation curves of each group demonstrated that the sequencing data and depth, and the sample size were sufficient (Supplementary Figure S1).

A Venn diagram was used to indicate the differences of bacterial populations between the two groups. The Venn diagram revealed that 592 OTUs were shared between the NEC and FPIAP groups, while 1550 and 102 OTUs were unique to NEC infants and FPIAP infants, respectively (Figure 2A). Suggesting that the richness of gut microbiota in NEC infants was higher than that of the FPIAP infants, and there were shared or specific gut microbiota between the two groups. As shown in the circos plot, Firmicutes, Proteobacteria, Actinobacteriota, and Bacteroidota were dominant in both NEC and FPIAP infants at the phylum level (Figure 2B). The community abundance on genus level of NEC and FPIAP infants was shown in Figures 2C, D, which showed noticeable discrepancies in community structure between infants with NEC and FPIAP (Figures 2C, D).

Microbial diversity

The α-phylogenetic diversity indexes were analyzed to explore the community richness and diversity in two groups. No significant differences were observed between the two groups

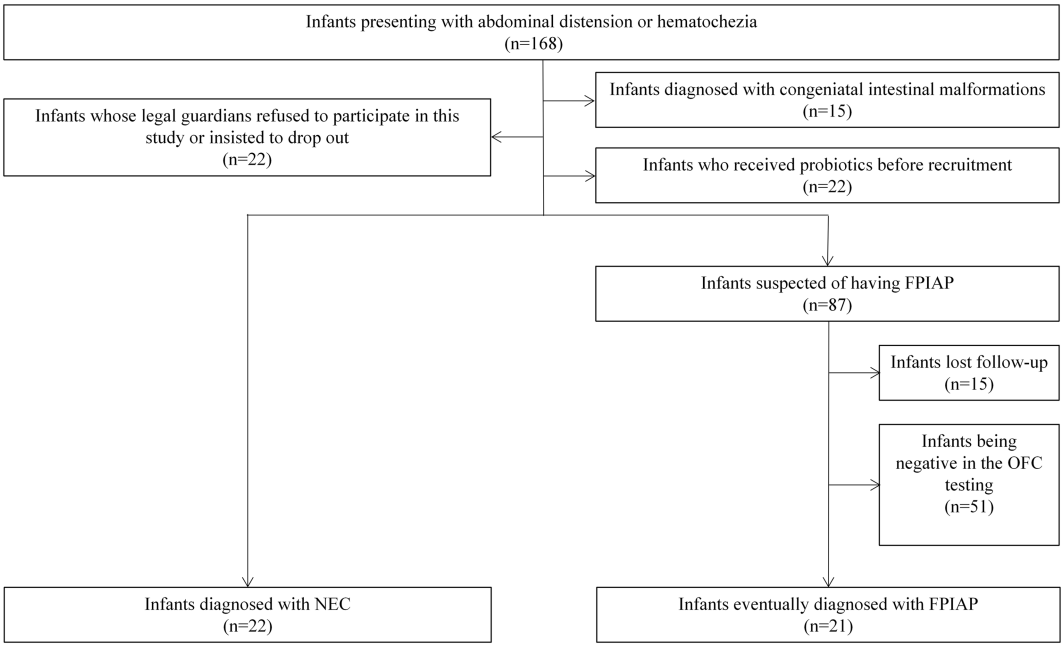


FIGURE 1
Flow diagram of the inclusion and exclusion processes of the study.

in terms of Shannon, Simpson, Ace, and Chao1 indexes (Figure 3A). The overall microbial structure was then analyzed in each group at the phylum and genus levels. The results showed that Firmicutes, Proteobacteria, Bacteroidota, and Actinobacteriota were the most abundant bacteria in the two groups and constituted over 90% of the total bacteria at the phylum level. Infants in the NEC group had significantly higher proportion of Actinobacteriota and reduced proportion of Bacteroidota compared with infants in the FPIAP group (P<;0.05, Figure 4A). While no significant difference was

TABLE 1 Baseline characteristics of the newborns between the NEC and FPIAP groups.

	NEC (n=22)	FPIAP (n=21)	*P* value
Gestational age (weeks)	35.5 ± 2.2	36.5 ± 1.4	0.105
Birth weight (g)	2542.9 ± 699.9	2715 ± 482.3	0.355
Male, n (%)	17 (77.3)	14 (66.7)	0.438
Age at sample collection (days)	11.6 (6.8-16.0)	15.2 (11.0-22.0)	0.114
Age at NEC/FPIAP diagnosis (days)	11.6 (6.8-16.0)	15.2 (11.0-22.0)	0.114
Rupture of membranes (>18h), n (%)	8 (36.4)	4 (19.0)	0.206
Meconium-staining amniotic fluid, n (%)	2 (9.1)	1 (4.8)	0.578
Intrauterine fetal distress, n (%)	4 (18.2)	2 (9.5)	0.413
Maternal hypertension, n (%)	3 (13.6)	0 (0)	0.079
Maternal diabetes, n (%)	5 (22.7)	2 (9.5)	0.241
Prenatal use of corticosteroids, n (%)	7 (31.8)	2 (9.5)	0.072
Vaginal birth, n (%)	17 (77.3)	14 (66.7)	0.438
Feeding pattern before the onset of symptoms			
Breast feeding, n (%)	2 (9.1)	4 (19.1)	0.412
CM-based formula, n (%)	16 (72.7)	13 (61.9)	0.526
Mixed feeding, n (%)	4 (18.2)	4 (19.0)	1.000
Antibiotic therapy before NEC/FPIAP diagnosis, n (%)	9 (40.9)	3 (14.3)	0.052

Data are mean (SD), median (IQR), or n (%), unless otherwise specified. CM, cow's milk; NEC, necrotizing enterocolitis; FPIAP, food protein-induced allergic proctocolitis.

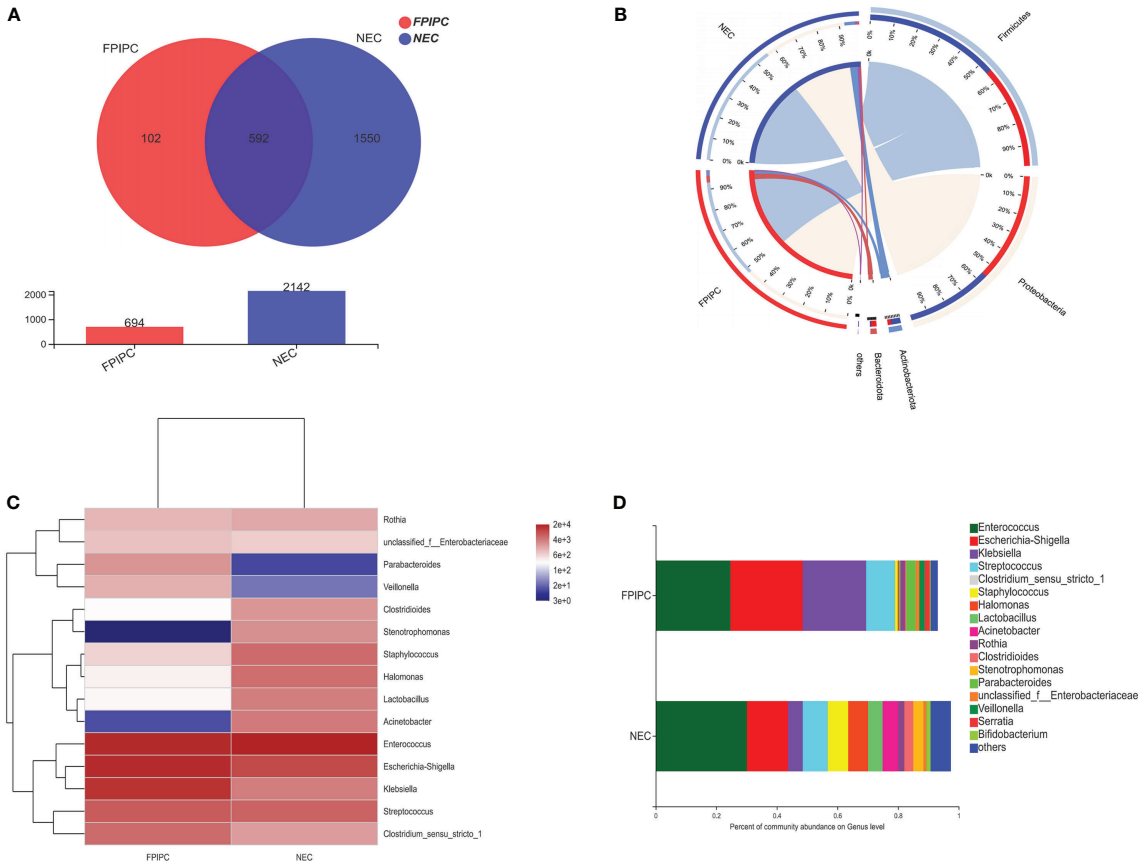


FIGURE 2
Shared and unique microbiota between the necrotizing enterocolitis (NEC) and food protein-induced allergic protocolitis (FPIAP) groups. **(A)** The Circos plot shows each main phyla between the NEC and FPIAP groups. Outer bars show the percentage of reads in a category that are connected to the category at the other end of the drawn band. **(B)** The heatmap displays genus-level changes (rows) between the samples of NEC and FPIAP groups (columns). The variation of each genus is indicated by a gradient of color from blue (decease) to red (increase). **(C)** Genus-level taxonomic composition of the NEC and FPIAP infants. Relative abundances are reported on the horizontal axis and the two groups on the vertical axis **(D)**.

observed between the two groups in terms of Firmicutes and Proteobacteria. At the genus level, infants in the NEC group had significantly higher proportions of *Halomonas, Acinetobacter, Bifidobacterium*, and *Stenotrophomonas* as compared to the infants of the FPIAP group ($P<;0.05$, Figure 4B).

Given these findings, principle coordinate analysis (PCoA) of unweighted UniFrac distances was used to estimate β-diversity of gut microbiota between the two groups. The NEC group had more variability compared with the FPIAP group. PCoA of unweighted UniFrac distance (quantitative, $R^2 = 0.0812$, $P = 0.001$) showed that the samples in the FPIAP group were ordinated closely, while the samples in the NEC group were separated obviously, indicating differences in bacterial structure in the NEC group (Figure 3B).

To determine the value of gut microbiota in identifying FPIAP and NEC in the early stage, ROC curves of Actinobacteriota, Bacteroidota, *Halomonas, Acinetobacter, Stenotrophomonas, and Bifidobacterium* were performed, and the area under curves (*AUCs)* were 0.6851, 0.7868, 0.8074, 0.8766, 0.7532, and 0.7814, respectively (Figure 5).

LEfSe analysis

Differential abundant phylotypes between the two groups were further evaluated by LEfSe using linear discriminant analysis (LDA) (LDA score≥4). This threshold guarantees that the meaningful taxa is compared and eliminates most of rare taxa. The figure generated in the LEfSe analysis (Figure 6A) shows the taxonomic groups with the largest differences between the two groups at various levels. The histogram (Figure 6B) shows the differences in 18 phylotypes between the two groups. At the family level, the abundance of Enterobacteriaceae in the fecal microbiota was higher in the FPIAP group, whereas the abundance of *Halomonadaceae, Lactobacillaceae, Moraxellaxceae*, and *Xanthomonadaceae* was

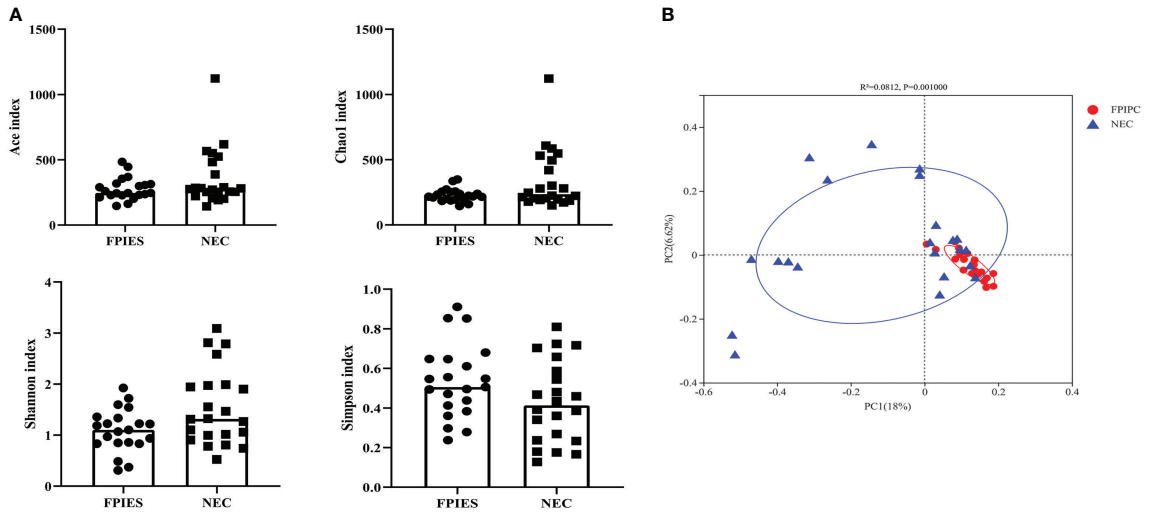


FIGURE 3
Alpha and beta diversity between the necrotizing enterocolitis (NEC) and food protein-induced allergic protocolitis (FPIAP) groups. There were no significant differences between the NEC and FPIAP groups in the ace index, Chao1 index, Shannon index, and Simpson index (P>0.05). **(A)** There was a significant difference in beta diversity between the NEC and FPIAP groups (P<;0.05). **(B)** N=22 for NEC and N=21 for FPIAP.

higher in the NEC group. There were four genus levels (*Halomonas, Lactobacillus, Acinetobacter*, and *Stenotrophomonas*) differences between the two groups, and the abundance of these four genus was higher in the NEC group as compared to the FPIAP group.

SCFAs production in NEC and FPIAP infants

In this study, GC-MS was used to investigate the concentrations of SCFAs in each sample. The results revealed that compared to the infants with FPIAP, infants with NEC had significantly lower levels of acetic acid, propionic acid, butyric acid, isovaleric acid, and total SCFAs, and higher level of hexanoic acid (all P<;0.05) (Figure 7).

ROC curves fore these metabolites were conducted to evaluate the value of SCFAs in the early identification of NEC and FPIAP. The results showed that the *AUCs* of acetic acid, butyric acid, hexanoic acid, isovaleric acid, propionic acid, and total SCFAs were 0.8398, 0.8593, 0.7576, 0.7641, 0.8680, and 0.8658, respectively (Figure 8).

Relationship between SCFAs and the gut microbiota

To explore the relationship between SCFAs and the gut microbiota, a heatmap was conducted as shown in Figures 9A, B. At the phylum level, propionic acid and butyric acid were all negatively correlated with Bacteroidota, Actinobacteriota, Proteobacteria, and Firmicutes, and acetic acid was negatively correlated with Firmucutes. In the contrary, propionic acid was positively correlated with Proteobacteria (P<;0.05) (Figure 9A). At the genus level, most SCFAs were negatively correlated with *Stenotrophomonas*, *Acinetobacter*, *Lactobacillus*, and *Halomonas*. In the contrary, acetic acid, propionic acid, and butyric acid were positively correlated with *Escherichia-Shigella*, and isobutyric acid was positively correlated with *Clostridioides* (P<;0.05) (Figure 9B).

Discussion

New biomarkers for the early identification of FPIAP and NEC are important. In this study, high throughput 16S rRNA gene sequencing and GC-MS techniques were used to compare the gut microbial profiles and diversity and metabolite characteristics in infants with NEC and FPIAP. The results confirmed that there are significant differences between the NEC and FPIAP groups in the main component of the gut microbiota, with differences between the two groups in species composition at different classification levels. In addition, significant differences were also observed between the two groups in terms of the fecal SCFAs concentrations, including acetic acid, propionic acid, butyric acid, isovaleric acid, hexanoic acid, and total SCFAs. These findings could provide value for the early identification of FPIAP and NEC in clinical settings.

At the phylum level, our study found that Firmicutes and Proteobacteria constituted the main component of intestinal microbiome in both NEC and FPIAP infants. As compared to

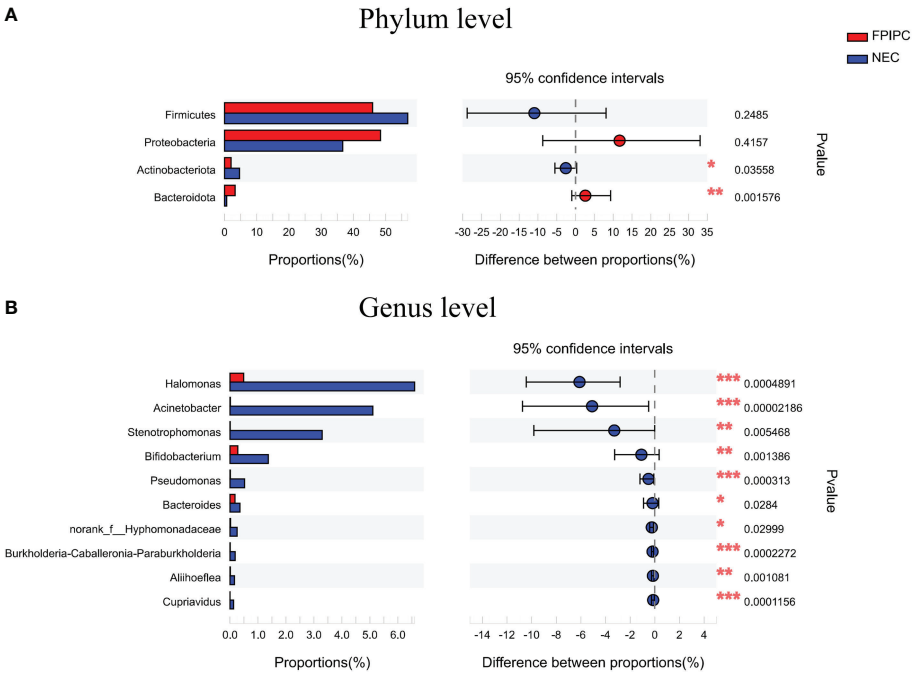


FIGURE 4
Relative abundances at the phylum level **(A)** and the genus level **(B)** in the necrotizing enterocolitis (NEC) and food protein-induced allergic protocolitis (FPIAP) groups. There were significant differences between the NEC and FPIAP groups in gut microbiota at the phylum and genus levels. *P<0.05, **P<0.01, ***, and P<0.001. Data was presented as means±SEM, N=22 for NEC and N=21 for FPIAP.

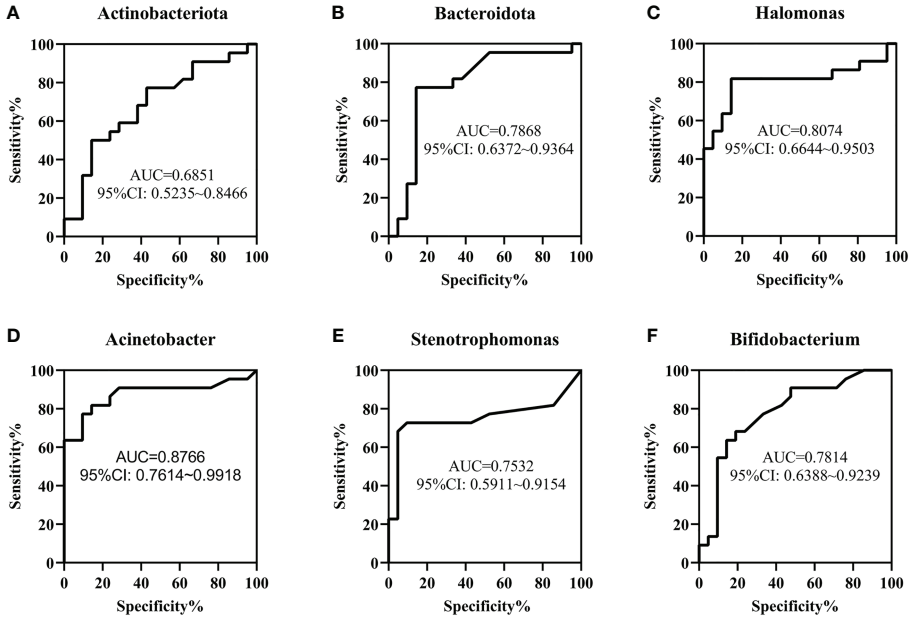


FIGURE 5
The value of gut microbiota in the early identification of necrotizing enterocolitis (NEC) and food protein-induced allergic protocolitis (FPIAP) by receiver operating characteristic (ROC) analysis. The area under curves (*AUCs)* of Actinobacteriota **(A)**, Bacteroidota **(B)**, *Halomonas* **(C)**, *Acinetobacter* **(D)**, *Stenotrophomonas* **(E)**, *and Bifidobacterium* **(F)** were 0.6851, 0.7868, 0.8074, 0.8766, 0.7532, and 0.7814, respectively. N=22 for NEC and N=21 for FPIAP.

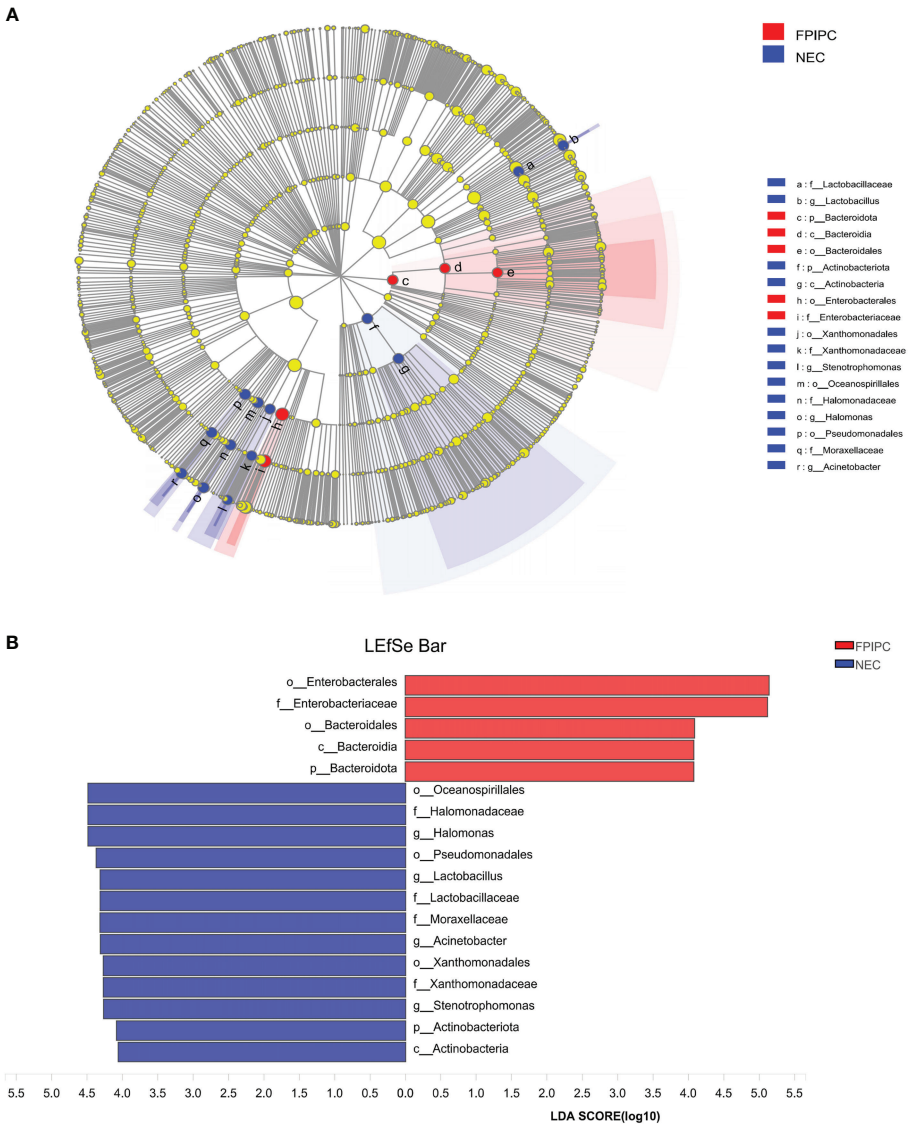


FIGURE 6
Differentially abundant taxa between the necrotizing enterocolitis (NEC) and food protein-induced allergic protocolitis (FPIAP) groups analyzed by Linear discriminant analysis (LDA) effect size (LEfSe) were shown in cladogram and histogram. **(A)** Comparative analysis of the gut microbiota by LEfSe: the cladogram shows bacterial taxa significantly higher in the group of infants of the same color, in the gut microbiota between NEC and FPIAP infants; **(B)** Gut microbiota analysis *via* LDA score between NEC and FPIAP infants. *N*=22 for NEC and *N*=21 for FPIAP.

the infants with FPIAP, infants in the NEC group had a remarkably lower number of Bacteroidota and higher number of Actinobacteriota. Previous studies have reported that the gut characteristic bacterial populations in healthy newborns are dominant in *Lactobacillus* and *Bifidobacterium* in term neonates, and *Enterobacteriaceae*, *Veillonella*, *Enterococcus*, and *Staphylococcus* in preterm neonates, respectively (Tirone et al., 2019), which are different from those in NEC or FPIAP infants of our study. There are four main phyla in the gut microbiota, including Firmicutes, Bacteroidota, Proteobacteria, and Actinobacteriota (Faith et al., 2013). The two dominant phyla, Firmicutes and Bacteroidota, represent over 90% of the total gut microbiota community (Magne et al., 2020), while members of Proteobacteria and Actinobacteriota are less abundant. Bacteroidota contain a large repertoire of genes involved in acquisition and metabolism of polysaccharides (Mahowald et al., 2009), and *Bacteroides* species are reported to be able to alter gut permeability (Curtis et al., 2014; Hua et al., 2016). Bacteroidota species have either anti-inflammatory effects or are involved in the process of proteolysis, however, some Bacteroidota species are pathogetic. The previous studies demonstrated that low level of Bacteroidota is associated with

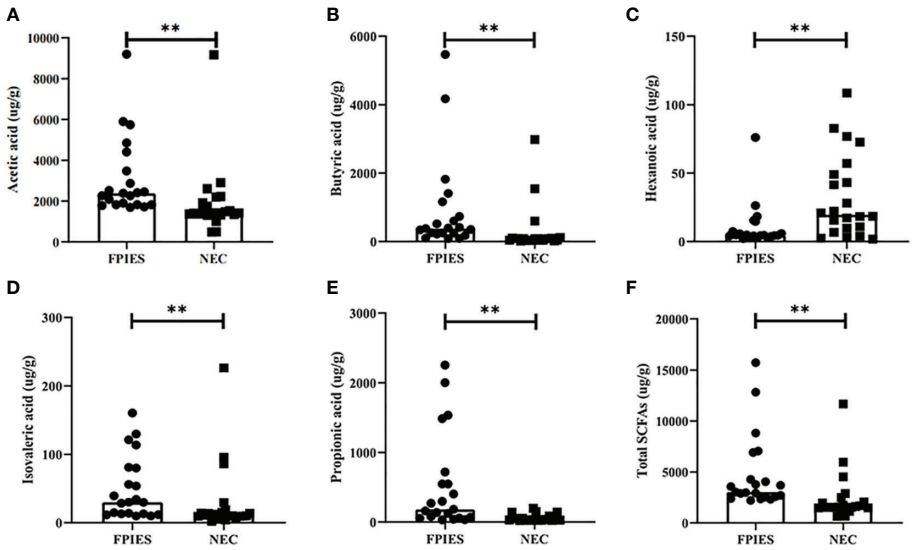


FIGURE 7
Gas chromatography-mass spectrometry (GC-MS) analysis of short chain fatty acids (SCFAs) in fecal samples from necrotizing enterocolitis (NEC) infants and food protein-induced allergic protocolitis (FPIAP). **(A)** acetic acid, **(B)** butyric acid, **(C)** hexanoic acid, **(D)** isovaleric acid, **(E)** propionic acid, and **(F)** Total SCFAs. $^{**}P<;0.01$. Data was presented as means±SEM, $N=22$ for NEC and $N=21$ for FPIAP.

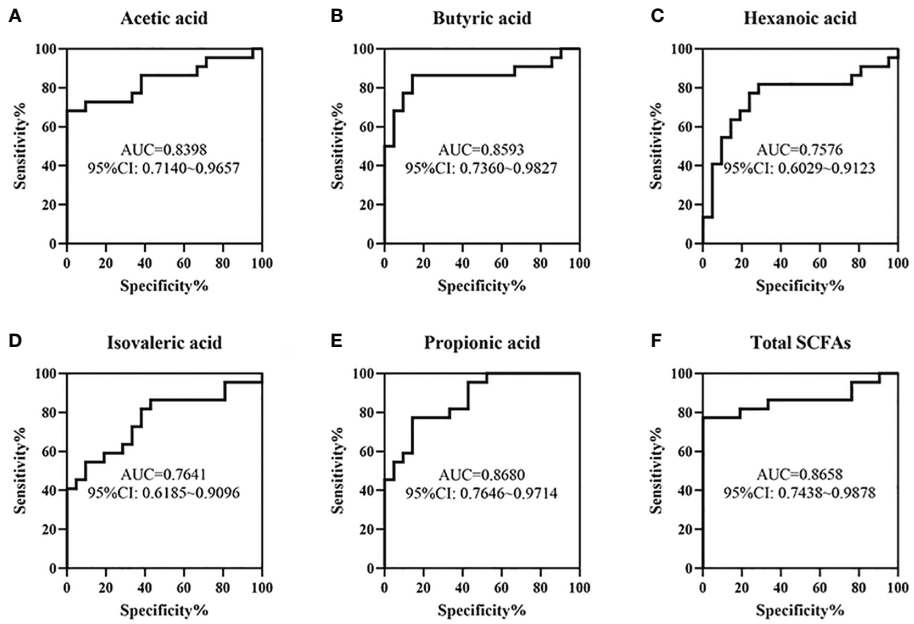


FIGURE 8
The value of some short chain fatty acids (SCFAs) in the early identification of necrotizing enterocolitis (NEC) and food protein-induced allergic protocolitis (FPIAP) by receiver operating characteristic (ROC) analysis. The area under curves (*AUCs)* of acetic acid **(A)**, butyric acid **(B)**, hexanoic acid **(C)**, isovaleric acid **(D)**, propionic acid **(E)**, and Total SCFAs **(F)** were 0.8398, 0.8593, 0.7576, 0.7641, 0.8680, and 0.8658, respectively. $N=22$ for NEC and $N=21$ for FPIAP.

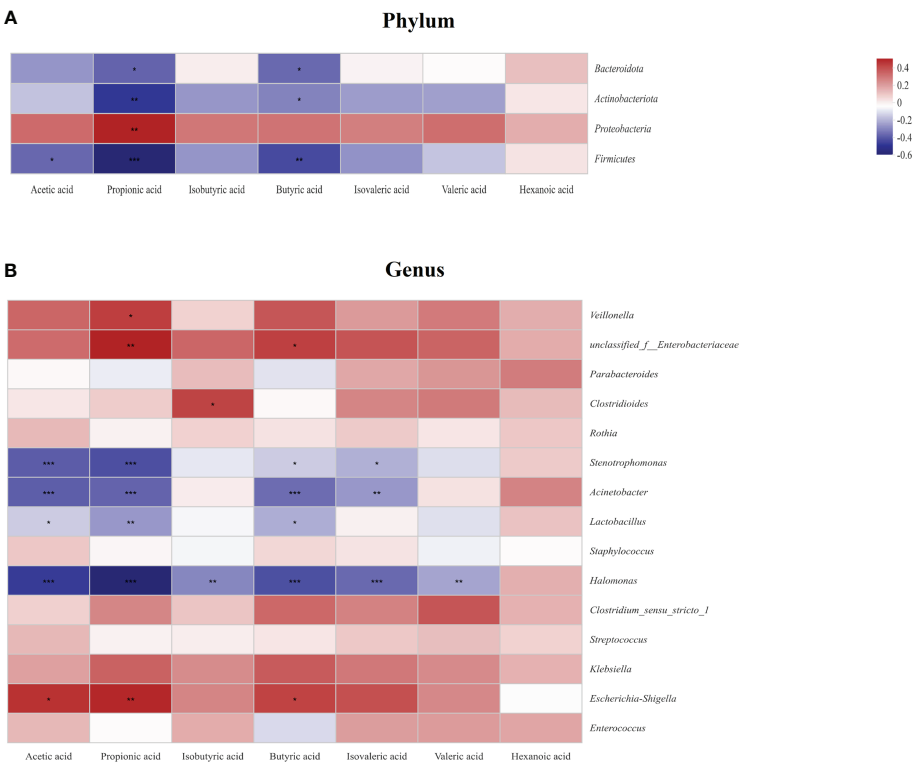


FIGURE 9
Relationship between the gut microbiota and short chain fatty acids (SCFAs) at the phylum level **(A)** and the genus level **(B)** in the study. Some flora and SCFAs were negatively related, represented in blue, and others were positively related, represented in red. The darker the color, the higher the correlation was. *P<0.05, **P<0.01, and ***P<0.001. Data was presented as means±SEM, N=22 for necrotizing enterocolitis (NEC) and N=21 for food protein-induced allergic protocolitis (FPIAP).

allergic disease and factors related to allergic disease, such as a Western lifestyle and cesarean section delivery (De Filippo et al., 2010; Abrahamsson et al., 2012). In contract, an enrichment of *Bacteroides* was found in non-IgE-mediated Cow's milk allergy children (Berni Canani et al., 2018b). Specifically, Kirjavainen et al. found high abundance of *Bacteroides* in the gut microbiome of infants with a high degree of milk allergy, early onset atopic eczema, and a strong family history of atopic disorders (Kirjavainen et al., 2002). A meta-analysis by Pammi et al. reported that fecal microbiome from infants with NEC had increased relative abundances of Proteobacteria and decreased relative abundances of Firmicutes and Bacteroidota (Pammi et al., 2017b), consistent with our results. In our study, the abundance of Actinobacteriota was higher in the NEC group as compared to the FPIAP group, which consistent with a previous study by Torrazza et al., in which a higher proportion of Actinobacteria was observed in NEC cases compared to controls (Torrazza et al., 2013). Actinobacteriota are Gram-negative bacteria with linear colonies and numerous species, such as *Bifidobacterium*, which are involved in immune modulation and metabolic activities. These findings indicated that dysbiosis of the gut microbiota plays an important role in the development of NEC and FPIAP.

At the genus level, our study also showed that differences in the gut microbiota at the genus level were notable between the NEC and FPIAP groups. The abundances of several genera of *Halomonas, Acinetobacter, Bifidobacterium*, and *Stenotrophomonas* were remarkably higher in the NEC group as compared to the FPIAP group. Belongs to the class Gammaproteobacteria and the family Halomonadaceae, *Halomonas* is a Gram-negative, aerobic, rod-shaped, extremely halotolerant bacteria, which has been found to have cytotoxic activity (Kim et al., 2013; Cheffi et al., 2021). *Stenotrophomonas* is an opportunistic pathogen of significant concern to susceptible patient populations, it can cause various nosocomial and community-acquired infections in humans and shows low susceptibility to many antibiotics (Sánchez, 2015; Brooke, 2021). To date, no studies have been carried out to explore the correlation between *Stenotrophomonas* and NEC or FPIPC infants. *Acinetobacter* is a gram-negative bacterum and is one of the most significant emerging multidrug-resistant pathogens. It is the cause of various hospital-acquired diseases

including septicemia, pneumonia, and wound infections (Geisinger et al., 2019). The genus *Bifidobacterium* is included within the phylum Actinobacteria and plays an important role in digestion and gut immunity. Previous studies have shown that some *Bifidobacterium* species have proteolytic activity (De Palma et al., ; de Almeida et al., 2020), which helps the protein absorption. Chen et al. found that the abundance of *Bifidobacterium* increased significantly in children with food protein allergy (Chen et al., 2016). Some studies have found that children received *Bifidobacterium* supplementation had significantly reduced allergic symptoms (Ismail et al., 2016; Liu et al., 2018). Decrease in the abundance of *Bifidobacterium* leads to a decrease in protein absorption and transformation, which may be the cause of FPIAP. Generally, the significant differences in abundance of *Halomonas*, *Acinetobacter*, *Bifidobacterium*, and *Stenotrophomonas* between the two groups may serve as biomarkers for NEC and FPIAP infants and suggest a role for the gut microbiota in the pathogenesis of the main symptoms of the disorder.

In this study, no significant differences were observed in mode of delivery, feeding patterns, and antibiotic exposure between the NEC and FPIAP groups, suggesting that the microbial diversity between the two groups in the present study may be associated with other factors. Substantial evidence suggested that the abundance and diversity of microbiota could be affected by multiple factors, including mode of delivery, feeding patterns (i.e., breast milk, formula, or both), and antibiotic exposure. Study reported that the microbial diversity was lower in infants delivered *via* C-section than in infants delivered vaginally (Korpela et al., 2018; Korpela et al., 2018; Lundgren et al., 2018). In fecal samples of infants born by vaginal delivery, the *Bifidobacterium* genus was predominant (Biasucci et al., 2008), followed by *Bacteroides* and *enterobacteria* (Fallani et al., 2010). In addition, Willers et al. showed that infants born by vaginal delivery had higher levels of S100 proteins as compared to infants born by cesarean delivery, which was associated with higher abundance of Actinobacteria and *Bifidobacteriaceae*, and lower abundance of Gammaproteobacteria-particularly opportunistic *Enterobacteriacea* (Willers et al., 2020). Investigating to the role of diet, Pammi et al. reported that significant differences in microbial diversity were observed among infants with different feeding types (Pammi et al., 2017a). In particular, formula-fed infants who developed NEC had more Proteobacteria and less Firmicutes compared to breast milk-fed controls (Pammi et al., 2017a). Investigating to antibiotic exposure, study also found that OTU richness between control infants who didn't received antibiotics and NEC infants who received antibiotics differed significantly (Pammi et al., 2017a). Antibiotic treatment decreases α-diversity of the individual's microbiome (Yassour et al., 2016). Besides, maternal treatment with antibiotics prior to delivery has also been related to a decrease in microbial diversity in infants, especially lacking *Bifidobacterium*, which is a genus regarded as favorable (Aloisio et al., 2014). The possible reason for the inconsistence between our findings and the above studies is due to the small sample size, and studies with a larger sample size are needed.

In the current study, NEC infants had significantly lower levels of acetic acid, propionic acid, butyric acid, isovaleric acid, and total SCFAs, and higher level of hexanoic acid compared with the FPIAP infants. SCFAs, which are produced by fermentation of dietary fibre by gut microbiota, are potential mediators involved in the intestinal immune function, including the inhibition of the production of pro-inflammatory factors and the maintenance of gut barrier function (Tedelind et al., 2007). Among the most common SCFAs, acetic acid, propionic acid, and butyric acid account for 90-95% of SCFAs in the human colon (Soldavini and Kaunitz, 2013). Some studies reported that acetic acid, propionic acid, and butyric acid can be produced by the fermentation of *Ruminococcus* (Lin et al., 2021; Liang et al., 2021). Besides, Some *Lactobacillus* strains, including *L. rhamnosus* GG, *L. gasseri* PA 16/8, *L. salivarius spp salcinius* JCM 1230, *L. agilis* JCM 1048, and *L. acidophilus* CRL 1014 were reported to participate in the production of acetic acid, propionic acid, and butyric acid (Markowiak-Kopeć and Śliżewska, 2020). Acetic acid is the most abundant SCFAs in the colon and constitutes over half of the total SCFAs content in the feces (Ziętek et al., 2021). Propionic acid is produced primarily by Bacteroidetes and Firmicutes (Russell et al., 2013). Acetic, propionic, and butyric acid have been shown to induce apoptosis (Kotunia et al., 2010), and butyric acid has been shown to exert the most significant anti-inflammatory property of all SCFAs and can improve the intestinal barrier function and mucosal immunity (Liu et al., 2018). Isovaleric acid and hexanoic acid are putrefactive acids generated by the unabsorbed amino acids or proteins reaching the intestines. Adequate balance of microbiota and metabolites could allow intestinal homeostasis and immunologic tolerance to food antigens, while imbalance of gut microbiota and their metabolites (SCFAs) possible influence key immunologic events that enhance allergic sensitization to food antigens. Therefore, the differences of the concentrations of SCFAs and the components of gut microbiota between the NEC and FPIAP groups could provide new strategies for the differential diagnosis of NEC and FPIPC.

In addition, we explored the value of the gut microbiota and SCFAs in the early identification of NEC and FPIAP and the results showed that the *AUCs* of Actinobacteriota, Bacteroidota, *Halomonas, Acinetobacter, Stenotrophomonas, Bifidobacterium*, acetic acid, propionic acid, butyric acid, isovaleric acid, hexanoic acid, and total SCFAs were 0.6851, 0.7868, 0.8074, 0.8766, 0.7532, 0.7814, 0.8398, 0.8680, 0.8593, 0.7641, 0.7576, and 0.8658, respectively. This finding suggests that they have moderate predictive value (Swets, 1988). Investigating to the heatmap, the production of metabolites was associated with the decline in Bacteriodota, Actinobacteriota, and Firmicutes, and

the increase in Proteobacteria, suggesting that it might be the joint work of the gut microbiota to produce metabolites.

This study has some limitations. Firstly, the samples were collected at a single hospital, and the cohort size was small. A large-scaled study is needed to further clarify the biomarkers in gut microbiota and SCFAs between the two groups. Secondly, as the gut microbiota composition was identified using 16S rRNA sequencing, we could not evaluate bacterial genomic functions or compare the composition at the species level. Detailed analysis using shotgun metagenomics would enable these evaluations.

Conclusions

The composition of gut microbiota and concentrations of SCFAs of NEC infants is different from that of FPIAP infants. NEC infants had higher abundances of Actinobacteria, *Halomonas, Acinetobacter, Bifidobacterium*, and *Stenotrophomonas*, and lower abundance of Bacteroidota, lower levels of acetic acid, propionic acid, butyric acid, isovaleric acid, and total SCFAs, and higher level of hexanoic acid as compared to FPIAP infants. The differences of gut microbiota composition and concentrations of SCFAs might represent suitable biomarker targets for early identification of NEC and FPIAP.

Data availability statement

The datasets of this study can be found in the NCBI (BioProject ID: PRJNA901061 https://www.ncbi.nlm.nih.gov/bioproject/901061).

Ethics statement

The studies involving human participants were reviewed and approved by Institutional review board for human studies of the Children's Hospital of Chongqing Medical University (project approval No. 2020104). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

All six authors made substantial contributions to the study and manuscript and met the criteria for authorship defined in the author instructions. JX drafted the manuscript, TY and X-SL collected the fecal samples and clinical data, X-CL worked on the basic sample processing, generated and analyzed the data. JX, LB, and L-QL conceived and designed the study. LB and L-QL supervised the project, contributed to the critical revision and final approval of the manuscript. All authors contributed to the article and approved the submitted version.

Funding

The study was supported by the National Natural Science Foundation of Chongqing, China (cstc2019jcyj-msxmX0169) and Project of Chongqing Science and Technology Commission (cstc2018jscx-msybX0027).

Acknowledgments

We thank the nurses in the Neonatal Diagnosis and Treatment Centre for collection of the fecal samples.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2022.1030588/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Rarefaction curves based on sobs index, Shannon curves based on Shannon index, and species accumulation curves between the necrotizing enterocolitis (NEC) and food protein-induced allergic protocolitis (FPIAP) groups. *N*=22 for NEC and *N*=21 for FPIAP.

References

Abrahamsson, T. R., Jakobsson, H. E., Andersson, A. F., Bjorksten, B., Engstrand, L., and Jenmalm, M. C. (2012). Low diversity of the gut microbiota in infants with atopic eczema. *J. Allergy Clin. Immunol.* 129 (2), 434–40, 440.e1-2. doi: 10.1016/j.jaci.2011.10.025

Aloisio, I., Mazzola, G., Corvaglia, L. T., Tonti, G., Faldella, G., Biavati, B., et al. (2014). Influence of intrapartum antibiotic prophylaxis against group b streptococcus on the early newborn gut composition and evaluation of the anti-streptococcus activity of bifidobacterium strains. *Appl. Microbiol. Biotechnol.* 98 (13), 6051–6060. doi: 10.1007/s00253-014-5712-9

Vermont Oxford Network database. *Manual of operations. part 2: Data definitions and data forms for infants born in 2013*. Available at: http://www.vtoxford.org/tools/ManualofOperationsPart2.pdf (Accessed 01 July 2014).

Bell, E. F., Hintz, S. R., Hansen, N. I., Bann, C. M., Wyckoff, M. H., DeMauro, S. B., et al. (2022). Mortality, in-hospital morbidity, care practices, and 2-year outcomes for extremely preterm infants in the US, 2013-2018. *JAMA* 327 (3), 248–263. doi: 10.1001/jama.2021.23580

Berni Canani, R., De Filippis, F., Nocerino, R., Paparo, L., Di Scala, C., Cosenza, L., et al. (2018a). Gut microbiota composition and butyrate production in children affected by non-IgE-Mediated cow's milk allergy. *Sci. Rep.* 8 (1), 12500. doi: 10.1038/s41598-018-30428-3

Berni Canani, R., De Filippis, F., Nocerino, R., Paparo, L., Di Scala, C., Cosenza, L., et al. (2018b). Gut microbiota composition and butyrate production in children affected by non-IgE-mediated cow's milk allergy. *Sci. Rep.* 8 (1), 12500. doi: 10.1038/s41598-018-30428-3

Biasucci, G., Benenati, B., Morelli, L., Bessi, E., and Boehm, G. (2008). Cesarean delivery may affect the early biodiversity of intestinal bacteria. *J. Nutr.* 138 (9), 1796S–1800S. doi: 10.1093/jn/138.9.1796S

Brooke, J. S. (2021). Advances in the microbiology of stenotrophomonas maltophilia. *Clin. Microbiol. Rev.* 34 (3), e0003019. doi: 10.1128/CMR.00030-19

Burks, A. W., Tang, M., Sicherer, S., Muraro, A., Eigenmann, P. A., Ebisawa, M., et al. (2012). ICON: food allergy. *J. Allergy Clin. Immunol.* 129 (4), 906–920. doi: 10.1016/j.jaci.2012.02.001

Calvert, W., Sampat, K., Jones, M., Baillie, C., Lamont, G., and Losty, P. D. (2021). Necrotising enterocolitis-a 15-year outcome report from a UK specialist centre. *Acta Paediatr.* 110 (2), 495–502. doi: 10.1111/apa.15510

Cheffi, M., Maalej, A., Mahmoudi, A., Hentati, D., Marques, A. M., Sayadi, S., et al. (2021). Lipopeptides production by a newly halomonas venusta strain: Characterization and biotechnological properties. *Bioorg. Chem.* 109, 104724. doi: 10.1016/j.bioorg.2021.104724

Chen, C. C., Chen, K. J., Kong, M. S., Chang, H. J., and Huang, J. L. (2016). Alterations in the gut microbiotas of children with food sensitization in early life. *Pediatr. Allergy Immunol.* 27 (3), 254–262. doi: 10.1111/pai.12522

Curtis, M. M., Hu, Z., Klimko, C., Narayanan, S., Deberardinis, R., and Sperandio, V. (2014). The gut commensal bacteroides thetaiotaomicron exacerbates enteric infection through modifcation of the metabolic landscape. *Cell. Host. Microbe* 16 (6), 759–769. doi: 10.1016/j.chom.2014.11.005

de Almeida, N. E. C., Esteves, F. G., Dos Santos-Pinto, J. R. A., Peres de Paula, C., da Cunha, A. F., Malavazi, I., et al. (2020). Digestion of intact gluten proteins by bifidobacterium species: Reduction of cytotoxicity and proinflammatory responses. *J. Agric. Food. Chem.* 68 (15), 4485–4492. doi: 10.1021/acs.jafc.0c01421

De Filippo, C., Cavalieri, D., Di Paola, M., Ramazzotti, M., Poullet, J. B., Massart, S., et al. (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc. Natl. Acad. Sci. U. S. A.* 107 (33), 14691–14696. doi: 10.1073/pnas.1005963107

De Palma, G., Cinova, J., Stepankova, R., Tuckova, L., and Sanz, Y. (2010) Pivotal advance: bifidobacteria and gram-negative bacteria differentially influence immune responses in the proinflammatory milieu of celiac disease. *J. Leukocyte Biol.* 87 (5), 765–778. doi: 10.1189/jlb.0709471

Elizur, A., Cohen, M., Goldberg, M. R., Rajuan, N., Cohen, A., Leshno, M., et al. (2012). Cow's milk associated rectal bleeding: a population based prospective study. *Pediatr. Allergy Immunol.* 23 (8), 766–770. doi: 10.1111/pai.12009

Faith, J. J., Guruge, J. L., Charbonneau, M., Subramanian, S., Seedorf, H., Goodman, A. L., et al. (2013). The long-term stability of the human gut microbiota. *Science* 341 (6141), 1237439. doi: 10.1126/science.1237439

Fallani, M., Young, D., Scott, J., Norin, E., Amarri, S., Adam, R., et al. (2010). Intestinal microbiota of 6-week-old infants across Europe: geographic influence beyond delivery mode, breast-feeding, and antibiotics. *J. Pediatr. Gastroenterol. Nutr.* 51 (1), 77–84. doi: 10.1097/MPG.0b013e3181d1b11e

Feuille, E., and Nowak-Węgrzyn, A. (2015). Food protein-induced enterocolitis syndrome, allergic proctocolitis, and enteropathy. *Curr. Allergy Asthma. Rep.* 15 (8), 50. doi: 10.1007/s11882-015-0546-9

Geisinger, E., Huo, W., Hernandez-Bird, J., and Isberg, R. R. (2019). Acinetobacter baumannii: Envelope determinants that control drug resistance, virulence, and surface variability. *Annu. Rev. Microbiol.* 73, 481–506. doi: 10.1146/annurev-micro-020518-115714

Hackam, D., and Caplan, M. (2018). Necrotizing enterocolitis: Pathophysiology from a historical context. *Semin. Pediatr. Surg.* 27 (1), 11–18. doi: 10.1053/j.sempedsurg.2017.11.003

Hua, X., Goedert, J. J., Pu, A., Yu, G., and Shi, J. (2016). Allergy associations with the adult fecal microbiota: analysis of the American gut project. *EBioMedicine* 3, 172–179. doi: 10.1016/j.ebiom.2015.11.038

Ismail, I. H., Boyle, R. J., Licciardi, P. V., Oppedisano, F., Lahtinen, S., Robins-Browne, R. M., et al. (2016). Early gut colonization by bifidobacterium breve and b. catenulatum differentially modulates eczema risk in children at high risk of developing allergic disease. *Pediatr. Allergy Immunol.* 27 (8), 838–846. doi: 10.1111/pai.12646

Kim, K. K., Lee, J. S., and Stevens, D. A. (2013). Microbiology and epidemiology of halomonas species. *Future. Microbiol.* 8 (12), 1559–1573. doi: 10.2217/fmb.13.108

Kirjavainen, P. V., Arvola, T., Salminen, S. J., and Isolauri, E. (2002). Aberrant composition of gut microbiota of allergic infants: a target of bifidobacterial therapy at weaning? *Gut* 51 (1), 51–55. doi: 10.1136/gut.51.1.51

Kliegman, R. M., and Walsh, M. C. (1987). Neonatal necrotizing enterocolitis: pathogenesis, classification, and spectrum of illness. *Current. Problems. pediatrics.* 17 (4), 213–288. doi: 10.1016/0045-9380(87)90031-4

Korpela, K., Costea, P., Coelho, L. P., Kandels-Lewis, S., Willemsen, G., Boomsma, D. I., et al. (2018). Selective maternal seeding and environment shape the human gut microbiome. *Genome. Res.* 28 (4), 561–568. doi: 10.1101/gr.233940.117

Korpela, K., Salonen, A., Hickman, B., Kunz, C., Sprenger, N., Kukkonen, K., et al. (2018). Fucosylated oligosaccharides in mother's milk alleviate the effects of caesarean birth on infant gut microbiota. *Sci. Rep.* 8 (1), 13757. doi: 10.1038/s41598-018-32037-6

Kotunia, A., Pietrzak, P., Guilloteau, P., and Zabielski, R. (2010). K Butyric acid in gastrointestinal tract. *Prz. Gastroenterol.* 5, 117–122. doi: 10.5114/pg.2010.14137

Liang, D., Zhang, L., Chen, H. Z., Zhang, H., Hu, H. H., and Dai, X. F. (2021). Potato resistant starch inhibits diet-induced obesity by modifying the composition of intestinal microbiota and their metabolites in obese mice. *Int. J. Biol. Macromol.* 180, 458–469. doi: 10.1016/j.ijbiomac.2021.02.209

Lindberg, T. P., Caimano, M. J., Hagadorn, J. I., Bennett, E. M., Maas, K., Brownell, E. A., et al. (2020). Preterm infant gut microbial patterns related to the development of necrotizing enterocolitis. *J. Matern. Fetal. Neonatal. Med.* 33 (3), 349–358. doi: 10.1080/14767058.2018.1490719

Lin, W., Wen, L. Y., Wen, J. P., and Xiang, G. D. (2021). Effects of sleeve gastrectomy on fecal gut microbiota and short-chain fatty acid content in a rat model of polycystic ovary syndrome. *Front. Endocrinol. (Lausanne).* 12. doi: 10.3389/fendo.2021.747888

Liu, Q., Jing, W., and Wang, W. (2018). Bifidobacterium lactis ameliorates the risk of food allergy in Chinese children by affecting relative percentage of treg and Th17 cells. *Can. J. Infect. Dis. Med. Microbiol.* 2018, 4561038. doi: 10.1155/2018/4561038

Liu, H., Wang, J., He, T., Becker, S., Zhang, G. L., Li, D. F., et al. (2018). Butyrate: A double-edged sword for health? *Adv. Nutr.* 9 (1), 21–29. doi: 10.1093/advances/nmx009

Lundgren, S. N., Madan, J. C., Emond, J. A., Morrison, H. G., Christensen, B. C., Karagas, M. R., et al. (2018). Maternal diet during pregnancy is related with the infant stool microbiome in a delivery mode-dependent manner. *Microbiome* 6 (1), 109. doi: 10.1186/s40168-018-0490-8

Lu, P., Sodhi, C. P., Jia, H., Shaffiey, S., Good, M., Branca, M. F., et al. (2014). Animal models of gastrointestinal and liver diseases. Animal models of necrotizing enterocolitis: pathophysiology, translational relevance, and challenges. *Am. J. Physiol. Gastrointest. Liver. Physiol.* 306 (11), G917–G928. doi: 10.1152/ajpgi.00422.2013

Magne, F., Gotteland, M., Gauthier, L., Zazueta, A., Pesoa, S., Navarrete, P., et al. (2020). The Firmicutes/Bacteroidetes ratio: A relevant marker of gut dysbiosis in obese patients? *Nutrients* 12 (5), 1474. doi: 10.3390/nu12051474

Mahowald, M. A., Rey, F. E., Seedorf, H., Turnbaugh, P. J., Fulton, R. S., Wollam, A., et al. (2009). Characterizing a model human gut microbiota composed of members of its two dominant bacterial phyla. *Proc. Natl. Acad. Sci. U. S. Axz.* 106 (14), 5859–5864. doi: 10.1073/pnas.0901529106

Markowiak-Kopeć, P., and Śliżewska, K. (2020). The effect of probiotics on the production of short-chain fatty acids by human intestinal microbiome. *Nutrients* 12 (4), 1107. doi: 10.3390/nu12041107

Martin, V. M., Virkud, Y. V., Seay, H., Hickey, A., Ndahayo, R., Rosow, R., et al. (2020). Prospective assessment of pediatrician-diagnosed food protein-induced allergic proctocolitis by gross or occult blood. *J. Allergy Clin. Immunol. Pract.* 8 (5), 1692–1699.e1. doi: 10.1016/j.jaip.2019.12.029

Meyer, R., Chebar Lozinsky, A., Fleischer, D. M., Vieira, M. C., Du Toit, G., Vandenplas, Y., et al. (2020). Diagnosis and management of non-IgE gastrointestinal allergies in breastfed infants-an EAACI position paper. *Allergy* 75 (1), 14–32. doi: 10.1111/all.13947

Nowak-Węgrzyn, A., Katz, Y., Mehr, S. S., and Koletzko, S. (2015). Non-IgE-mediated gastrointestinal food allergy. *J. Allergy Clin. Immunol.* 135 (5), 1114–1124. doi: 10.1016/j.jaci.2015.03.025

Ohtsuka, Y. (2015). Food intolerance and mucosal inflammation. *Pediatr. Int.* 57 (1), 22–29. doi: 10.1111/ped.12546

Olm, M. R., Bhattacharya, N., Crits-Christoph, A., Firek, B. A., Baker, R., Song., Y. S., et al. (2019). Necrotizing enterocolitis is preceded by increased gut bacterial replication, klebsiella, and fimbriae-encoding bacteria. *Sci. Adv.* 5 (12), eaax5727. doi: 10.1126/sciadv.aax5727

Pammi, M., Cope, J., Tarr, P. I., Warner, B. B., Morrow, A. L., Mai, V., et al. (2017a). Intestinal dysbiosis in preterm infants preceding necrotizing enterocolitis: a systematic review and meta-analysis. *Microbiome* 5 (1), 31. doi: 10.1186/s40168-017-0248-8

Pammi, M., Cope, J., Tarr, P. I., Warner, B. B., Morrow, A. L., Mai, V., et al. (2017b). Intestinal dysbiosis in preterm infants preceding necrotizing enterocolitis: a systematic review and meta-analysis. *Microbiome* 5 (1), 31. doi: 10.1186/s40168-017-0248-8

Russell, W. R., Hoyles, L., Flint, H. J., and Dumas, M. E. (2013). Colonic bacterial metabolites and human health. *Curr. Opin. Microbiol.* 16, 246–254. doi: 10.1016/j.mib.2013.07.002

Sánchez, M. B. (2015). Antibiotic resistance in the opportunistic pathogen stenotrophomonas maltophilia. *Front. Microbiol.* 6. doi: 10.3389/fmicb.2015.00658

Savage, J. H., Lee-Sarwar, K. A., Sordillo, J., Bunyavanich, S., Zhou, Y., O'Connor, G., et al. (2018). A prospective microbiome-wide association study of food sensitization and food allergy in early childhood. *Allergy* 73 (1), 145–152. doi: 10.1111/all.13232

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., et al. (2009). Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* 75 (23), 7537–7541. doi: 10.1128/AEM.01541-09

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., et al. (2011). Metagenomic biomarker discovery and explanation. *Genome. Biol.* 12 (6), R60. doi: 10.1186/gb-2011-12-6-r60

Senocak, N., Ertugrul, A., Ozmen, S., and Bostanci, I. (2022). Clinical features and clinical course of food protein-induced allergic proctocolitis: 10-year experience of a tertiary medical center. *J. Allergy Clin. Immunol. Pract.* 10 (6), 1608–1613. doi: 10.1016/j.jaip.2022.02.013

Soldavini, J., and Kaunitz, J. D. (2013). Pathobiology and potential therapeutic value of intestinal short-chain fatty acids in gut inflammation and obesity. *Dig. Dis. Sci.* 58 (10), 2756–2766. doi: 10.1007/s10620-013-2744-4

Sowden, M., van Weissenbruch, M. M., Bulabula, A. N. H., van Wyk, L., Twisk, J., and van Niekerk, E. (2022). Effect of a multi-strain probiotic on the incidence and severity of necrotizing enterocolitis and feeding intolerances in preterm neonates. *Nutrients* 14 (16), 3305. doi: 10.3390/nu14163305

Swets, J. A. (1988). Measuring the accuracy of diagnostic systems. *Science* 240 (4857), 1285–1293. doi: 10.1093/advances/nmx009

Tarracchini, C., Milani, C., Longhi, G., Fontana, F., Mancabelli, L., Pintus, R., et al. (2021). Unraveling the microbiome of necrotizing enterocolitis: Insights in novel microbial and metabolomic biomarkers. *Microbiol. Spectr.* 9 (2), e0117621. doi: 10.1128/Spectrum.01176-21

Tedelind, S., Westberg, F., Kjerrulf, M., and Vidal, A. (2007). Anti-inflammatory properties of the short-chain fatty acids acetate and propionate: a study with relevance to inflammatory bowel disease. *World. J. Gastroenterol.* 13 (20), 2826–2832. doi: 10.3748/wjg.v13.i20.2826

Thänert, R., Keen, E. C., Dantas, G., Warner, B. B., and Tarr, P. I. (2021). Necrotizing enterocolitis and the microbiome: Current status and future directions. *J. Infect. Dis.* 223 (12 Suppl 2), S257–S263. doi: 10.1093/infdis/jiaa604

Thompson, E. C., Brown, M. F., Bowen, E. C., Smith, L. M., and vander Griten, D. (1996). Causes of gastrointestinal hemorrhage in neonates and children. *South. Med. J.* 89 (4), 370–374. doi: 10.1097/00007611-199604000-00003

Tirone, C., Pezza, L., Paladini, A., Tana, M., Aurilia, C., Lio, A., et al. (2019). Gut and lung microbiota in preterm infants: Immunological modulation and implication in neonatal outcomes. *Front. Immunol.* 10, 2910. doi: 10.3389/fimmu.2019.02910

Torrazza, R. M., Ukhanova, M., Wang, X., Sharma, R., Hudak, M. L., Neu, J., et al. (2013). Intestinal microbial ecology and environmental factors affecting necrotizing enterocolitis. *PLoS. One* 8 (12), e83304. doi: 10.1371/journal.pone.0083304

Willers, M., Ulas, T., Völlger, L., Vogl, T., Heinemann, A. S., Pirr, S., et al. (2020). S100A8 and S100A9 are important for postnatal development of gut microbiota and immune system in mice and infants. *Gastroenterology* 159 (6), 2130–2145.e5. doi: 10.1053/j.gastro.2020.08.019

Yassour, M., Vatanen, T., Siljander, H., Hämäläinen, A. M., Härkönen, T., and Ryhänen, S. J. (2016). Natural history of the infant gut microbiome and impact of antibiotic treatment on bacterial strain diversity and stability. *Sci. Transl. Med.* 8 (343), 343ra81. doi: 10.1126/scitranslmed.aad0917

Ziętek, M., Celewicz, Z., and Szczuko, M. (2021). Short-chain fatty acids, maternal microbiota and metabolism in pregnancy. *Nutrients* 13 (4), 1244. doi: 10.3390/nu13041244

frontiers | Frontiers in Cellular and Infection Microbiology

TYPE Review
PUBLISHED 01 December 2022
DOI 10.3389/fcimb.2022.1075201

OPEN ACCESS

EDITED BY
Xiangtian Yu,
Shanghai Jiao Tong University, China

REVIEWED BY
Wei Wu,
Guangdong Provincial Center for Disease Control and Prevention, China
Haiming Fang,
Second Hospital of Anhui Medical University, China

*CORRESPONDENCE
Kaijian Hou
kaijianhou@126.com
Liping Li
lpli@stu.edu.cn

SPECIALTY SECTION
This article was submitted to Intestinal Microbiome, a section of the journal Frontiers in Cellular and Infection Microbiology

RECEIVED 20 October 2022
ACCEPTED 15 November 2022
PUBLISHED 01 December 2022

CITATION
Zhang S, Deng F, Chen J, Chen F, Wu Z, Li L and Hou K (2022) Fecal microbiota transplantation treatment of autoimmune-mediated type 1 diabetes: A systematic review. *Front. Cell. Infect. Microbiol.* 12:1075201.
doi: 10.3389/fcimb.2022.1075201

Fecal microbiota transplantation treatment of autoimmune-mediated type 1 diabetes: A systematic review

Shuo Zhang[1,2], Feiying Deng[1,2], Jingxian Chen[3,4], Fengwu Chen[2,3], Zezhen Wu[1,2], Liping Li[4]* and Kaijian Hou[3,4]*

[1]Shantou University Medical College, Shantou, China, [2]Department of Endocrine and Metabolic Diseases, The First Affiliated Hospital of Shantou University Medical College, Shantou, China, [3]Department of Endocrine and Metabolic Diseases, Longhu People's Hospital, Shantou, China, [4]School of Public Health, Shantou University, Shantou, China

There is a strong link between fecal microbiota and the development of type 1 diabetes. As an emerging therapeutic modality, fecal microbiota transplantation has been shown to be safe and effective in the treatment of many intestinal and extraintestinal diseases. Various studies have found that fecal microbiota transplantation can treat diseases by correcting patients' immune disorders. Besides, many studies have found that fecal microbiota transplantation can improve glycemic control and insulin resistance in diabetic patients. Therefore, this paper reviews the mechanism of action of fecal microbiota transplantation on autoimmune-mediated T1DM and the current research progress, feasibility, and issues that need to be addressed in the future development of fecal microbiota transplantation in the treatment of autoimmune-mediated T1DM.

KEYWORDS

Autoimmune-mediated type 1 diabetes, gut microbiota, fecal microbiota transplantation, chronic inflammatory state, insulin resistance

Introduction

Current status of type 1 diabetes mellitus

Diabetes mellitus is a group of diseases characterized by chronic hyperglycemia caused by multiple etiologies, which can cause damage to multiple systems of the body and bring about various acute and chronic complications (Hou et al., 2021). The most common types of diabetes mellitus are type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), other specific diabetes mellitus, and gestational diabetes, and they can all be summarized as a chronic inflammatory state (American Diabetes Association

Professional Practice C. 2, 2022). T1DM is also known as a type of insulin-dependent diabetes mellitus, which mostly starts in adolescence or childhood. According to the International Diabetes Federation (IDF), 536.6 million adults aged 20–79 years in 215 countries and territories have been diagnosed as diabetes patients by 2021, and 1.2 million children and adolescents under 20 years of age have been diagnosed with type 1 diabetes, and there will probably be 149,500 children and adolescents with type 1 diabetes by 2045 (Sun et al., 2022). The systemic neurological and vascular dysfunction caused by T1DM can affect the cardiac vessels, nerves, eyes, and kidneys (Nicholson et al., 2012). Its complications and mortality account for approximately 5-10% of the global diabetes financial burden (Mobasseri et al., 2020). The current incidence of T1DM is increasing at 3% to 5% per year, which will cause a severe social and economic burden (Wang et al., 2017), and global diabetes-related health expenditures are estimated to reach $1,054 billion by 2045 (Sun et al., 2022). At present, the dominant treatment for T1DM is still to reduce blood glucose by injecting insulin, but this is only a symptomatic treatment.

T1DM etiology and influencing factors

At present, T1DM is composed of two subtypes, including the autoimmune type (T1A) and the non-autoimmune type (also known as idiopathic type 1 diabetes mellitus (T1B), of which T1A accounts for the majority (American Diabetes Association Professional Practice C. 2, 2022). On the one hand, T1DM patients are in an autoimmune-mediated inflammatory state, producing a variety of inflammatory factors, such as TNF- α, IL-1, and IL-6 (Vatanen et al., 2016); on the other hand, innate and adaptive immunity mediates the production of autoantibodies, such as glutamic acid decarboxylase antibodies and zinc transporter 8 antibodies, both of which will lead to the damage of β cell function and insulin secretion (Abdellatif and Sarvetnick, 2019). Genetic factors, harmful factors in the environment, bacteria, fungi, and viral infections all might make pancreatic β cells exhausted and eventually fail due to a secondary autoimmune destruction (Rewers and Ludvigsson, 2016). In the population, different seasons and geographical locations (Samuel et al., 2008; Kimura et al., 2013), changes in diet and delivery methods, and the use of antibiotics will also affect the incidence of T1DM (Krauss, 2004). For example, in early life, exposure to environmental chemicals and air pollution can affect the development of the immune system, and the function and survival of β cells leading to an increase in the incidence rate of T1DM (Malmqvist et al., 2015). The different components of drinking water in different regions will also affect the incidence rate of T1DM, it has been found that the content of some metal elements, barium, and nickel, is negatively correlated with the incidence rate of T1DM (Chafe et al., 2018). Viruses mainly include enteroviruses and Coxsackie B viruses. Human infection with related viruses can induce pancreatitis or produce substances similar to islet autoantigens, thus activating the immune system and leading to or accelerating the progress of T1DM (Stankov et al., 2013). Among genetic factors, more than 50 T1DM-susceptible genes have been found through family linkage analysis and genome-wide association studies. Different genes will lead to variable effects on T1DM susceptibility, among which HLA-DR and DQ genes are the most closely related, accounting for 40% to 50% of the pathogenic risk factors (Ziegler and Nepom, 2010). Although HLA-DR risk alleles increase the susceptibility of high-risk children to T1DM, only 5% or fewer genes will lead to the development of T1DM (Krischer et al., 2019). Nongenetic modification factors such as diet, gut microbiota, pressure, and chemical and environmental factors play an essential role in the occurrence and development of T1DM (Mullaney et al., 2018). Therefore, the limited residual of T1DM patients is retained through nongenetic factors β Cell function is crucial for the quality of life and prognosis of patients (Wang and Jia, 2016). More and more studies have found that gut microbiota plays a crucial role in the occurrence and disease progression of T1DM. The transplantation treatment around gut microbiota can effectively improve the gut microbiota imbalance in patients, which holds promise for improving glycemic control and insulin resistance in patients with T1DM (Steffes et al., 2003).

T1DM and gut microbiota

More and more studies show that gut microbiota (GM) is closely related to the occurrence and development of T1DM. The pathophysiological changes of T1DM are related to the changes in GM. The GM can affect the progress of T1DM in many aspects (de Goffau et al., 2014; Davis-Richardson and Triplett, 2015; de Groot et al., 2017). “Gut microbiota” refers to more than 1014 kinds of bacteria, fungi, viruses, and others residing in the gastrointestinal tract and performing various functions in the gastrointestinal tract. The “microbiota” is considered the genome of the entire microbiota (Abdellatif and Sarvetnick, 2019). The intestinal tract of humans is composed of about 100 trillion bacterial cells, 10 times the total number of human cells. The microbiota weighs 1.5 kg and has more than 3.3 million genes, which is 150 times the human gene (Pitocco et al., 2020), showing that GM can play an essential role in our body. GM is mainly divided into four types at the phyla level. The first is Firmicutes (gram-positive), which constitutes 60-80% of the microbiota, including more than 200 genera (the most important ones are Rumen coccus, Clostridium, and Lactobacillus); the second is Bacteroides (gram-negative, including Bacteroides, Prevotella, and Trichoderma), accounting for 20-30% of the microbiota; the next is actinomycetes (gram-positive), accounting for about 10%

of the microbiota (mainly Bifidobacterium); and finally, Proteus, such as Escherichia coli and Enterobacteriaceae (Hou et al., 2022). Therefore, we can see the close relationship between gut microbiota and T1DM, and the feasibility of fecal microbiota transplantation with gut microbiota as the therapeutic target.

Insulin resistance is influenced by gut microbiota

The insulin resistance is the leading risk factor and feature of T2DM. Although the main cause of T1DM is the absolute lack of insulin secretion, most patients have insulin resistance at the same time, and this feature runs through the beginning of the disease and the subsequent insulin treatment process. Some patients have a trend of increasing insulin demand in the subsequent clinical treatment, which reflects the rising insulin resistance index (Homa IR) (Pedersen et al., 2016). Repiso et al. (Gutierrez-Repiso et al., 2020) analyzed gut microbiota composition in 46 patients with low Home-IR, high Home-IR, and T2DM patients treated with metformin. The results showed that compared with the low HOMA-IR group, the high HOMA-IR group had significantly higher flora abundance (q5.011) in Proteus (W52), Fusobacterium (W52), and Bacteroides (W51). It was also found that some gut microbiota, such as Prevotella copri and Bacteroides vulgatus, can affect the insulin resistance of diabetic patients by synthesizing branched-chain amino acids (BCAAs)-leucine, isoleucine, and valine (White and Newgard, 2019). In contrast, the content of BCAAs in the serum metabolic group of patients with insulin resistance increases, and the increase in BCAAs intake in food is associated with a higher risk of insulin resistance. Reducing BCAAs intake can improve postprandial insulin sensitivity, so BCAAs intake is considered an indicator of insulin resistance and a predictor of diabetes development (Shou et al., 2019). Thus, we can conclude that the change in gut microbiota can affect the insulin resistance of diabetic patients.

Diabetic patients experience an inflammatory state caused by a disruption in their gut microbiota

Diabetic patients are in a chronic inflammatory state. T1DM is a proinflammatory problem, leading to islet βcell crushing and the loss of insulin production (Rodriguez-Valera et al., 2009). In T2DM, the proinflammatory state can lead to insulin resistance (Scheithauer et al., 2016), and the gut microbiota can mediate the occurrence and development of this inflammatory state in many ways. First of all, lipopolysaccharide (LPS) is one of the components of the outer membrane of gram-negative bacteria. LPS and LPS cytokines, such as IL-1 and IL-6, can combine with their toll-like receptor 4 (TLR4) to increase proinflammatory molecules. The receptor is found in cells from a variety of organs and tissues, including human adipose tissue, the brain, the liver, muscle, and the pancreas. Therefore, when the intestinal gram-negative bacteria change, it can affect the inflammatory state in the body. At the same time, some cytokines, such as IL-10 and IL-22, can play an anti-inflammatory role, while Enterobacter, Bacteroides fragilis, Achmania mucophilus, and Lactobacillus plantarum can induce the production of these cytokines (Zhu et al., 2018; Chen et al., 2022). Secondly, GM can affect intestinal barrier function. LPS can destroy the tight junction between epithelial cells, thus reducing the tight junction proteins (occludin and occlusive zone-1) and CB2 (Hasain et al., 2020). The decomposition products of GM can be used as the energy substrate of the intestinal epithelium to promote the renewal metabolism and damage repair of the intestinal epithelium (De Vadder et al., 2014). Short-chain fatty acids (SCFAs), mainly propionic acid, butyric acid, etc.), the products of cellulose and carbohydrate decomposed by GM, can help maintain the integrity of the intestinal epithelium by inducing mucin synthesis and improve the intestinal barrier by promoting tight connection assembly (Burger-van Paassen et al., 2009). When the balance of gut microbiota is broken, the intestinal barrier function is reduced, which can leak whole bacteria, fatty acids, and lipopolysaccharides and transfer them to all body parts through blood transport. Thus, TLR4 is activated, which leads to metabolic inflammation and accelerates the progression of diabetes (Que et al., 2021). Meanwhile, bacteria entering the body stimulate the immune system and produce antibodies against them. These antibodies will cross-react with islet cell surface antigens, and the cross-reaction of T cells will mediate the destruction of islet cells and the formation of T1DM (Cole et al., 2016). In addition, the anti-inflammatory properties of short-chain fatty acids can also be shown by directly inhibiting the transport of harmful bacteria through epithelial cells (Macfarlane and Macfarlane, 2011). Butyrate can regulate the function of macrophages to reduce the expression of proinflammatory mediators, promote the differentiation of regulatory T cells, and thus enhance anti-inflammatory properties (Qin et al., 2016). Besides, GM makes the host resistant to the colonization of pathogenic bacteria by occupying the host niche, which plays a vital role in preventing infection (Backhed et al., 2012).

Gut microbiota affects energy intake and absorption

Gubat et al. sequenced and analyzed the GM of normal-weight children and overweight children respectively (Golloso-Gubat et al., 2020). The results showed that Bifidobacterium, Turicibacter, and Clostridiaceae were higher in normal-weight children, and Lachnospira was higher in overweight children. Studies have shown that inulin and other prebiotic fibers can

prevent overeating related to energy-intensive diet intake in rodents (Chassaing et al., 2015). We can see that GM and its metabolites can affect the energy intake and absorption of T1DM patients through multiple channels, which can affect the appetite and total energy intake of patients as well as the consumption and metabolism of carbohydrates, fats, and other dietary components through peripheral and central channels (Rowland et al., 2018). In the gastrointestinal tract, GM and its metabolites, such as SCFAs, Peptide Y(PYY) and indole derivatives, can combine with vagal afferent neurons to transmit information to the nucleus tractus solitarius to affect the body's sense of satiety (Tolhurst et al., 2012; Raybould and Zumpano, 2021). In addition, GM can also improve the sensitivity of the body to leptin by affecting the release of cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1), and then affect satiety through the gut-brain axis (Kałużna-Czaplińska et al., 2017). Some anti-diabetes drugs have been proven to be able to control patients' blood glucose through the above ways (Holmes, 2016). Besides, we have mentioned that when the GM of patients is disordered, the body will be in an inflammatory state. This inflammatory state will also appear in the central solar tract and hypothalamus, thus affecting gut-brain feedback, appetite, and energy consumption, which may be related to microglia in the central system (Heiss and Olofsson, 2018). GM can also regulate the reward pathway of the central nervous system. Carbohydrate compounds can bring pleasure to people by promoting the production of dopamine, allowing people to increase their intake of carbohydrate foods. Inulin can reduce the activation of reward-related regions in the brain, thus reducing the attraction of carbohydrates to the body, thereby reducing their intake (Walker et al., 2018). In addition, GM can also regulate adipose tissue distribution and vitamin synthesis (Rinninella et al., 2019; Kumar et al., 2020).

The interaction between gut microbiota and the immune system

The pathogenesis of both T1DM and T2DM involves the immune system, including inflammation and autoimmunity dysfunction (Moffa et al., 2019). The abnormality of the immune system plays a significant role in the occurrence and development of T1DM, and the gut microbiota plays a crucial role in the regulation and function of the immune system (Salazar et al., 2020). After the appearance of T1DM-related autoantibody, the GM produced insufficient butyric acid-producing bacteria, and the bacterial diversity and community stability were low (Dedrick et al., 2020). de Goffau et al. (2013) performed pyrophosphate sequencing of the fecal specimens retained from 18 children positive for at least two diabetes-related autoantibodies while setting up a control group of 18 healthy children to match them. Compared to the autoantibody-negative group, the two most predominant bifidobacterial species observed in the observation group, namely Bifidobacterium adolescentis and Bifidobacterium pseudostreptum were deficient. At the same time, the number of Bacteroidetes spp. was increased. Bell et al. (2022) concluded that remodeling the GM can significantly affect the immune system of patients with T1DM. Therefore, we can see that GM is related to the autoimmune status of diabetic patients, and the adaptive immune system of patients can be regulated by regulating GM. In addition, GM can affect the occurrence of diabetes by affecting the innate immune system. In the T1DM anode mouse model, the deletion of MyD88, the primary response gene for medullary differentiation of the innate immune adapter, provides disease-dependent protection for the microbiota: under sterile (GF) conditions, MyD88 negative mice, but under specific pathogen-free conditions, do not develop diseases; in GF mice containing multiple GM, colon cancer reduced the occurrence of T1DM in MyD88 negative but nonwild type NOD mice (Burrows et al., 2015). At the same time, GM can help the development of intestinal-associated lymphoid tissue and lymphocytes, which plays a vital role in lymphocyte function, leading to inflammation or immune tolerance (Kamada et al., 2013). Under normal circumstances, immune cells such as macrophages in the body have low reactivity to normal symbiotic bacteria in the intestinal tract and will not produce an obvious proinflammatory reaction. GM plays a vital role in this immune tolerance. When GM is dysfunctional, some bacteria can induce the expression of IL-1β, leading to the disorder of the immune system in the body (Franchi et al., 2012) and accelerating the progression of diabetes. In addition, GM can also make immune cells react faster to infection by regulating the immune response in the intestine to maintain homeostasis in the intestine (Gomes et al., 2018).

Above all, we can see the critical role of GM in the development of diabetes, it is also the mechanism of fecal microbiota transplantation in the treatment of T1DM (Figure 1A). Fecal microbiota transplantation (FMT) have been conducted to treat various internal and external intestinal diseases by oral probiotics, prebiotics with satisfactory results (Al-Jameel, 2021).

Feasibility of FMT in the treatment of T1DM patients

FMT can help to modify blood glucose and insulin resistance

FMT is an aggressive and effective therapeutic approach to alter the microbiome in a limited clinical setting. FMT involves the transfer of gut microbiota from a healthy donor screened for pathogens to a recipient through oral capsules, enemas, or transnasal intestinal tube infusion of bacterial fluids, thus providing colonization resistance, producing beneficial metabolites, and restoring interaction with the mucosal

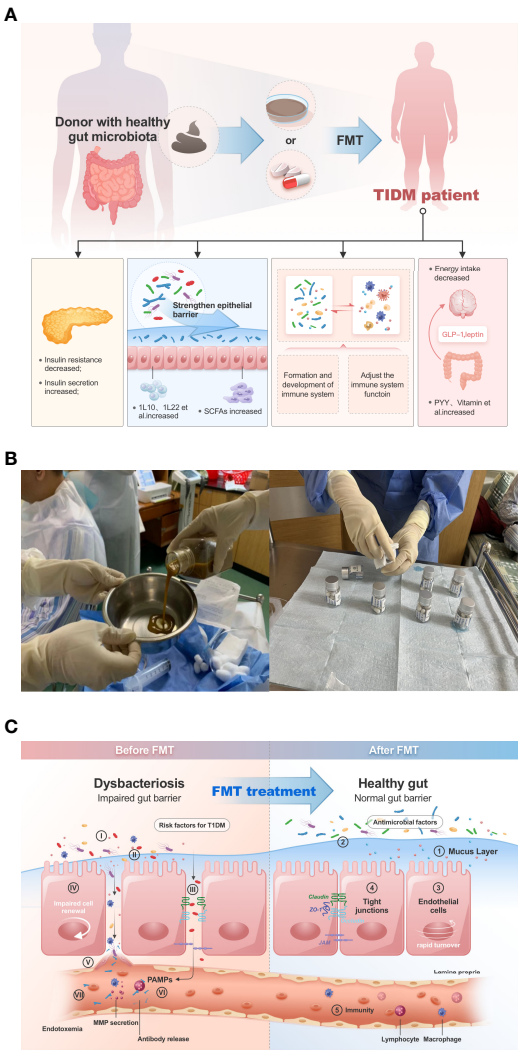


FIGURE 1
(A) FMT can improve the disease progression of T1DM patients in several ways. **(B)**, in our clinical trial study, patients were given gut microbiota transplantation by injection of bacterial solution and oral capsules (Clinical Registration Number: ChiCTR2100045789, Chinese Clinical Trail Registry: http://www.chictr.org.cn/showprojen.aspx?proj=125179). **(C)**, FMT improves dysbiosis of gut microbiota in type 1 diabetic patients.

immune system interactions (Sorbara and Pamer, 2022). During FMT, the role includes not only GM but also virulent fungi in the bacterial fluid, metabolites of the GM, the restoration of the mucosal immune system, and short-chain fatty acids play a crucial role (Leiva-Gea et al., 2018). Mankind has never stopped exploring FMT for the treatment of internal and external intestinal diseases from ancient times to the present. According to ancient biblical records, as early as 3000 years ago, some Indians applied cow dung to treat gastrointestinal diseases; in 400 BC, Chinese medical sage Li Shizhen used "Huang Long Tang" (a mixture of fresh feces and water) to treat patients with chronic diarrhea (Oprita et al., 2016). In Western countries, gut microbiota transplantation has also been explored for the treatment of intestinal and external diseases since the mid-20th century (Khoruts et al., 2015). In the past, due to limited medical technology, the treatment of FMT was initially performed by transplanting the whole feces of a healthy person into the patient, i.e., "swallowing feces by mouth". It seemed to be an unacceptable treatment for many people. This treatment was also not in line with the concept of precision medicine, and there were infections and immune rejection for the transplanted patients. It is also a risk of infection and immune rejection for transplant recipients (Halaweish et al., 2022). Later, with the development of microbiology, people started to culture colonies that might benefit patients, but many bacteria and fungi require harsh culture environments and long culture cycles, which also hindered the development of colony transplantation techniques. It was not until the

emergence of biotechnologies such as macrogenome sequencing, 16sRNA, and human gut microarrays that the study of gut microbiota became more operational and free from dependence on bacterial culture (Lee and Rho, 2022); the analysis of gut microbiota became more accurate and comprehensive, and the analysis of flora could be studied down to the species level and even the strain level of microorganisms, and the interactions between different species of microorganisms, the association between microorganisms and the environment, etc. could be explored. This has led to the rapid development of FMT technology (Org et al., 2017). In 2011, Time Magazine listed flora technology as one of the top ten breakthroughs in medicine, and since then more research has been conducted around FMT for the treatment of diseases.

The basic steps of FMT are as followings. First is the selection of donor and recipient. Recipients have different inclusion and exclusion criteria according to different research categories, but the general exclusion principles are severe cardiac, hepatic, renal, and other vital organ insufficiencies; leukopenia, the manifestation of autoimmune disease or diagnosed autoimmune disease within the past three months; other gastrointestinal diseases that may affect drug absorption; use of other hormones, antibiotics, and patients treated with probiotic prebiotics (He et al., 2022). Regarding the selection of flora donors, the inclusion criteria for autologous donors are equivalent to the inclusion criteria for recipients. As for allogeneic donors, it is necessary to exclude that they have hereditary diseases, autoimmune diseases, infectious diseases, diabetes, and gastrointestinal diseases, have not taken hormones, antibiotics and proton pump inhibitors in the past 3 months, and have not received vaccines and other tested drugs in the past 6 months (Zhang et al., 2019). Donors also should have a good and healthy psychological state, free from anxiety, depression and traumatic stress, and can be assessed by the international self-rating anxiety scale (SAS) and self-rating depression scale (SDS) (Mollayeva et al., 2016). Allogeneic donors are required to have a body mass index (BMI) less than 30 Kg/m2 (Woodworth et al., 2017). Allogeneic donors should be older than 18 years of age. There are no special rules regarding the upper age limit, but elderly people are excluded because of the combination of chronic diseases such as dysfunction of vital organs and diabetes. There is no special requirement for donor gender, but the donor should maintain good lifestyle habits, such as a reasonable diet (regular eating and healthy diet structure) and moderate exercise (Anand et al., 2017). Stool samples collected during pre-FMT matching and post-FMT clinical follow-up are often preserved in preservative solution, which is suitable for clinical studies because of the low requirements for equipment and the preservation environment. There is a special reagent tube for collecting stool samples from patients, which contains stool sample preservation solution and a sampling spoon. After sampling with the sampling spoon, put the fecal sample into the bottom of the sampling tube so that the fecal sample is completely immersed in the sample storage solution, and then screw the tube cover tightly and shake it well. The fecal sample can be stored at room temperature or in a household refrigerator for up to 12 months. Among them, there are many kinds of fecal preservation solutions, and the commonly used preservatives are ethanol, RNAlate, EDTA salt, sodium citrate, and other substances (Guan et al., 2021). The next step is the testing of blood samples and stool specimens from the donor, and these basic tests can be done in general hospital outpatient clinics. After the screening of the donor and recipient, the second step of FMT is to analyze the fecal specimens of both parties, type them, and make the flora capsules, liquid or oil. Stool specimens prepared for FMT were preserved in Maltodextrin-trehalose containing cryoprotectants and then stored in a standard freezer at -80°C. Preservation did not require strict anaerobic conditions, only the removal of air above the specimen. Stool specimens were analyzed by 16S rRNA, metabolomic fingerprinting, and flow cytometry assays to retain optimal recovery potential over a 3-month observation period (Burz et al., 2019).Then the flora preparations are resuscitated in a water bath to about 37°C before transplantation to avoid discomfort to the patient (Smits et al., 2018). The method of flora transplantation usually includes oral capsules, nasogastric tube, and nasojejunal tube injection of bacterial solution or oil, etc. After the transplantation, clinicians need to observe the patients for any adverse reactions and follow up with the patients for a certain period to observe the clinical benefits and gut microbiota changes after receiving FMT (Figure 1B).

FMT is now used to treat a variety of diseases. First, FMT is a safe treatment modality, and in all available clinical cases of FMT, the most common adverse effects are mild clinical symptoms, including diarrhea, gastrointestinal cramps, nausea, bloating, flatulence, constipation, and fever (Allegretti et al., 2019). Second, FMT can effectively alter the gut microbiota of patients, and the effects of flora alteration have been found to be sustained in later clinical follow-ups. It was found in FMT for recurrent C. difficile infection that the distribution of GM in transplanted recipients may be broadly similar to that of healthy donors, and this effect has been shown to persist for up to one-year (Weingarden et al., 2015). In a randomized double-blind trial, 22 obese patients were enrolled in the study and divided into two groups receiving capsule FMT and placebo capsules, and the results could be seen in the patients receiving the capsule FMT group sustained changes in the gut microbiome and bile acid profile that were similar to those of the lean donor (Allegretti et al., 2020). FMT has shown significant clinical efficacy in the treatment of a variety of diseases, and in the treatment of intestinal diseases, FMT has an internationally recognized role in the treatment of refractory C. difficile infections, with efficiency rates exceeding those of advanced antibiotics such as vancomycin and cure rates of more than 85%, FMT becomes a recommended therapy for recurrent CDI by the American College of Gastroenterology and the Infectious

Diseases Society of America (Smillie et al., 2018). Given the close relationship between GM and the endocrine and immune systems, more and more studies have focused on immuno-metabolic and diabetes-related diseases. It has been found that the structure and function of the intestinal barrier can be restored by FMT, which can alleviate the chronic inflammatory state in diabetic patients, improving their clinical symptoms and slowing down the progression of the disease (Ganesan et al., 2018).

Many studies have found that FMT significantly improves insulin resistance, islet secretion, and dysbiosis in mice with non-obese diabetes (NOD) (Vrieze et al., 2012). We have already mentioned that FMT can restore short-chain fatty acids in the intestine (Allegretti et al., 2019), which we have already mentioned can control the progression of diabetes in several ways (Hanssen et al., 2021). FMT can also improve insulin sensitivity and control the progression of diabetes by affecting the autoimmune status of patients (Allegretti et al., 2020). The bacteriophage component of the GM can enter the brain through the intestinal and blood-brain barriers (Chen et al., 2022). Gabanyi et al. (2022) studied mice lacking the pattern recognition receptor Nod2, it was found that intestinal bacterial cell wall debris can cross the intestinal barrier into the brain through the blood circulation and bind to Nod2 in specific neurons in the hypothalamus thereby regulating appetite and body weight; in addition, GM can affect the autonomic nerves in the gut leading to changes in satiety and mood, thus FMT can modulate the patients' gut-brain axis to control insulin resistance and body weight (Hartstra et al., 2020). It was also found that the SCFA-producing microbiota was reduced in T1DM mice (Hanssen et al., 2021), and the addition of propionic acid-producing mucilaginous Ackermania or probiotics that significantly increased SCFA production to the gut of non-obese diabetic (NOD) mice resulted in a reduced incidence of T1DM in NOD mice; Hui W et al. (Wang et al., 2019) performed FMT on a T2DM mouse model established by a high-fat diet combined with streptozotocin and found that insulin resistance and islet β-cell function were improved after FMT, and the inflammatory response of mouse pancreatic tissue was also decreased and apoptosis of islet β-cells was somewhat inhibited. Enterobacteriaceae is a genus of opportunistic endotoxin-producing pathogenic bacteria in mice, with 35% of the gut bacteria in morbidly obese volunteers with diabetes and severe metabolic disorders (Cani et al., 2007). Fei N et al. (Fei and Zhao, 2013) organized a 23-week cereal plus probiotic diet for volunteers. The result showed that after the intervention, the volunteers' body weight was effectively reduced; the abundance of Enterobacteriaceae was reduced from 35% to undetectable, and hyperinsulinemia, insulin resistance, and hyperglycemic states were alleviated. To some extent, probiotic intake also belongs to FMT, and this study mentions the effectiveness of FMT in improving glycemic control and insulin resistance in patients. A case has been reported in which a female patient with an 8-year history of diabetes mellitus with poor glycemic control under the control of glucose-lowering medication had an excellent clinical response and control of glycemia and related diabetic complications after receiving two FMTs within 3 months (Cai et al., 2018). In the treatment of a study enrolling 38 patients with metabolic syndrome, who were divided into two groups receiving allogeneic FMT from lean donors and autologous FMT from their fecal infusion, it was found after 6 weeks that patients receiving allogeneic FMT had increased insulin sensitivity and that this clinical change may be associated with an increase in mucinous Ackermania in the intestine (Kootte et al., 2017). Mocanu et al. found in a randomized, double-blind controlled trial of 61 patients with obesity and metabolic syndrome, FMT supplemented with low fermentable fiber significantly improved insulin resistance in patients and that this metabolic benefit was associated with improved intestinal endocrine function, altered GM abundance, and increased donor bacteria (Mocanu et al., 2021). Above all, FMT can improve the disease progression of diabetic patients in several ways (Figure 1C).

Our study

Two groups of subjects underwent autologous FMT and allogeneic FMT in a randomized controlled trial of new-onset T1DM patients within 6 months; the results showed that FMT stabilized residual -β cell function and optimized glycemic control in patients with new-onset T1DM and that the GM of patients changed at the phylum, genus, and species levels, with a lot of flora such as D. pigerand, B. stercoris, Prevotella spp, and S. oralis correlating with the progression of the treatment course correlated (de Groot et al., 2021). Based on the known metabolic benefits of FMT in patients with autoimmune type 1 diabetes, we treated two adolescent patients with autoimmune type 1 diabetes with FMT (He et al., 2022). First, we performed multiple FMT at different nodes in two T1DM patients; second, we followed both patients clinically for 34 and 19 weeks, respectively, during which stool and serum samples were collected. No adverse events were observed in either patient during our clinical study. Based on the macrogenome sequencing of the stool samples, we concluded that FMT resulted in the colonization of beneficial bacteria in T1DM patients and that the colonization of these flora persisted during the long-term follow-up after the finish of the FMT treatment. Based on the colonization of beneficial bacteria, the clinical outcomes of both patients were significantly improved, and they stopped the use of insulin and some oral hypoglycemic agents. Their blood glucose levels remained. The clinical outcomes were also significantly improved in two patients who had discontinued insulin and some oral hypoglycemic agents, and whose blood glucose levels remained at a more optimal level. Although the number of patients included in this clinical trial is small, it provides strong

theoretical and practical support for us to conduct more clinical research and treatment of T1DM patients with FMT. In addition, we also identified several characteristic bacteria that may be related to the progress of T1DM. According to the analysis of the correlation between the gut microbiota and clinical indicators of patients at the level of genus and species, Faecalibacterium and Butyricimonas were negatively correlated with the insulin resistance (Homa IR) of patients, while Blautia and Anaerostipes were positively correlated with insulin resistance. P. Successives, P. faecium may improve the insulin secretion of patients. L. bacterium GAM79, Clostridium bone, and B. caccae are negatively correlated with insulin secretion index.

Current development status of FMT

Safety and Limitations of FMT

Several studies have shown that FMT appears safe, and patients are less likely to experience adverse reactions. A meta-analysis that included 61 studies after searching to identify 378 reference articles (Rapoport et al., 2022) showed that less than 1% of the 5099 patients who underwent FMT experienced FMT-related serious adverse effects (SAEs). However, on June 13, 2019, the US Food and Drug Administration (FDA) issued a warning about the risks of FMT when they reported two cases of patients who transferred antibiotic-resistant microorganisms [specifically, broad-spectrum β-lactamase-producing Escherichia coli (E. coli)] *via* FMT, causing the patients to develop transplant-related sepsis and leading to death in one of the patients (Battaglioli et al., 2018), in which none of the donor's stool was screened for this resistant antibiotic, and the recipients were immunocompromised patients. DeFilippo et al. (DeFilipp et al., 2019) also reported drug-resistant E. coli bacteremia transmitted by fecal microbiota transplantation.

With the introduction of macroeconomic analysis, it has become clear that, in addition to bacteria, the fecal microbiota contains considerable numbers of viruses, fungi, and phages, as well as intact shed colonic cells. One study reported that feces contained 1011 bacteria/g, 107 intact colonocytes/g, 108 viruses/g, and 108 archaea/g. Although bacteria dominate the intestinal population, other components, such as viruses and miRNAs, cannot be excluded from influencing host physiology (Liu et al., 2016). Since the transfer of unidentified microbial communities may pose some risk, Ott et al. asked whether sterile fecal microbial filtrates would also have beneficial biological effects and investigated this. Their team performed FMT on five patients with relapsed CDI using a filtered (small particles and bacteria removed) fecal solution and found that this sterile (containing bacterial debris, proteins, DNA, antimicrobial compounds, metabolites, and viruses) fecal microbial filtrate could alter the patients' gastrointestinal microbiota and eliminate their gastrointestinal symptoms (Ott et al., 2017), suggesting that non-bacterial elements may play a more important role than previously recognized. In line with this, Zuo et al. (2018) recently reported that phage transfer during FMT could affect the progression of CDI. Similarly, Conceiço-Neto et al. (Conceicao-Neto et al., 2018) suggested that eukaryotic viral groups are associated with successfully treating ulcerative colitis by FMT. These studies suggest that bacterial fractions, metabolites, or phages can mediate the transfer of whole fecal microorganisms. Therefore, in gut microbiota transplantation, non-bacterial components of donor feces may also be transferred through FMT, and the role that these known and unknown components play in transplantation and the impact they bring are also unknown.

Likewise, the development of FMT in the clinical setting is often limited. Firstly, some patients and even clinicians have questioned the effectiveness of FMT in treating T1DM, which greatly hinders the development of therapeutic studies of FMT in the clinical setting. Secondly, many autoimmune-mediated T1DM patients are adolescents, and it is not ethical to conduct clinical studies on patients who are too young. For older minor patients, their lack of cooperation with FMT treatment, lifestyle modifications such as diet structure, late clinical follow-up, and the lack of awareness of their patients also make it more difficult to develop the technology. In addition, we have already mentioned that GM can be affected by many factors, such as the external environment (different air quality and composition of drinking water), different dietary structures, the use of probiotics, antibiotics, and personal habits of smoking and drinking (Hanssen et al., 2021). This leads to a certain degree of limitation of the clinical data and conclusions from the existing FMT clinical studies for the reference of subsequent studies. The clinical benefits of GM for one region or even for one patient may not necessarily be the same for other patients.

Lack of consistency in the supervision of FMT

The European Commission decided that member countries are free to regulate FMT at the national level, which has led to regulatory haphazardness among member states and even a lack of any regulatory standards for FMT in some countries (Verbeke et al., 2017). Lack of standardized regulation can create confusion, while overly restrictive regulation may hinder access to fecal bacteria and research on FMT (Allegretti et al., 2019). Restricting the use of FMT through regulation may lead to some unintended consequences. It becomes less difficult to perform FMT in an environment without medical supervision. People can perform FMT with impunity, and patients can search the Internet for instructions and methods of home FMT and perform self-transplantation, leading to a significant increase in the number of self-FMT (Segal et al., 2018). The lack of

regulation, its unclear donor source, the acquisition process, and the lack of rigor in the transplantation process can significantly increase the risk of transplantation-related diseases (e.g., infectious diseases). On the other hand, the excessive regulatory restrictions have also caused significant problems for those wishing to conduct clinical studies on FMT, and the complex applications and approvals required to conduct clinical trials have discouraged many researchers, incredibly discouraging many clinical workers and preventing them from engaging in such studies (Bunnik et al., 2017).

The effectiveness and efficiency of FMT cannot be determined

Microbial diversity was found to be a reliable predictor of FMT success by comparing the gut flora characteristics of different donors (Kump et al., 2018). Patients who achieve a clinical response to FMT generally show higher microbial diversity than non-responders (Paramsothy et al., 2017b). We suggest that the success of FMT can be considered a two-step process: first, requiring transplanting the transplanted microbiome into a new host and increasing the local commensal community, and then clinical improvement may be observed. The selection of a suitable fecal donor is a critical factor in the success of FMT (Vermeire et al., 2016). However, other factors, such as genetics and environment can also influence the success of FMT. It has been suggested that remission rates can be improved by pooling donor stools together, thereby limiting patients' chances of receiving only ineffective stools (Kazerouni and Wein, 2017). This approach was studied in a cohort of 85 patients with mild to moderate ulcerative colitis in Australia (Paramsothy et al., 2017a), and patients in the treatment group received a mixture of stools containing up to seven different donors in the hope that the donor-dependent effects could be homogenized. In addition, a more intensive dosing regimen was used, with initial FMT by colonoscopy followed by fecal enemas five times a week for eight weeks. Despite the multiple donors and intensive dosing approach, Paramsothy et al. achieved a remission rate after FMT (27% for FMT versus 8% for placebo, $p = 0.02$), similar to that reported previously. Therefore, although the effectiveness of FMT is related to microbial diversity, simply increasing the biodiversity of the donor does not guarantee the effectiveness of transplantation.

Discussion

Autoimmune-mediated T1DM patients have a significant alteration of gut microbiota, and gut microbiota, as the "second genome" of humans, can affect the disease progression of T1DM in many ways (Abdellatif and Sarvetnick, 2019). FMT can improve the glycemic control and insulin resistance of T1DM patients by adjusting the imbalance of gut microbiotaT1DM patients. Many studies have shown that GM is closely related to the occurrence and development of T1DM. In a case-control study, the feces of 16 T1DM children and 16 healthy children were analyzed for GM. It was found that there were significant differences in gut microbial structure between the two groups of children. The abundance of actinomycetes and chlamydia, and the proportion of chlamydia and Bacteroides in T1DM children were lower than those in healthy children, and the number of Clostridium, Bacteroides, and micro-venous bacilli in the intestine of T1DM children was higher (Murri et al., 2013). It was also found that the microbiome of healthy children was more diversified and stable than that of children with T1DM. After the occurrence of autoimmune diseases, the level of Firmicum decreased, and the level of bacteroids increased (Giongo et al., 2011). Stewart et al. (2018) analyzed the fecal specimens through 16s rRNA and macroeconomic sequencing retained from 903 patients with T1DM aged 3 months to 46 months and the healthy control group patients; the abundance of genera of bacteria changed between the observation and control groups. They found higher levels of streptococcus and lactococcus in T1DM. It could protect the intestinal mucosal barrier function, enhance intestinal integrity, and reduce the chronic inflammatory state in diabetic patients (Brown et al., 2011). Many drugs have induced changes in the GM of diabetic patients while controlling blood glucose. Metformin is the first-line cornerstone drug for glycemic control in diabetic patients, and Bryrup et al. (2019) found that after six weeks of continuous oral administration of metformin, patients experienced significant changes in gut microbiota richness, with a decrease in the abundance of Enterobacter spp. and Clostridium spp. and an increase in the abundance of Salmonella spp. and Shigella spp. and Biliophage spp. Gu et al. (2017) demonstrated that acarbose treatment altered the composition of the GM, with increased concentrations of Lactobacillus and Bifidobacterium and decreased concentrations of Bacteroidetes and Clostridium, both of which could improve insulin resistance in patients by modulating bile acid metabolism. Therefore, we can see that T1DM patients do have gut microbiota disorders and the improvement of blood glucose levels in diabetic patients is closely related to the change in gut microbiota. The effectiveness of FMT in improving patients' glycemic control and insulin resistance can also be seen in the clinical treatments around FMT listed in the previous section, but there are still two major problems. Firstly, many clinicians are skeptical about FMT for T1DM, they believe that gut microbiota plays a limited role in the progression of T1DM and deny that FMT can bring significant glycemic control benefits to T1DM patients, and they believe that the safety of FMT is still to be investigated, which largely hinders the development and application of FMT treatment technology. Secondly, the clinical studies on the treatment of T1DM by FMT are limited,

and there is a lack of large-scale clinical trials with many subjects and no case-control trials with different nodes transplanted for comparison, and the mode, number, and time point of transplantation have not yet been fully standardized. We have not yet demonstrated how often and how many repeat transplants are needed to achieve the best clinical benefit for patients. We also cannot specifically control the effect of other food medications on the efficacy of FMT. Many questions need to be addressed in the future development of FMT treatment technology.

In the future development of FMT treatment technology, many questions must be solved. Firstly, its safety should be further improved. The technology should be able to effectively screen out pathogenic intestinal bacteria and try to avoid serious adverse events, such as infection, aggravation of gut microbiota dysbiosis, and immune rejection-related events. Secondly, the National Health Organization should establish and improve the standardized system of FMT for disease treatment and clarify the relevant indications, contraindications, transplantation methods, and related expenses. Third, the implementation of "precise transplantation technology" is the transplantation of specific disordered microbiota related to the disease to restore normal intestinal homeostasis and then regulate the immune and metabolic disorders in the patient's body. Fourthly, we should be aware of the importance of clinicians in the development of FMT for T1DM. Therefore, FMT-related studies could be added to the mini-lessons in hospitals to complement the knowledge gaps of clinicians in this field, so that they can see the clinical benefits of FMT for T1DM patients and thus facilitate more therapeutic studies. Fifth, at present, the specific mechanism of FMT in the treatment of autoimmune-mediated T1DM has not been completely revealed. Many researchers believe that the benefits of FMT in T1DM patients such as the change of autoimmune status and the improvement of beta-cell function are related to SCFAs (Jacob et al., 2020). However, another study found that oral SCFAs did not improve the innate immunity and islet autoimmunity of T1DM patients (de Groot et al., 2020). Therefore, the mechanism of FMT intervention in the treatment of T1DM remains to be further explored. Finally, there are still many questions about the transplantation process: for example, how can oral capsules or nasal-intestinal tube injections achieve better therapeutic effects through the transplantation method? Are multiple transplants better than single transplants? If multiple transplants are performed, what is the optimal time interval between each transplant, the duration of FMT, and how to extend the duration of treatment? How much will the use of antibiotics, hormones, immunosuppressants, and other probiotics affect FMT's efficacy, and can the adverse effects be avoided? These unknowns will hopefully be further addressed in the future development of FMT technology.

Author contributions

Conceptualization, KH and LL. Writing—original draft preparation, SZ, ZW, and FC. Writing— review and editing, FD and JC. Supervision, KH. Funding acquisition, LL. All authors have read and agreed to the published version of the manuscript.

Funding

This work was supported by grants from the Guangdong Science and Technology Special Fund (No.210629086900260), Longhu People's Hospital, Shantou, China.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Abdellatif, A. M., and Sarvetnick, N. E. (2019). Current understanding of the role of gut dysbiosis in type 1 diabetes. *J. Diabetes* 11 (8), 632–644. doi: 10.1111/1753-0407.12915

Al-Jameel, S. S. (2021). Association of diabetes and microbiota: An update. *Saudi J. Biol. Sci.* 28 (8), 4446–4454. doi: 10.1016/j.sjbs.2021.04.041

Allegretti, J. R., Kassam, Z., Mullish, B. H., Chiang, A., Carrellas, M., Hurtado, J., et al. (2020). Effects of fecal microbiota transplantation with oral capsules in obese patients. *Clin. Gastroenterol. Hepatol.* 18 (4), 855–63.e2. doi: 10.1016/j.cgh.2019.07.006

Allegretti, J. R., Mullish, B. H., Kelly, C., and Fischer, M. (2019). The evolution of the use of faecal microbiota transplantation and emerging therapeutic indications. *Lancet* 394 (10196), 420–431. doi: 10.1016/S0140-6736(19)31266-8

American Diabetes Association Professional Practice C. 2 (2022). Classification and diagnosis of diabetes: Standards of medical care in diabetes-2022. *Diabetes Care* 45 (Suppl 1), S17–S38. doi: 10.2337/dc22-S002

Anand, R., Song, Y., Garg, S., Girotra, M., Sinha, A., Sivaraman, A., et al. (2017). Effect of aging on the composition of fecal microbiota in donors for FMT and its

impact on clinical outcomes. *Dig Dis. Sci.* 62 (4), 1002–1008. doi: 10.1007/s10620-017-4449-6

Backhed, F., Fraser, C. M., Ringel, Y., Sanders, M. E., Sartor, R. B., Sherman, P. M., et al. (2012). Defining a healthy human gut microbiome: current concepts, future directions, and clinical applications. *Cell Host Microbe* 12 (5), 611–622. doi: 10.1016/j.chom.2012.10.012

Battaglioli, E. J., Hale, V. L., Chen, J., Jeraldo, P., Ruiz-Mojica, C., Schmidt, B. A., et al. (2018). Clostridioides difficile uses amino acids associated with gut microbial dysbiosis in a subset of patients with diarrhea. *Sci. Transl. Med.* 10 (464), eaam7019. doi: 10.1126/scitranslmed.aam7019

Bell, K. J., Saad, S., Tillett, B. J., McGuire, H. M., Bordbar, S., Yap, Y. A., et al. (2022). Metabolite-based dietary supplementation in human type 1 diabetes is associated with microbiota and immune modulation. *Microbiome.* 10 (1), 9. doi: 10.1186/s40168-021-01193-9

Brown, C. T., Davis-Richardson, A. G., Giongo, A., Gano, K. A., Crabb, D. B., Mukherjee, N., et al. (2011). Gut microbiome metagenomics analysis suggests a functional model for the development of autoimmunity for type 1 diabetes. *PloS One* 6 (10), e25792. doi: 10.1371/journal.pone.0025792

Bryrup, T., Thomsen, C. W., Kern, T., Allin, K. H., Brandslund, I., Jorgensen, N. R., et al. (2019). Metformin-induced changes of the gut microbiota in healthy young men: results of a non-blinded, one-armed intervention study. *Diabetologia.* 62 (6), 1024–1035. doi: 10.1007/s00125-019-4848-7

Bunnik, E. M., Aarts, N., and Chen, L. A. (2017). Physicians must discuss potential long-term risks of fecal microbiota transplantation to ensure informed consent. *Am. J. Bioeth* 17 (5), 61–63. doi: 10.1080/15265161.2017.1299816

Burger-van Paassen, N., Vincent, A., Puiman, P. J., van der Sluis, M., Bouma, J., Boehm, G., et al. (2009). The regulation of intestinal mucin MUC2 expression by short-chain fatty acids: implications for epithelial protection. *Biochem. J.* 420 (2), 211–219. doi: 10.1042/BJ20082222

Burrows, M. P., Volchkov, P., Kobayashi, K. S., and Chervonsky, A. V. (2015). Microbiota regulates type 1 diabetes through toll-like receptors. *Proc. Natl. Acad. Sci. U S A.* 112 (32), 9973–9977. doi: 10.1073/pnas.1508740112

Burz, S. D., Abraham, A. L., Fonseca, F., David, O., Chapron, A., Beguet-Crespel, F., et al. (2019). A guide for ex vivo handling and storage of stool samples intended for fecal microbiota transplantation. *Sci. Rep.* 9 (1), 8897. doi: 10.1038/s41598-019-45173-4

Cai, T. T., Ye, X. L., Yong, H. J., Song, B., Zheng, X. L., Cui, B. T., et al. (2018). Fecal microbiota transplantation relieve painful diabetic neuropathy: A case report. *Med. (Baltimore)* 97 (50), e13543. doi: 10.1097/MD.0000000000013543

Cani, P. D., Amar, J., Iglesias, M. A., Poggi, M., Knauf, C., Bastelica, D., et al. (2007). Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes.* 56 (7), 1761–1772. doi: 10.2337/db06-1491

Chafe, R., Aslanov, R., Sarkar, A., Gregory, P., Comeau, A., and Newhook, L. A. (2018). Association of type 1 diabetes and concentrations of drinking water components in Newfoundland and Labrador, Canada. *BMJ Open Diabetes Res. Care* 6 (1), e000466. doi: 10.1136/bmjdrc-2017-000466

Chassaing, B., Miles-Brown, J., Pellizzon, M., Ulman, E., Ricci, M., Zhang, L., et al. (2015). Lack of soluble fiber drives diet-induced adiposity in mice. *Am. J. Physiol. Gastrointest Liver Physiol.* 309 (7), G528–G541. doi: 10.1152/ajpgi.00172.2015

Chen, F., He, L., Li, J., Yang, S., Zhang, B., Zhu, D., et al. (2022). Polyethylene glycol loxenatide injection (GLP-1) protects vascular endothelial cell function in middle-aged and elderly patients with type 2 diabetes by regulating gut microbiota. *Front. Mol. Biosci.* 9, 879294. doi: 10.3389/fmolb.2022.879294

Chen, F., Hou, K., and Chen, Z. S. (2022). Gut microbes regulate the feeding center: a new discovery of gut brain axis. *Signal Transduct Target Ther.* 7 (1), 284. doi: 10.1038/s41392-022-01117-5

Cole, D. K., Bulek, A. M., Dolton, G., Schauenberg, A. J., Szomolay, B., Rittase, W., et al. (2016). Hotspot autoimmune T cell receptor binding underlies pathogen and insulin peptide cross-reactivity. *J. Clin. Invest.* 126 (9), 3626. doi: 10.1172/JCI89919

Conceicao-Neto, N., Deboutte, W., Dierckx, T., Machiels, K., Wang, J., Yinda, K. C., et al. (2018). Low eukaryotic viral richness is associated with faecal microbiota transplantation success in patients with UC. *Gut.* 67 (8), 1558–1559. doi: 10.1136/gutjnl-2017-315281

Davis-Richardson, A. G., and Triplett, E. W. (2015). A model for the role of gut bacteria in the development of autoimmunity for type 1 diabetes. *Diabetologia.* 58 (7), 1386–1393. doi: 10.1007/s00125-015-3614-8

Dedrick, S., Sundaresh, B., Huang, Q., Brady, C., Yoo, T., Cronin, C., et al. (2020). The role of gut microbiota and environmental factors in type 1 diabetes pathogenesis. *Front. Endocrinol. (Lausanne)* 11, 78. doi: 10.3389/fendo.2020.00078

DeFilipp, Z., Bloom, P. P., Torres Soto, M., Mansour, M. K., Sater, M. R. A., Huntley, M. H., et al. (2019). Drug-resistant e. coli bacteremia transmitted by fecal microbiota transplant. *N Engl. J. Med.* 381 (21), 2043–2050. doi: 10.1056/NEJMoa1910437

de Goffau, M. C., Fuentes, S., van den Bogert, B., Honkanen, H., de Vos, W. M., Welling, G. W., et al. (2014). Aberrant gut microbiota composition at the onset of type 1 diabetes in young children. *Diabetologia.* 57 (8), 1569–1577. doi: 10.1007/s00125-014-3274-0

de Goffau, M. C., Luopajarvi, K., Knip, M., Ilonen, J., Ruohtula, T., Harkonen, T., et al. (2013). Fecal microbiota composition differs between children with beta-cell autoimmunity and those without. *Diabetes.* 62 (4), 1238–1244. doi: 10.2337/db12-0526

de Groot, P. F., Belzer, C., Aydin, O., Levin, E., Levels, J. H., Aalvink, S., et al. (2017). Distinct fecal and oral microbiota composition in human type 1 diabetes, an observational study. *PloS One* 12 (12), e0188475. doi: 10.1371/journal.pone.0188475

de Groot, P. F., Nikolic, T., Imangaliyev, S., Bekkering, S., Duinkerken, G., Keij, F. M., et al. (2020). Oral butyrate does not affect innate immunity and islet autoimmunity in individuals with longstanding type 1 diabetes: a randomised controlled trial. *Diabetologia.* 63 (3), 597–610. doi: 10.1007/s00125-019-05073-8

de Groot, P., Nikolic, T., Pellegrini, S., Sordi, V., Imangaliyev, S., Rampanelli, E., et al. (2021). Faecal microbiota transplantation halts progression of human new-onset type 1 diabetes in a randomised controlled trial. *Gut.* 70 (1), 92–105. doi: 10.1136/gutjnl-2020-322630

De Vadder, F., Kovatcheva-Datchary, P., Goncalves, D., Vinera, J., Zitoun, C., Duchampt, A., et al. (2014). Microbiota-generated metabolites promote metabolic benefits *via* gut-brain neural circuits. *Cell* 156 (1-2), 84–96. doi: 10.1016/j.cell.2013.12.016

Fei, N., and Zhao, L. (2013). An opportunistic pathogen isolated from the gut of an obese human causes obesity in germfree mice. *ISME J.* 7 (4), 880–884. doi: 10.1038/ismej.2012.153

Franchi, L., Kamada, N., Nakamura, Y., Burberry, A., Kuffa, P., Suzuki, S., et al. (2012). NLRC4-driven production of IL-1beta discriminates between pathogenic and commensal bacteria and promotes host intestinal defense. *Nat. Immunol.* 13 (5), 449–456. doi: 10.1038/ni.2263

Gabanyi, I., Lepousez, G., Wheeler, R., Vieites-Prado, A., Nissant, A., Wagner, S., et al. (2022). Bacterial sensing via neuronal Nod2 regulates appetite and body temperature. *Science* 376 (6590), eabj3986. doi: 10.1126/science.abj3986

Ganesan, K., Chung, S. K., Vanamala, J., and Xu, B. (2018). Causal relationship between diet-induced gut microbiota changes and diabetes: A novel strategy to transplant faecalibacterium prausnitzii in preventing diabetes. *Int. J. Mol. Sci.* 19 (12), 3720. doi: 10.3390/ijms19123720

Giongo, A., Gano, K. A., Crabb, D. B., Mukherjee, N., Novelo, L. L., Casella, G., et al. (2011). Toward defining the autoimmune microbiome for type 1 diabetes. *ISME J.* 5 (1), 82–91. doi: 10.1038/ismej.2010.92

Golloso-Gubat, M. J., Ducarmon, Q. R., Tan, R. C. A., Zwittink, R. D., Kuijper, E. J., Nacis, J. S., et al. (2020). Gut microbiota and dietary intake of normal-weight and overweight Filipino children. *Microorganisms* 8 (7), 1015. doi: 10.3390/microorganisms8071015

Gomes, A. C., Hoffmann, C., and Mota, J. F. (2018). The human gut microbiota: Metabolism and perspective in obesity. *Gut Microbes* 9 (4), 308–325. doi: 10.1080/19490976.2018.1465157

Guan, H., Pu, Y., Liu, C., Lou, T., Tan, S., Kong, M., et al. (2021). Comparison of fecal collection methods on variation in gut metagenomics and untargeted metabolomics. *mSphere.* 6 (5), e0063621. doi: 10.1128/mSphere.00636-21

Gutierrez-Repiso, C., Moreno-Indias, I., Martin-Nunez, G. M., Ho-Plagaro, A., Rodriguez-Canete, A., Gonzalo, M., et al. (2020). Mucosa-associated microbiota in the jejunum of patients with morbid obesity: alterations in states of insulin resistance and metformin treatment. *Surg. Obes. Relat. Dis.* 16 (10), 1575–1585. doi: 10.1016/j.soard.2020.04.008

Gu, Y., Wang, X., Li, J., Zhang, Y., Zhong, H., Liu, R., et al. (2017). Analyses of gut microbiota and plasma bile acids enable stratification of patients for antidiabetic treatment. *Nat. Commun.* 8 (1), 1785. doi: 10.1038/s41467-017-01682-2

Halaweish, H. F., Boatman, S., and Staley, C. (2022). Encapsulated fecal microbiota transplantation: Development, efficacy, and clinical application. *Front. Cell Infect. Microbiol.* 12, 826114. doi: 10.3389/fcimb.2022.826114

Hanssen, N. M. J., de Vos, W. M., and Nieuwdorp, M. (2021). Fecal microbiota transplantation in human metabolic diseases: From a murky past to a bright future? *Cell Metab.* 33 (6), 1098–1110. doi: 10.1016/j.cmet.2021.05.005

Hartstra, A. V., Schuppel, V., Imangaliyev, S., Schrantee, A., Prodan, A., Collard, D., et al. (2020). Infusion of donor feces affects the gut-brain axis in humans with metabolic syndrome. *Mol. Metab.* 42, 101076. doi: 10.1016/j.molmet.2020.101076

Hasain, Z., Mokhtar, N. M., Kamaruddin, N. A., Mohamed Ismail, N. A., Razalli, N. H., Gnanou, J. V., et al. (2020). Gut microbiota and gestational diabetes mellitus: A review of host-gut microbiota interactions and their therapeutic potential. *Front. Cell Infect. Microbiol.* 10, 188. doi: 10.3389/fcimb.2020.00188

He, L., Chen, R., Zhang, B., Zhang, S., Khan, B. A., Zhu, D., et al. (2022). Fecal microbiota transplantation treatment of autoimmune-mediated type 1 diabetes mellitus. *Front. Immunol.* 13, 930872. doi: 10.3389/fimmu.2022.930872

Heiss, C. N., and Olofsson, L. E. (2018). Gut microbiota-dependent modulation of energy metabolism. *J. Innate Immun.* 10 (3), 163–171. doi: 10.1159/000481519

Holmes, D. (2016). Gut microbiota: Antidiabetic drug treatment confounds gut dysbiosis associated with type 2 diabetes mellitus. *Nat. Rev. Endocrinol.* 12 (2), 61. doi: 10.1038/nrendo.2015.222

Hou, K., Wu, Z. X., Chen, X. Y., Wang, J. Q., Zhang, D., Xiao, C., et al. (2022). Microbiota in health and diseases. *Signal Transduct Target Ther.* 7 (1), 135. doi: 10.1038/s41392-022-00974-4

Hou, K., Zhang, S., Wu, Z., Zhu, D., Chen, F., Lei, Z. N., et al. (2021). Reconstruction of intestinal microecology of type 2 diabetes by fecal microbiota transplantation: Why and how. *Bosn J. Basic Med. Sci.* 22 (3), 315–325. doi: 10.17305/bjbms.2021.6323

Jacob, N., Jaiswal, S., Maheshwari, D., Nallabelli, N., Khatri, N., Bhatia, A., et al. (2020). Butyrate induced tregs are capable of migration from the GALT to the pancreas to restore immunological tolerance during type-1 diabetes. *Sci. Rep.* 10 (1), 19120. doi: 10.1038/s41598-020-76109-y

Kałużna-Czaplińska, J., Gątarek, P., Chartrand, M. S., Dadar, M., and Bjørklund, G. (2017). Is there a relationship between intestinal microbiota, dietary compounds, and obesity? *Trends Food Sci. Technol.* 70, 105–113. doi: 10.1016/j.tifs.2017.10.010

Kamada, N., Seo, S. U., Chen, G. Y., and Nunez, G. (2013). Role of the gut microbiota in immunity and inflammatory disease. *Nat. Rev. Immunol.* 13 (5), 321–335. doi: 10.1038/nri3430

Kazerouni, A., and Wein, L. M. (2017). Exploring the efficacy of pooled stools in fecal microbiota transplantation for microbiota-associated chronic diseases. *PloS One* 12 (1), e0163956. doi: 10.1371/journal.pone.0163956

Khoruts, A., Sadowsky, M. J., and Hamilton, M. J. (2015). Development of fecal microbiota transplantation suitable for mainstream medicine. *Clin. Gastroenterol. Hepatol.* 13 (2), 246–250. doi: 10.1016/j.cgh.2014.11.014

Kimura, I., Ozawa, K., Inoue, D., Imamura, T., Kimura, K., Maeda, T., et al. (2013). The gut microbiota suppresses insulin-mediated fat accumulation *via* the short-chain fatty acid receptor GPR43. *Nat. Commun.* 4, 1829. doi: 10.1038/ncomms2852

Kootte, R. S., Levin, E., Salojarvi, J., Smits, L. P., Hartstra, A. V., Udayappan, S. D., et al. (2017). Improvement of insulin sensitivity after lean donor feces in metabolic syndrome is driven by baseline intestinal microbiota composition. *Cell Metab.* 26 (4), 611–9.e6. doi: 10.1016/j.cmet.2017.09.008

Krauss, R. M. (2004). Lipids and lipoproteins in patients with type 2 diabetes. *Diabetes Care* 27 (6), 1496–1504. doi: 10.2337/diacare.27.6.1496

Krischer, J. P., Liu, X., Vehik, K., Akolkar, B., Hagopian, W. A., Rewers, M. J., et al. (2019). Predicting islet cell autoimmunity and type 1 diabetes: An 8-year TEDDY study progress report. *Diabetes Care* 42 (6), 1051–1060. doi: 10.2337/dc18-2282

Kumar, M., Singh, P., Murugesan, S., Vetizou, M., McCulloch, J., Badger, J. H., et al. (2020). Microbiome as an immunological modifier. *Methods Mol. Biol.* 2055, 595–638. doi: 10.1007/978-1-4939-9773-2_27

Kump, P., Wurm, P., Grochenig, H. P., Wenzl, H., Petritsch, W., Halwachs, B., et al. (2018). The taxonomic composition of the donor intestinal microbiota is a major factor influencing the efficacy of faecal microbiota transplantation in therapy refractory ulcerative colitis. *Aliment Pharmacol. Ther.* 47 (1), 67–77. doi: 10.1111/apt.14387

Lee, S. J., and Rho, M. (2022). Multimodal deep learning applied to classify healthy and disease states of human microbiome. *Sci. Rep.* 12 (1), 824. doi: 10.1038/s41598-022-04773-3

Leiva-Gea, I., Sanchez-Alcoholado, L., Martin-Tejedor, B., Castellano-Castillo, D., Moreno-Indias, I., Urda-Cardona, A., et al. (2018). Gut microbiota differs in composition and functionality between children with type 1 diabetes and MODY2 and healthy control subjects: A case-control study. *Diabetes Care* 41 (11), 2385–2395. doi: 10.2337/dc18-0253

Liu, S., da Cunha, A. P., Rezende, R. M., Cialic, R., Wei, Z., Bry, L., et al. (2016). The host shapes the gut microbiota *via* fecal MicroRNA. *Cell Host Microbe* 19 (1), 32–43. doi: 10.1016/j.chom.2015.12.005

Macfarlane, G. T., and Macfarlane, S. (2011). Fermentation in the human large intestine: its physiologic consequences and the potential contribution of prebiotics. *J. Clin. Gastroenterol.* 45 Suppl, S120–S127. doi: 10.1097/MCG.0b013e31822fecfe

Malmqvist, E., Larsson, H. E., Jonsson, I., Rignell-Hydbom, A., Ivarsson, S. A., Tinnerberg, H., et al. (2015). Maternal exposure to air pollution and type 1 diabetes–accounting for genetic factors. *Environ. Res.* 140, 268–274. doi: 10.1016/j.envres.2015.03.024

Mobasseri, M., Shirmohammadi, M., Amiri, T., Vahed, N., Hosseini Fard, H., and Ghojazadeh, M. (2020). Prevalence and incidence of type 1 diabetes in the world: a systematic review and meta-analysis. *Health Promot Perspect.* 10 (2), 98–115. doi: 10.34172/hpp.2020.18

Mocanu, V., Zhang, Z., Deehan, E. C., Kao, D. H., Hotte, N., Karmali, S., et al. (2021). Fecal microbial transplantation and fiber supplementation in patients with severe obesity and metabolic syndrome: a randomized double-blind, placebo-controlled phase 2 trial. *Nat. Med.* 27 (7), 1272–1279. doi: 10.1038/s41591-021-01399-2

Moffa, S., Mezza, T., Cefalo, C. M. A., Cinti, F., Impronta, F., Sorice, G. P., et al. (2019). The interplay between immune system and microbiota in diabetes. *Mediators Inflamm.* 2019, 9367404. doi: 10.1155/2019/9367404

Mollayeva, T., Thurairajah, P., Burton, K., Mollayeva, S., Shapiro, C. M., and Colantonio, A. (2016). The Pittsburgh sleep quality index as a screening tool for sleep dysfunction in clinical and non-clinical samples: A systematic review and meta-analysis. *Sleep Med. Rev.* 25, 52–73. doi: 10.1016/j.smrv.2015.01.009

Mullaney, J. A., Stephens, J. E., Costello, M. E., Fong, C., Geeling, B. E., Gavin, P. G., et al. (2018). Type 1 diabetes susceptibility alleles are associated with distinct alterations in the gut microbiota. *Microbiome.* 6 (1), 35. doi: 10.1186/s40168-018-0417-4

Murri, M., Leiva, I., Gomez-Zumaquero, J. M., Tinahones, F. J., Cardona, F., Soriguer, F., et al. (2013). Gut microbiota in children with type 1 diabetes differs from that in healthy children: a case-control study. *BMC Med.* 11, 46. doi: 10.1186/1741-7015-11-46

Nicholson, J. K., Holmes, E., Kinross, J., Burcelin, R., Gibson, G., Jia, W., et al. (2012). Host-gut microbiota metabolic interactions. *Science* 336 (6086), 1262–1267. doi: 10.1126/science.1223813

Oprita, R., Bratu, M., Oprita, B., and Diaconescu, B. (2016). Fecal transplantation - the new, inexpensive, safe, and rapidly effective approach in the treatment of gastrointestinal tract diseases. *J. Med. Life.* 9 (2), 160–162.

Org, E., Blum, Y., Kasela, S., Mehrabian, M., Kuusisto, J., Kangas, A. J., et al. (2017). Relationships between gut microbiota, plasma metabolites, and metabolic syndrome traits in the METSIM cohort. *Genome Biol.* 18 (1), 70. doi: 10.1186/s13059-017-1194-2

Ott, S. J., Waetzig, G. H., Rehman, A., Moltzau-Anderson, J., Bharti, R., Grasis, J. A., et al. (2017). Efficacy of sterile fecal filtrate transfer for treating patients with clostridium difficile infection. *Gastroenterology* 152 (4), 799–811.e7. doi: 10.1053/j.gastro.2016.11.010

Paramsothy, S., Kamm, M. A., Kaakoush, N. O., Walsh, A. J., van den Bogaerde, J., Samuel, D., et al. (2017a). Multidonor intensive faecal microbiota transplantation for active ulcerative colitis: a randomised placebo-controlled trial. *Lancet* 389 (10075), 1218–1228. doi: 10.1016/S0140-6736(17)30182-4

Paramsothy, S., Paramsothy, R., Rubin, D. T., Kamm, M. A., Kaakoush, N. O., Mitchell, H. M., et al. (2017b). Faecal microbiota transplantation for inflammatory bowel disease: A systematic review and meta-analysis. *J. Crohns Colitis.* 11 (10), 1180–1199. doi: 10.1093/ecco-jcc/jjx063

Pedersen, H. K., Gudmundsdottir, V., Nielsen, H. B., Hyotylainen, T., Nielsen, T., Jensen, B. A., et al. (2016). Human gut microbes impact host serum metabolome and insulin sensitivity. *Nature* 535 (7612), 376–381. doi: 10.1038/nature18646

Pitocco, D., Di Leo, M., Tartaglione, L., De Leva, F., Petruzziello, C., Saviano, A., et al. (2020). The role of gut microbiota in mediating obesity and diabetes mellitus. *Eur. Rev. Med. Pharmacol. Sci.* 24 (3), 1548–1562. doi: 10.26355/eurrev_202002_20213

Qin, B., Viera, A. J., Muntner, P., Plassman, B. L., Edwards, L. J., Adair, L. S., et al. (2016). Visit-to-Visit variability in blood pressure is related to late-life cognitive decline. *Hypertension.* 68 (1), 106–113. doi: 10.1161/HYPERTENSIONAHA.116.07494

Que, Y., Cao, M., He, J., Zhang, Q., Chen, Q., Yan, C., et al. (2021). Gut bacterial characteristics of patients with type 2 diabetes mellitus and the application potential. *Front. Immunol.* 12, 722206. doi: 10.3389/fimmu.2021.722206

Rapoport, E. A., Baig, M., and Puli, S. R. (2022). Adverse events in fecal microbiota transplantation: a systematic review and meta-analysis. *Ann. Gastroenterol.* 35 (2), 150–163. doi: 10.20524/aog.2022.0695

Raybould, H. E., and Zumpano, D. L. (2021). Microbial metabolites and the vagal afferent pathway in the control of food intake. *Physiol. Behav.* 240, 113555. doi: 10.1016/j.physbeh.2021.113555

Rewers, M., and Ludvigsson, J. (2016). Environmental risk factors for type 1 diabetes. *Lancet.* 387 (10035), 2340–2348. doi: 10.1016/S0140-6736(16)30507-4

Rinninella, E., Raoul, P., Cintoni, M., Franceschi, F., Miggiano, G. A. D., Gasbarrini, A., et al. (2019). What is the healthy gut microbiota composition? a changing ecosystem across age, environment, diet, and diseases. *Microorganisms* 7 (1), 14. doi: 10.3390/microorganisms7010014

Rodriguez-Valera, F., Martin-Cuadrado, A. B., Rodriguez-Brito, B., Pasic, L., Thingstad, T. F., Rohwer, F., et al. (2009). Explaining microbial population genomics through phage predation. *Nat. Rev. Microbiol.* 7 (11), 828–836. doi: 10.1038/nrmicro2235

Rowland, I., Gibson, G., Heinken, A., Scott, K., Swann, J., Thiele, I., et al. (2018). Gut microbiota functions: metabolism of nutrients and other food components. *Eur. J. Nutr.* 57 (1), 1–24. doi: 10.1007/s00394-017-1445-8

Salazar, J., Angarita, L., Morillo, V., Navarro, C., Martinez, M. S., Chacin, M., et al. (2020). Microbiota and diabetes mellitus: Role of lipid mediators. *Nutrients* 12 (10), 3039. doi: 10.3390/nu12103039

Samuel, B. S., Shaito, A., Motoike, T., Rey, F. E., Backhed, F., Manchester, J. K., et al. (2008). Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. *Proc. Natl. Acad. Sci. U S A.* 105 (43), 16767–16772. doi: 10.1073/pnas.0808567105

Scheithauer, T. P., Dallinga-Thie, G. M., de Vos, W. M., Nieuwdorp, M., and van Raalte, D. H. (2016). Causality of small and large intestinal microbiota in weight regulation and insulin resistance. *Mol. Metab.* 5 (9), 759–770. doi: 10.1016/j.molmet.2016.06.002

Segal, J. P., Abbasi, F., Kanagasundaram, C., and Hart, A. (2018). Does the Internet promote the unregulated use of fecal microbiota transplantation: a potential public health issue? *Clin. Exp. Gastroenterol.* 11, 179–183. doi: 10.2147/CEG.S159609

Shou, J., Chen, P. J., and Xiao, W. H. (2019). The effects of BCAAs on insulin resistance in athletes. *J. Nutr. Sci. Vitaminol (Tokyo)* 65 (5), 383–389. doi: 10.3177/jnsv.65.383

Smillie, C. S., Sauk, J., Gevers, D., Friedman, J., Sung, J., Youngster, I., et al. (2018). Strain tracking reveals the determinants of bacterial engraftment in the human gut following fecal microbiota transplantation. *Cell Host Microbe* 23 (2), 229–240.e5. doi: 10.1016/j.chom.2018.01.003

Smits, L. P., Kootte, R. S., Levin, E., Prodan, A., Fuentes, S., Zoetendal, E. G., et al. (2018). Effect of vegan fecal microbiota transplantation on carnitine- and choline-derived trimethylamine-N-Oxide production and vascular inflammation in patients with metabolic syndrome. *J. Am. Heart Assoc.* 7 (7), e008342. doi: 10.1161/JAHA.117.008342

Sorbara, M. T., and Pamer, E. G. (2022). Microbiome-based therapeutics. *Nat. Rev. Microbiol.* 20 (6), 365–380. doi: 10.1038/s41579-021-00667-9

Stankov, K., Benc, D., and Draskovic, D. (2013). Genetic and epigenetic factors in etiology of diabetes mellitus type 1. *Pediatrics* 132 (6), 1112–1122. doi: 10.1542/peds.2013-1652

Steffes, M. W., Sibley, S., Jackson, M., and Thomas, W. (2003). Beta-cell function and the development of diabetes-related complications in the diabetes control and complications trial. *Diabetes Care* 26 (3), 832–836. doi: 10.2337/diacare.26.3.832

Stewart, C. J., Ajami, N. J., O'Brien, J. L., Hutchinson, D. S., Smith, D. P., Wong, M. C., et al. (2018). Temporal development of the gut microbiome in early childhood from the TEDDY study. *Nature* 562 (7728), 583–588. doi: 10.1038/s41586-018-0617-x

Sun, H., Saeedi, P., Karuranga, S., Pinkepank, M., Ogurtsova, K., Duncan, B. B., et al. (2022). IDF diabetes atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res. Clin. Pract.* 183, 109119. doi: 10.1016/j.diabres.2021.109119

Tolhurst, G., Heffron, H., Lam, Y. S., Parker, H. E., Habib, A. M., Diakogiannaki, E., et al. (2012). Short-chain fatty acids stimulate glucagon-like peptide-1 secretion *via* the G-protein-coupled receptor FFAR2. *Diabetes.* 61 (2), 364–371. doi: 10.2337/db11-1019

Vatanen, T., Kostic, A. D., d'Hennezel, E., Siljander, H., Franzosa, E. A., Yassour, M., et al. (2016). Variation in microbiome LPS immunogenicity contributes to autoimmunity in humans. *Cell* 165 (6), 1551. doi: 10.1016/j.cell.2016.05.056

Verbeke, F., Janssens, Y., Wynendaele, E., and De Spiegeleer, B. (2017). Faecal microbiota transplantation: a regulatory hurdle? *BMC Gastroenterol.* 17 (1), 128. doi: 10.1186/s12876-017-0687-5

Vermeire, S., Joossens, M., Verbeke, K., Wang, J., Machiels, K., Sabino, J., et al. (2016). Donor species richness determines faecal microbiota transplantation success in inflammatory bowel disease. *J. Crohns Colitis.* 10 (4), 387–394. doi: 10.1093/ecco-jcc/jjv203

Vrieze, A., Van Nood, E., Holleman, F., Salojarvi, J., Kootte, R. S., Bartelsman, J. F., et al. (2012). Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology.* 143 (4), 913–6.e7. doi: 10.1053/j.gastro.2012.06.031

Walker, D. M., Cates, H. M., Loh, Y. E., Purushothaman, I., Ramakrishnan, A., Cahill, K. M., et al. (2018). Cocaine self-administration alters transcriptome-wide responses in the brain's reward circuitry. *Biol. Psychiatry* 84 (12), 867–880. doi: 10.1016/j.biopsych.2018.04.009

Wang, J., and Jia, H. (2016). Metagenome-wide association studies: fine-mining the microbiome. *Nat. Rev. Microbiol.* 14 (8), 508–522. doi: 10.1038/nrmicro.2016.83

Wang, H., Lu, Y., Yan, Y., Tian, S., Zheng, D., Leng, D., et al. (2019). Promising treatment for type 2 diabetes: Fecal microbiota transplantation reverses insulin resistance and impaired islets. *Front. Cell Infect. Microbiol.* 9, 455. doi: 10.3389/fcimb.2019.00455

Wang, Z., Xie, Z., Lu, Q., Chang, C., and Zhou, Z. (2017). Beyond genetics: What causes type 1 diabetes. *Clin. Rev. Allergy Immunol.* 52 (2), 273–286. doi: 10.1007/s12016-016-8592-1

Weingarden, A., Gonzalez, A., Vazquez-Baeza, Y., Weiss, S., Humphry, G., Berg-Lyons, D., et al. (2015). Dynamic changes in short- and long-term bacterial composition following fecal microbiota transplantation for recurrent clostridium difficile infection. *Microbiome* 3, 10. doi: 10.1186/s40168-015-0070-0

White, P. J., and Newgard, C. B. (2019). Branched-chain amino acids in disease. *Science* 363 (6427), 582–583. doi: 10.1126/science.aav0558

Woodworth, M. H., Neish, E. M., Miller, N. S., Dhere, T., Burd, E. M., Carpentieri, C., et al. (2017). Laboratory testing of donors and stool samples for fecal microbiota transplantation for recurrent clostridium difficile infection. *J. Clin. Microbiol.* 55 (4), 1002–1010. doi: 10.1128/JCM.02327-16

Zhang, F., Zhang, T., Zhu, H., and Borody, T. J. (2019). Evolution of fecal microbiota transplantation in methodology and ethical issues. *Curr. Opin. Pharmacol.* 49, 11–16. doi: 10.1016/j.coph.2019.04.004

Zhu, C., Song, K., Shen, Z., Quan, Y., Tan, B., Luo, W., et al. (2018). Roseburia intestinalis inhibits interleukin17 excretion and promotes regulatory T cells differentiation in colitis. *Mol. Med. Rep.* 17 (6), 7567–7574. doi: 10.3892/mmr.2018.8833

Ziegler, A. G., and Nepom, G. T. (2010). Prediction and pathogenesis in type 1 diabetes. *Immunity.* 32 (4), 468–478. doi: 10.1016/j.immuni.2010.03.018

Zuo, T., Wong, S. H., Lam, K., Lui, R., Cheung, K., Tang, W., et al. (2018). Bacteriophage transfer during faecal microbiota transplantation in clostridium difficile infection is associated with treatment outcome. *Gut* 67 (4), 634–643. doi: 10.1136/gutjnl-2017-313952

frontiers | Frontiers in Cellular and Infection Microbiology

TYPE Original Research
PUBLISHED 04 January 2023
DOI 10.3389/fcimb.2022.1089991

OPEN ACCESS

EDITED BY
Qinglong Wu,
Baylor College of Medicine,
United States

REVIEWED BY
Bo Zhu,
University of Texas MD Anderson Cancer Center, United States
Ravi Verma,
Department of Pathology and Immunology, Baylor College of Medicine, United States

*CORRESPONDENCE
Kaijian Hou
kaijianhou@126.com
Yongsong Chen
yongsongchen@126.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Intestinal Microbiome, a section of the journal Frontiers in Cellular and Infection Microbiology

RECEIVED 04 November 2022
ACCEPTED 12 December 2022
PUBLISHED 04 January 2023

CITATION
Wu Z, Zhang B, Chen F, Xia R, Zhu D, Chen B, Lin A, Zheng C, Hou D, Li X, Zhang S, Chen Y and Hou K (2023) Fecal microbiota transplantation reverses insulin resistance in type 2 diabetes: A randomized, controlled, prospective study.
Front. Cell. Infect. Microbiol. 12:1089991.
doi: 10.3389/fcimb.2022.1089991

Fecal microbiota transplantation reverses insulin resistance in type 2 diabetes: A randomized, controlled, prospective study

Zezhen Wu[1,2,3†], Bangzhou Zhang[2,4†], Fengwu Chen[1,2†], Rongmu Xia[5†], Dan Zhu[2], Baolong Chen[2], Aiqiang Lin[2], Chuyan Zheng[2], Ducheng Hou[2], Xiaoyu Li[2], Shuo Zhang[1,2,3], Yongsong Chen[1*] and Kaijian Hou[1,2,6*]

[1]Department of Endocrine and Metabolic Diseases, The First Affiliated Hospital of Shantou University Medical College, Shantou, China, [2]Department of Endocrine and Metabolic Diseases, Longhu Hospital, Shantou, China, [3]Graduate School of Shantou University Medical College, Shantou, Guangdong, China, [4]School of Pharmacy, Fujian University of Traditional Chinese Medicine, Fuzhou, China, [5]Clinical Research Center, The Second Affiliated Hospital of Fujian University of Traditional Chinese Medicine, Fuzhou, China, [6]School of Public Health, Shantou University, Shantou, China

Objectives: Recent studies have shown that fecal microbiota transplantation (FMT) improved the metabolic profiles of patients with type 2 diabetes mellitus (T2DM), yet the effectiveness in reversing insulin resistance and increasing metformin sensitivity in T2DM patients have not been reported. In this study, we evaluated the improvements of T2DM patients and their gut microbiota by FMT alone and FMT plus metformin.

Methods: A total of 31 patients with newly diagnosed T2DM were randomized to intervention by metformin, FMT, or FMT plus metformin in the study. Patients were followed up at baseline and week 4 after treatment. Blood and stool samples were collected and subject to analyze clinical parameters and microbial communities by metagenomic sequencing, respectively.

Results: FMT alone and FMT plus metformin significantly improved the clinical indicators HOMA-IR and BMI in T2DM, besides fasting blood glucose, postprandial blood glucose, and hemoglobin A1c that were also controlled by metformin. Donor microbiota effectively colonized in T2DM with slightly higher colonization ration in FMT than FMT plus metformin within 4 weeks, resulting in increased microbial diversity and community changes from baseline after treatment. A total of 227 species and 441 species were significantly alerted after FMT and FMT plus metformin, respectively. FMT were significantly associated with the clinical parameters. Among them, *Chlorobium phaeovibrioides, Bifidibacterium adolescentis* and *Synechococcus* sp.*WH8103* were potential due to their significantly negative correlations with HOMA-IR.

Conclusions: FMT with or without metformin significantly improve insulin resistance and body mass index and gut microbial communities of T2DM patients by colonization of donor-derived microbiota.

KEYWORDS

fecal microbiota transplantation, type 2 diabetes mellites, metformin, metagenomics, microbiota colonization

1 Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disease characterized by a decrease in pancreatic β-cell mass and function, and represents a failure to compensate for the high insulin demand of homeostatic model assessment of insulin resistant (HOMA-IR) states (Aguayo-Mazzucato et al., 2019). The occurrence of HOMA-IR is a key predictor of the development of T2DM (Wallace et al., 2019). The global prevalence of T2DM is alarmingly high with an estimated population of 370 million, which is predicted to be doubled by 2030 (Wild et al., 2004). This dramatic increase in T2DM poses an immense public health crisis and medical challenge. Recent research showed that intestinal dysbiosis is a key factor in the development of metabolic endotoxemia and T2DM (Zhao et al., 2018; Thingholm et al., 2019; Wu et al., 2022).

The human intestines harbor a complex community of intestinal bacteria (Skelly et al., 2019), viruses (Ingle et al., 2019), fungi (Li et al., 2019), and protists (Chudnovskiy et al., 2016). Recent data confirmed that intestinal dysbiosis was associated with the development of metabolic syndrome, especially T2DM (Karlsson et al., 2013; Que et al., 2021; Hou et al., 2022). The composition and quantity of intestinal microbiota in diabetic patients have been found to differ from healthy individuals (Marchesi et al., 2016; Chen et al., 2019). Current studies showed that intestinal microbiota is involved in the development of obesity and insulin resistance in diabetes mellitus *via* different mechanisms, and many hypoglycemic drugs result in changes of intestinal microbiota (Su et al., 2015; Li et al., 2017). Metformin is now widely used in T2DM treatment, and recent evidence suggests that the intestinal microbiota serves as a metformin action site (Pollak, 2017; Rodriguez et al., 2018; Foretz et al., 2019). Sun et al. indicated that metformin acted partially *via* a B. fragilis-glycoursodeoxycholic acid (GUDCA)–intestinal farnesoid-X receptor (FXR) axis to improve metabolic dysfunction (Sun et al., 2018). The therapeutic potential of fecal microbiota transplantation (FMT) in diabetes has been discussed in many papers (Wang et al., 2019; Aron-Wisnewsky et al., 2019; Ng et al., 2022; Hou et al., 2022). For example, Groot et al. revealed that FMT could halt the decline in endogenous insulin production and featured intestinal microbiota were linked to remaining beta cell function of type 1 diabetes (T1DM) patients (de Groot et al., 2021). Siew et al. reported that repeated FMTs enhance the level and duration of microbiota engraftment in obese patients with T2DM (Ng et al., 2022). However, no study has reported the application of FMT in assisting the efficacy of metformin in T2DM treatment. Thus the aim of our work was to evaluate adjunctive FMT with metformin in southeast Chinese population with T2DM.

We proposed that FMT would alter T2DM patients' microbial ecology and thereafter improve the blood glucose and insulin sensitivity. An FMT clinical trial for the intervention of T2DM patients with metformin, FMT alone, and FMT plus metformin was initiated. The primary outcome was the evaluation of changes in insulin sensitivity (HOMA-IR and HOMA-HBCI), postprandial blood glucose (PBG), fasting blood glucose (FBG), hemoglobin A1c (HbA1c), and BMI between baseline and after 4 weeks of intervention. The secondary outcomes were the proportion of subjects acquiring least 20% of microbiota from the donor after FMT at week 4.

2 Materials and methods

2.1 Study population

We recruited 29 adult T2DM patients, following the diagnostic criteria of the American Diabetes Association (ADA) for T2DM in 2019. We obtained written informed consent from all patients before screening. All patients volunteered to participate in the trial and exhibited good compliance and did not replace diabetic drugs in the study cycle. Patients were excluded if they had other diagnoses: 1) acute and chronic infectious diseases, gastrointestinal diseases, severe heart insufficiency, severe liver and kidney insufficiency, and/or other diseases or complications; 2) other gastrointestinal diseases that may affect drug absorption; 3) pregnant and lactating women; 4) people with allergies; 5) patients who have used other hormone therapy within the past three months; 6) Leukopenia or abnormal granulocytes; 7) cardiovascular and cerebrovascular diseases that first occurred in the past three

months; 8) Participants in other clinical trials during the same period; 9) a history of human immunodeficiency virus (HIV) seropositivity after laboratory screening; and 10) hepatitis B virus surface antigen (HBsAg) positive or hepatitis C virus antibody (HCV-Ab) history after laboratory screening. During this period, the research team instructed all participants to maintain their original eating habits before and after the intervention, including total calories, types, diet culture, etc., and to maintain light to moderate physical activity (the same intensity) and avoid heavy physical activity. The study has been approved by the Longhu Hospital, The First Affiliated Hospital of Shantou University Medical College Ethics Committee in Shantou, China(Ethics number:LHLL2019001), and was registered at Chinses Clinical Trial Registry.(Registration number: ChiCTR1900024636).(http://www.chictr.org.cn/showprojen.aspx?proj=41166).

2.2 Research plan and outcomes

This study used FMT as an auxiliary method to compare the therapeutic effects of solely metformin, solely FMT and FMT combined with metformin onT2DM patients. Eight T2DM patients received FMT plus metformin treatment, 9 patients underwent FMT alone and 12 patients received solely metformin treatment. The primary research objective was to evaluate the efficacy of FMT in assisting the metformin treatment in T2DM adult patients from the aspects of blood sugar control and insulin resistance. The secondary research objective was to observe the influence of FMT on the bacterial engraftment from donor microbiota during baseline inspection and week 4 intervention. We classified microbiota species identified in the recipients into four types and mainly focused on the donor-associated species as previously defined (Ng et al., 2022).

2.3 Intervention procedures

Screening of study donors was based on previous reports (Wu et al., 2020; He et al., 2021). The gut microbiota of ten qualified-donors was isolated automatically using the fecal microbiota extractor TG-01 (Treat-gut company, Guangzhou, China). The procedures involved mixing of stool with saline solution and multiple filtrations with different pore sizes, which were completed in Xiamen Treat-gut Biotechnology Co. ltd. We studied the effects of FMT *via* nasojejunal feeding tubes on clinical phenotypes and intestinal microbiota before and 4 weeks after intervention. The interventions consisted of metformin, FMT alone, and FMT plus metformin. For the FMT, before transplantation, patients had to be confirmed their stomach remained empty for more than 4 hours, then 200 mL of FMT solution containing 50g bacterial sludge was injected *via* the nasointestinal tube to the anterior jejunum. The position was confirmed by X-ray. Two hours after FMT, the participants were allowed to have a small amount of liquid diet. Blood and stool samples of all participants were collected at baseline (week 0) and week 4 after intervention for biochemical and microbiota assessments.

2.4 Fecal microbiota analysis by metagenomic sequencing

Fecal samples from donor and T2DM patients were collected on the day of the medical examination and immediately frozen at −80°C. Fecal genomic DNA was extracted using the QIAamp Fast DNA Stool Mini Kit (Qiagen, CA, USA). DNA samples were stored at −20°C before use as templates for next-generation sequencing library preparation. Samples were fragmented to an average insert size of 400 bp and sequenced by Illumina Nova seq with PE 150 reagents. Reads were trimmed using KneadData with default parameters to filter the sequencing adapter, low-quality reads, and the human genome. The taxonomic composition was processed using kraken2 (Erem et al., 2014) with default parameters.

2.5 Statistical analysis

Microbiota alpha diversity Shannon and Chao1 were calculated using the R program package 'vegan' (version 2.5.6). β-diversity metrics were obtained with rda and PERMANOVA with the adonis function. Principal Components analysis (PCA) was performed using the package vegan. Different analysis was performed to identify taxa with differentiating abundance in the different groups (Krentz and Bailey, 2005). The corr.test function was used to analyze the correlation between the microbial taxa and clinical indexes. Statistical significance was taken as p value <0.05.

3 Results

3.1 Characteristics of the study population

A total of 36 patients with T2DM were assessed for eligibility, of whom 31 were recruited and randomized to either FMT plus metformin, FMT alone, or metformin from July 2019 to Oct 2021. One of participants in both FMT plus metformin and FMT alone group withdraw after FMT infusion. Finally, 29 patients allocated to FMT plus metformin (n=8), FMT alone (n=9), or metformin (n=12) completed their follow-up assessment at both baseline and week 4 (Figure 1). Demographic characteristics of patients in the three groups were comparable. Males constituted 48.3% of the patients

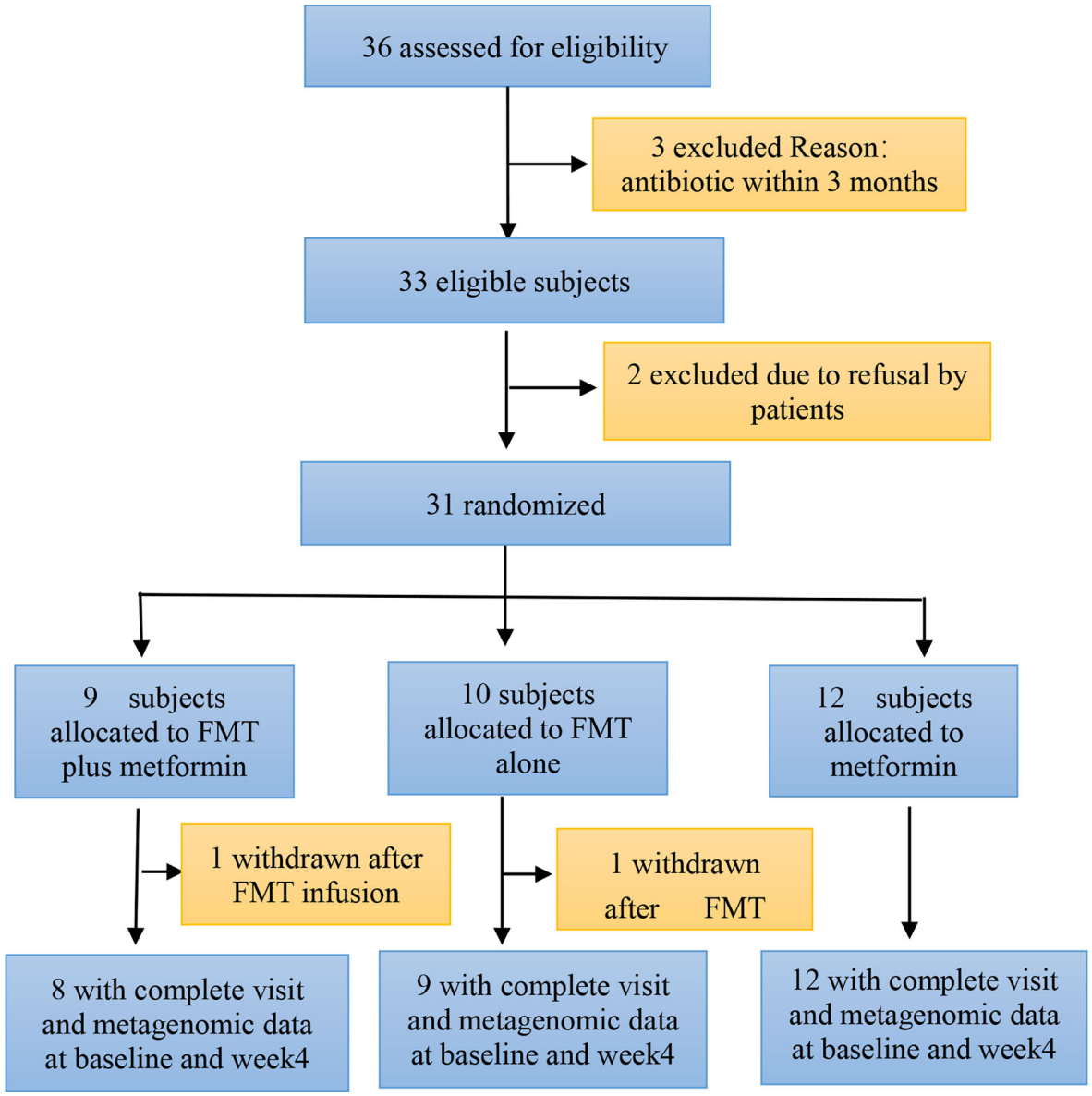


FIGURE 1
Consort diagram of the study flow. FMT, faecal microbiota transplantation.

(n=14), and the median BMIs for FMT plus metformin, FMT alone, and the metformin groups were 27.46, 27.29, and 27.01 kg/m^2, respectively. Ten healthy donors (90% male, median BMI: 21.56kg/m^2) provided stool for the FMT solution.

3.2 Blood glucose, insulin resistance, and BMI improvement after intervention

Participants in the three treatment groups had significant ($P<0.05$) improvement in fasting blood glucose (FBG); postprandial blood glucose (PBG), hemoglobin A1c (HbA1c), and HOMA-HBCI at week 4 after intervention compared with the baseline (Table 1). More importantly, patients in FMT alone and FMT plus metformin had significantly decreased HOMA-IR ($p<0.05$) and BMI ($p<0.05$) at week 4 after FMT intervention, while no differences were observed for those in metformin group (Table 1). Significantly lower UA, TG, and Globulin were observed in FMT plus metformin group (Figure 2).

We further evaluated the magnitude of change among the three groups. Percentages of improvements in PBG, FBG, HOMA-IR, BMI, AST/ALT and ALP were significantly higher

10.3389/fcimb.2022.1089991

TABLE 1 Clinical data included in this study.

	Metformin (n=12)			FMT (n=9)			FMT plus metformin (n=8)		
Index	Week 0	Week 4	*p* value	Week 0	Week 4	*p* value	Week 0	Week 4	*p* value
FBG	9.08±2.02	8.37±1.98	**0**	14.3±1.99	8.12±0.72	**0**	9.24±1.92	6.78±1.68	**0.01**
PBG	13.96±2.61	11.35±1.92	**0**	18.48±5.59	11.34±2.2	**0**	14.21±4.77	9.08±2	**0.01**
HbA1c	8.56±1.13	8.29±1.09	**0**	10.75±1.85	8.57±1.97	**0.01**	9.1±2.14	8.76±2.21	**0.01**
HOMA-HBCI	41.55±20.25	58.04±37.21	**0.03**	22.9±16.06	44.65±23.03	**0**	41.86±19.77	86.47±109.22	**0.01**
BMI	27.51±0.89	27.42±0.99	0.2	27.2±0.95	26.37±0.87	**0**	27.27±1.07	26.46±1.28	**0.01**
HOMA-IR	3.99±1.39	4.51±2.03	0.52	6.73±2.88	3.55±1.58	**0**	5.57±5.91	3.61±4.15	**0.01**
PCP	4.58±2.09	4±1.26	0.13	3.85±2.19	4.5±2.35	0.13	3.86±3.21	4.62±4.21	0.84
FINS	10.08±3.03	12.21±5.34	0.27	11.05±5.25	9.93±4.63	0.07	12.33±9.65	12.04±14.81	0.25
AFU	30.32±4.02	28.49±3.43	0.09	25.88±5.75	24.5±4.24	0.25	19.69±7.16	24.23±7.58	**0.03**
FCP	1.89±1.12	1.87±0.72	0.62	1.57±0.99	1.76±0.71	0.43	1.68±1.22	1.99±0.93	0.74
Creatinine	87.67±94.69	75±39.53	0.4	64.67±11.07	68±12.45	0.11	66.38±12.39	72.25±14.66	0.09
GGT	30.93±13.1	33.6±16.84	0.73	39.03±56.67	35.1±51.57	0.36	31.27±17.9	23.32±12.02	0.15
D-BIL	4.21±1.09	3.61±1.45	0.08	3.96±1.37	3.66±1.04	0.43	3.12±1.43	3.7±1.24	0.53
ALP	83.75±15.79	67.33±11.24	**0**	73.22±29.39	69.89±16.71	1	68.88±11.64	67.12±14.19	0.36
TBA	6.82±2.31	3.36±2.24	**0**	6.28±7.42	3.53±1.68	0.31	3.99±2.68	4.22±3.42	0.95
TBIL	11.4±2.77	11.08±4.37	0.62	12.71±4.1	12.19±3.81	0.82	13.04±7.3	13.26±4.71	0.95
APO-B	0.86±0.24	0.97±0.18	0.08	1.03±0.35	1.11±0.26	0.65	0.84±0.21	0.83±0.21	0.74
PINS	37.38±22.24	31.74±14.88	0.2	35.72±24.36	35.23±28.05	0.82	31.31±21.07	14.3±14.47	0.04
LDL-C	2.96±1.09	3.12±0.74	0.57	2.98±0.56	3.52±0.79	0.16	2.78±1.12	2.74±0.75	0.74
BUN	5.22±2.46	5.77±2.41	0.13	5.21±0.81	5.64±1.2	0.25	5.68±1.26	5.01±2.1	0.55
ALT	30.23±34.83	35.03±46.76	0.46	22.8±11.11	21.43±5.86	0.73	29.55±19.71	24.2±6.66	0.38
HDL-C	1.19±0.24	1.25±0.27	0.14	1.13±0.28	1.16±0.2	0.64	1.32±0.52	1.27±0.46	0.2
CHE	9196.42 ±1539.21	9511.5 ±1510.36	0.47	9830.11 ±2714.23	9413.89 ±1709.88	0.73	6051.88 ±3700.28	6019.25 ±3762.15	0.74
UA	366.42±81.33	374.5±67.24	0.73	4061.56 ±126.12	366.11±74.71	0.57	377.38±59.76	360.12±60.41	**0.01**
TG	1.74±0.57	1.52±0.46	0.23	3.84±5.5	2.3±1.36	0.57	2.18±1.44	1.26±0.29	**0.04**
TC	4.72±1.35	4.87±0.79	0.64	5.37±1.02	5.48±0.36	0.73	4.8±1.13	4.44±0.71	0.64
AST/ALT	1.17±0.38	0.86±0.24	**0.01**	1±0.31	1.02±0.23	0.83	1.12±0.6	1.06±0.24	0.94
TP	75.22±5.45	72.91±4.03	0.11	73.51±10.05	74.67±3.6	0.57	72.36±11.66	68.65±6.16	0.31
MAO	4.25±0.97	4.25±1.14	1	6.67±6.86	4.44±2.3	1	5.55±1.44	7.06±2.48	0.14
I-BIL	6.8±1.87	7.27±2.96	0.62	8.76±2.91	8.94±2.34	1	9.92±6.87	9.03±4.17	0.64
Globulin	26.82±3.85	26.95±4	0.97	26.37±6.58	27.88±3.26	0.43	27.89±6.11	23.92±3.38	**0.04**
AST	27.88±13.51	24.23±17.34	**0.03**	20.29±6.06	21.59±5.57	0.91	25.24±7.82	22.88±6.27	0.23

(Continued)

TABLE 1 Continued

	Metformin (n=12)			FMT (n=9)			FMT plus metformin (n=8)		
Index	Week 0	Week 4	*p* value	Week 0	Week 4	*p* value	Week 0	Week 4	*p* value
APO-A	1.28±0.21	1.23±0.16	0.24	1.43±0.29	1.35±0.15	0.82	1.4±0.19	1.31±0.41	0.38
Albumin	48.44±2.89	46.36±1.99	**0.02**	47.04±3.77	47.3±2.48	0.91	44.47±6.43	44.86±5.87	0.89

The data are shown as the mean±SD. N=12 in the metformin group, N=9 in the FMT group and n=8 in the FMT plus metformin group for all outcomes. FMT, fecal microbiota transplantation; FBG, fasting blood glucose; PBG, postprandial blood glucose; HbA1c, hemoglobin A1c.
HOMA-HBCI=20×FINS/(FBG-3.5); BMI, body mass index; HOMA-IR=(FBG×FINS)/22.5; PCP, postprandial c-peptide; FINS, fasting insulin; AFU, α-L-fucosidase; FCP, fasting c-peptide; GGT, γ-glutamyl transpeptadase; D-BIL, direct bilirubin; ALP, alkaline phosphatase; TBA, total bile acid; TBIL, total bilirubin; APO-B, apolipoprotein B; PINS, postprandial insulin; LDL, low-density lipoprotein; BUN, urea nitrogen; ALT, alanine aminotransferase; HDLC, high-density lipoprotein; CHE, cholinesterase;UA, uric acid; TG, triglyceride; TC, total cholesterol;TP, total protein; MAO, monoamine oxidase; I-BIL, indirect bilirubin; AST, aspartate aminotransferase; APOA, apolipoprotein A.
Bold values represents statistically significant indicators.

in the FMT alone and FMT plus metformin than in metformin group (p<0.05) (Figure 3). Improvements in HOMA-HBCI, HbA1c, AST, TBA and TP were observed significantly higher in FMT alone than metformin group. Additionally, FBG and HbA1c decreased more in FMT plus metformin than in FMT alone. No significant differences in changes of PBG, PINS, HOMA-IR, HOMR-HBCI, AST/ALT, ALP, TBA, TP and BMI were observed between the two FMT-associated treatments (p>0.05) (Figure 3).

3.3 Microbiota alterations associated with FMT intervention

Microbial richness (observed taxa and Chao1) and Shannon diversity were obviously (P < 0.05) improved at week 4 after FMT compared with the baseline, although the significance is marginal. Moreover, the evenness was significantly (P<0.05) increased by FMT in FMT alone group at week 4 (Figure 4A). No obvious changes in diversity indexes were observed in metformin group. As expected, Bacteroidetes, Firmicutes, and Proteobacteria were the dominant taxa in donor alone or overall samples (Figure 4B). Relative abundance of Bacteroidetes was decreased, while Firmicutes increased after intervention in both FMT alone and FMT plus metformin groups. An uncommon microbial composition was observed at week 4 after metformin treatment, with a surprising high proportion of Proteobacteria and almost absence of Bacteroidetes.

3.4 β-diversity and microbial colonization

The results of β-diversity based on Euclidean distance showed that the intestinal microbiota changed at week 4 compared with week 0 with the three treatments. The gut microbial communities in FMT plus metformin group were

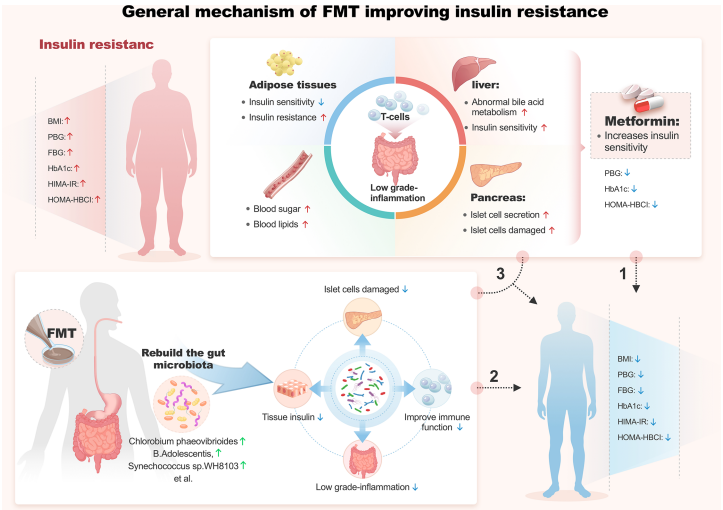


FIGURE 2
General mechanism of FMT improving insulin resistance.

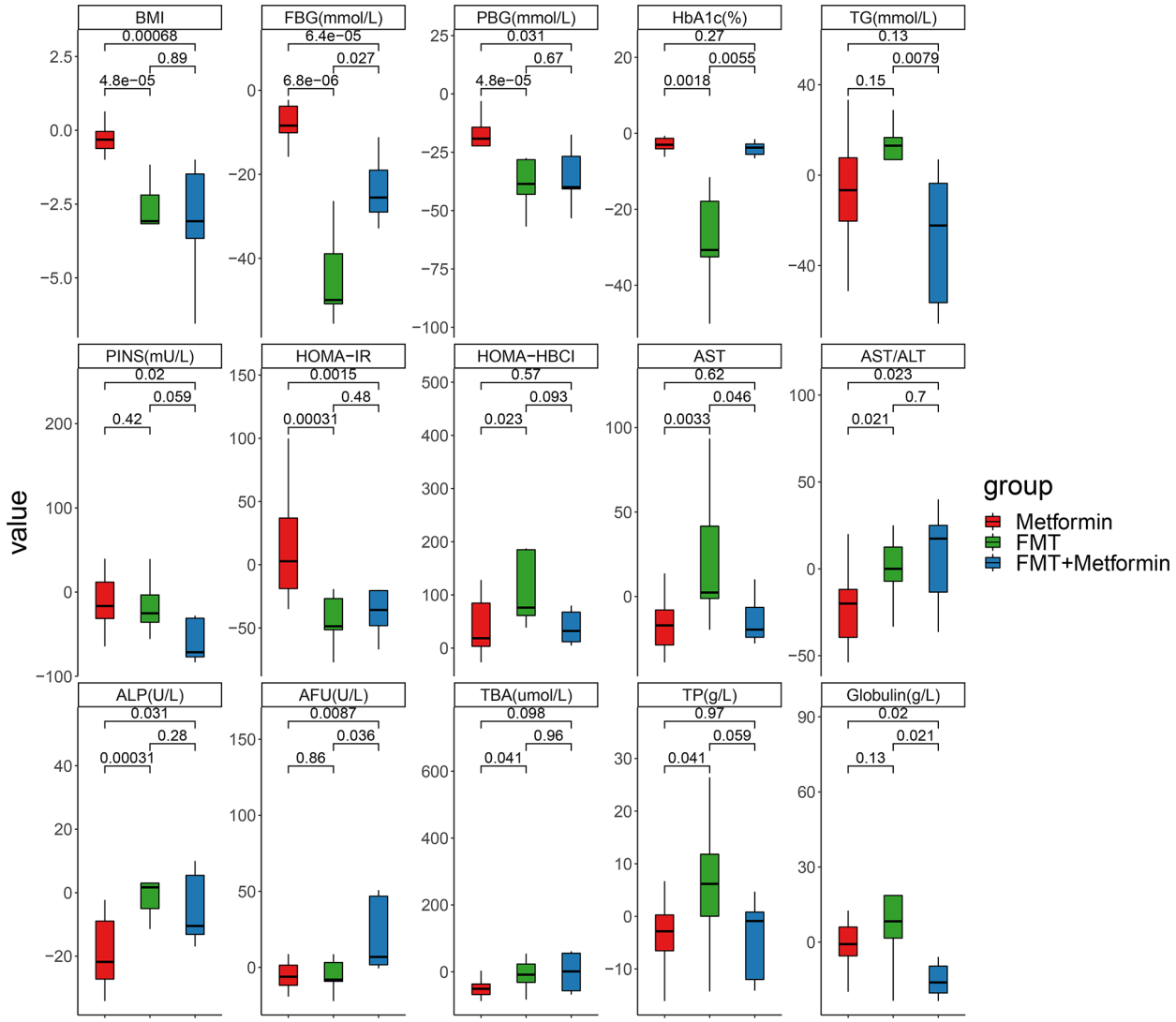


FIGURE 3
Fold changes of clinical indexes based on week 4 divided by week 0 in T2DM patients with different interventions. Pairwise comparisons between groups were conducted using the Wilcox test. Metformin, n=12 patients; FMT, n=9 patients; FMT plus metformin, n=8 patients. $p<0.05$ is defined as statistically significant.

significantly different (PERMANOVA, $p < 0.05$) between week 4 and week 0, while there was no significant difference in FMT alone nor in metformin group (Figure 4C). Among the three groups, the metformin group had the smallest change in beta diversity after 4 weeks, followed by the FMT group, and that the FMT plus metformin group had the largest change, although significant difference were observed among the three groups (Figure 4E). Similarly, the Euclidean distance between week 4 from each of the three groups and the donor was calculated. The results showed that the distance between the metformin group and the donor was the largest, and the FMT plus metformin group was the smallest, indicating that the gut microbiota in the FMT plus metformin group was similar to that of the donor after treatment (Figure 4G, Figure 5).

The colonization of donor-derived microbial species was analyzed and detected in post-FMT samples from all recipients in both FMT alone and FMT plus metformin groups, with percentage ranging from 3.1% to 73.7%.The results showed that 6 patients (66.7%) in the FMT group and 5 patients (62.5%) in the FMT plus metformin group achieved ≥20% donor-derived microbial species, which was considered as effective colonization. However, there was no significant

10.3389/fcimb.2022.1089991

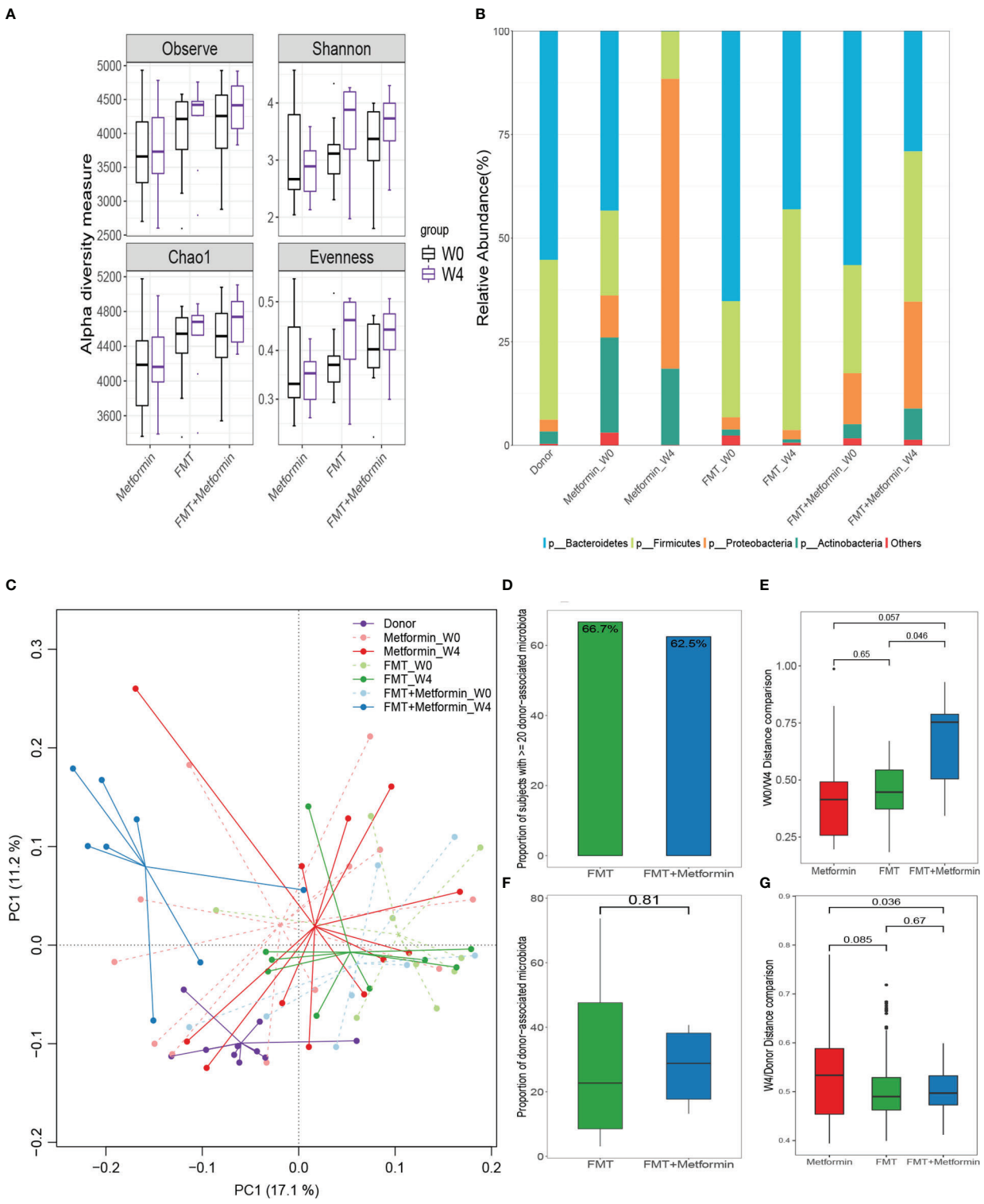


FIGURE 4
Changes in gut microbiota between week 0 and week 4 in T2DM patients after interventions. **(A)** Differences in alpha diversity indexes, microbial richness (Observed index and Chao1 index), Shannon diversity, and evenness. **(B)** Relative abundances of the top phyla in the these groups; Changes in gut microbiota and microbial colonization of donor-derived microbiota in subjects with type 2 diabetes. **(C)** Differences in beta diversity between baseline (W0) and week 4 (W4) after intervention by metformin, FMT, or FMT plus metformin, visualized by PCA. **(D, F)** The colonization of donor-derived microbiota in FMT alone and FMT plus metformin groups. **(E)** Changes of Euclidean distance between W0 and W4. **(G)** Changes of Euclidean distance between W4 and donors.

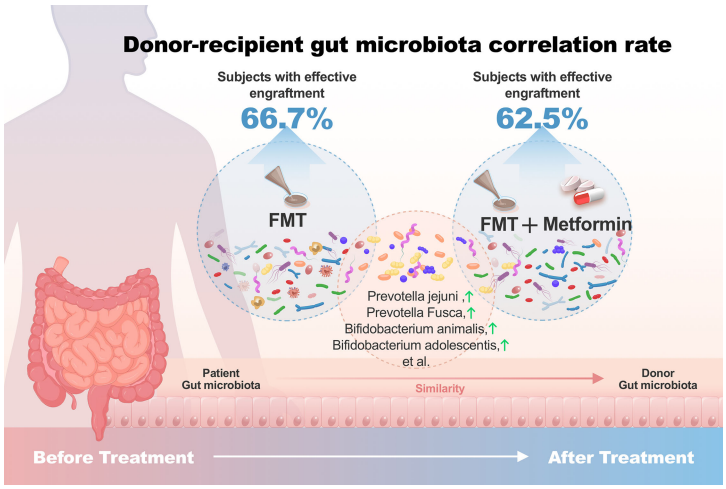


FIGURE 5
Donor-recipient gut microbiota correlation rate. Subjects with effective engraftment: Percentage of subjects with ≥20% donor-associated microbiota.

difference in colonization rate between the two groups ($p > 0.05$) (Figures 4D, F).

3.5 Taxa significantly associated with clinical improvements

To explore the taxa associated with improvement in clinical efficacy, the differences between baseline and week 4 after intervention in FMT alone and FMT plus metformin groups were analyzed by Wilcoxon-rank sum test. A total of 7 phyla, 57 families, and 133 genera level were significantly ($p < 0.05$) different after intervention in FMT alone group, while the numbers were 10, 63 and 206 in the FMT plus metformin group (Table S1). With cut-off of relative abundance bigger than 0.001%, there were 227 species and 441 species that were significantly different between baseline and week 4 in in FMT alone and FMT plus metformin groups, respectively. Among them, 89 species were shared in these two groups (Figure 6). The species with increased relative abundance at week 4 after treatment mainly belonged to the genera *Prevotella* and *Bifidobacterium*, including *Prevotella jejuni*, *Prevotella Fusca*, *Bifidobacterium animalis*, and *Bifidobacterium adolescentis*. Correlation analysis based on the samples from these two groups showed that *Provetella* were positively correlated with ALP and TP, while *Bifidobacterium* were negatively correlated with CHE, FBG, PBG, TC and PINS (Figure 7A). Additionally, *Collinella aerofaciens* was strongly negatively correlated with FBG, while PGB was strongly positively correlated with *Clostridium bolteae* and HOMA-IR was strongly positively correlated with *Dysosomobacter Wekbionis* ($p < 0.05$).

Since improvement in HOMA-IR is urgent in treatment of T2D, we further explored the species by conducting correlation analysis between HOMAR-IR and the species significantly different either after FMT or FMT plus metformin, based on samples from T2DM patients at baseline and week 4 after intervention. The results showed that most of the significantly associated species were positively correlated with HOMA-IR (Figure 7B), including *Lactobacillus ruminis*, *D. welbiois* and *Xylanimicrobium* sp. *FW10M-9*, while *Chlorobium phaeovibrioides*, *B. adolescentis* and *Synechococcus* sp.*WH8103* were strongly negatively correlated with HOMA-IR, which were increased at week 4 after intervention by FMT or FMT plus metformin (Figure 7C).

4 Discussion

This study aimed to evaluate the improvements of T2DM patients and their gut microbiota by FMT alone and FMT plus metformin, compared with metformin. Results showed that FMT alone and FMT plus metformin significantly improved insulin resistance (HOMA-IR), HOMA-HBCI, BMI, FBG, and PBG within 4 weeks after intervention, and modified gut microbial communities by colonization of donor-derived microbiota. Correlation analysis revealed that *B. adolescents*, *C. phaeovibrioides*, and *S.* sp.*WH8103* were significantly negatively correlated with HOMA-IR, the urgent indicator in treatment of T2DM. In short, this study support that FMT alone and FMT plus metformin can improve insulin resistance and other indicators of patients T2DM by colonizing donor-derived microbes and modifying gut microbiota in diversity and specific species.

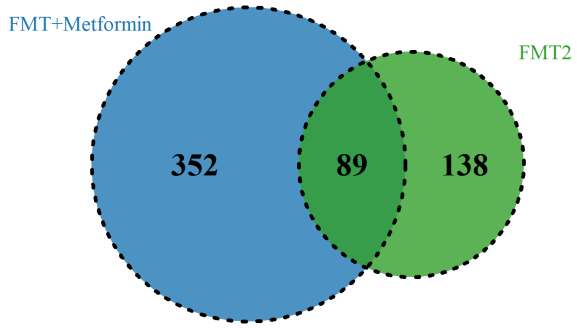


FIGURE 6
The commonly and significantly different species of between the FMT alone and FMT plus metformin groups.

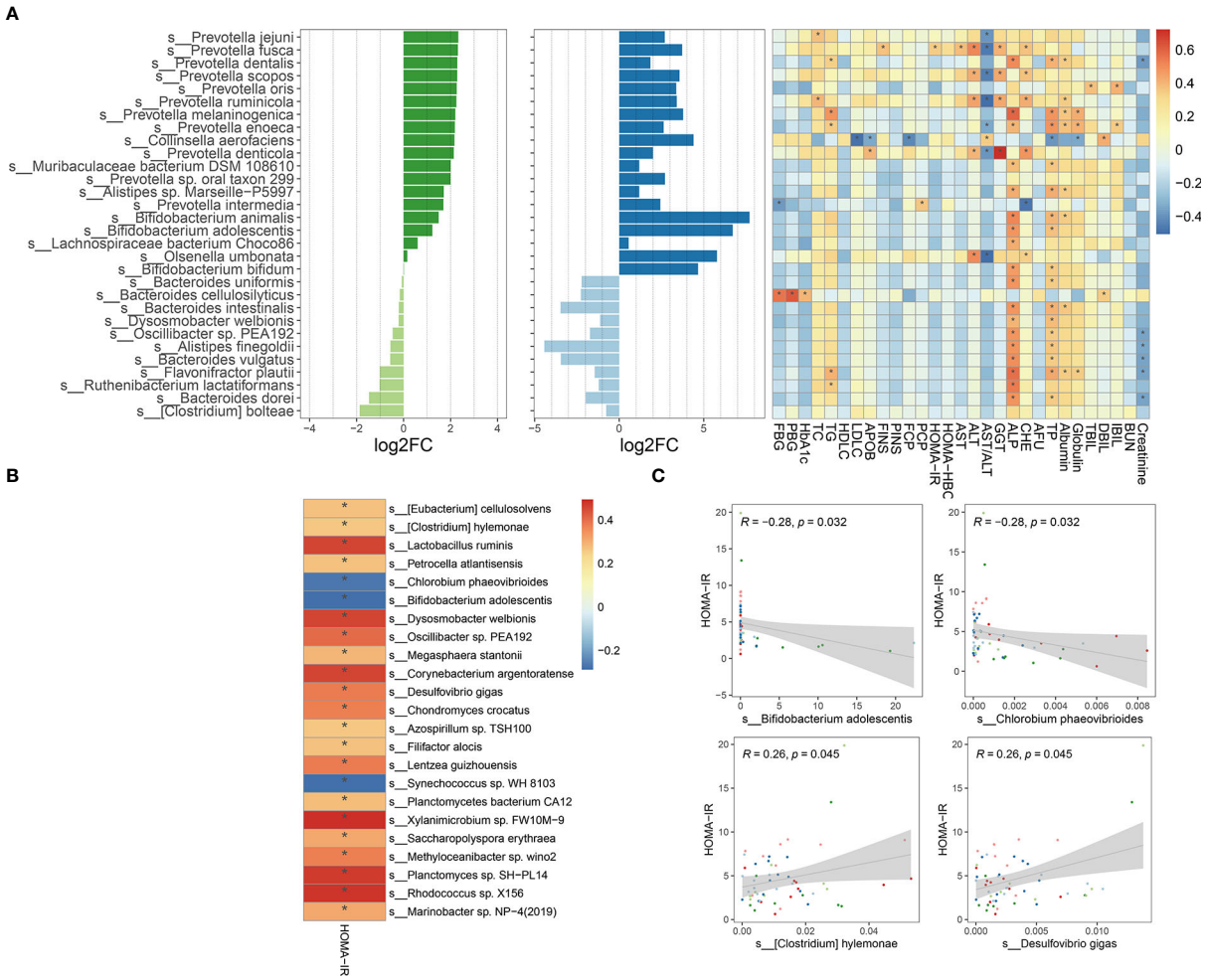


FIGURE 7
Microbial species significantly changed after intervention and their correlation with clinical indicators. **(A)** The top 30 significantly changed species after FMT or FMT plus metformin and their correlation with clinical indicators based on samples from W0 and W4 of these two groups. **(B)** Significant corrections between HOMA-IR and significantly changed species with relative abundance greater than 0.001%, based on all samples from T2DM patients at W0 and W4 in metformin, FMT alone, and FMT plus metformin group. **(C)** 4 gut microbes significantly related to HOMA-IR. "*" means that P value is < 0.05.

Metformin is currently widely used as the first-line drug for the treatment of T2DM patients, as recommended by clinical guidelines, due to the improved glycemic profile and reduction in cardiovascular mortality without the risk of hypoglycemia and/or body weight gains (Buse et al., 2016; Foretz et al., 2019). Previous research showed that metformin decreased BMI, HbA1c, and FBG during an initial 4-month study period (Shin et al., 2014). In another 52 week study, newly diagnosed T2DM patients who used metformin for blood glucose intervention had significantly lower BMI, FPG, PPG and HbA1c after treatment compared with baseline in the MET group (DeFronzo et al., 2016). The present study also observed the improvements in HbA1c and FBG of T2DM after intervention of metformin, but not BMI and other indicators including HOMA-IR, which was improved by FMT alone and FMT plus metformin. This may be related to FMT peripheral insulin sensitivity and other mechanisms to improve blood glucose control, while MET mainly plays a role in the liver by reducing liver glucose output (Wu et al., 2017). More and more studies have shown that the process of metformin's role has a certain relationship with gut microbiota. Intravenous metformin does not improve blood sugar (de la Cuesta-Zuluaga et al., 2017). However, the level of metformin in the intestine is 100-300 times higher than that in the serum, making the intestine the main reservoir of dimethyl lenediamine for human (Carvalho and Saad, 2013; Duparc et al., 2017; Depommier et al., 2020). In addition, metformin can also change the composition of gut microbiota by increasing *Akkermansiamuciniphila*, a microbiota that stimulates SCFA production (short chain fatty acids), which is degraded by mucin (Ma et al., 2019). The production and regulation of SCFA is considered to be one of the mechanisms of probiotics to promote health results (Hartstra et al., 2015). In our experiment, the FBG and HbA1c in the FMT alone group decreased more than those in the FMT plus metformin group, which may be related to the improvement of microbial population structure promoted by metformin.

The patients included in our cohort were diagnosed with T2DM and were not receiving prior regular drug treatment or dietary intervention. These T2DM patients had poorly controlled blood glucose or serious insulin resistance and received no other medications for the treatment of other diseases. During the observation period, the enrolled patients did not perform any physical exercise other than daily life activities and received generally consistent dietary regulation. This design enabled us to diminish the effects of major confounding factors that have a known impact on the gut microbiome. Clinically, not all T2DM patients benefit from the use of metformin or respond to metformin quickly. For example, some patients exhibit strong insulin resistance and weak insulin secretion function despite metformin treatment. Therefore, FMT was used to assist metformin treatment in these patients to quickly improve their sensitivity to metformin.

The present study demonstrated that, although metformin could improve the blood glucose, the addition of FMT promoted the improvement further. Many studies have shown that gut microbiota are closely related to glucose metabolism (Musso et al., 2011), insulin resistance (Lee et al., 2019), and insulin secretion (Kootte et al., 2017). The mounting evidence of a causal role of the gut microbiota in T2DM has led to the development of targeted therapeutic approaches that are designed to alter the microbial composition (Vrieze et al., 2012; Rinott et al., 2021). Fecal microflora transplantation (FMT) is a method to treat diseases by rebuilding gut microbiota (Wu et al., 2011). FMT has consistently demonstrated a capability to overcome dysbiosis *via* a profound sustained effect on the gut microbiome, which may become a new way to treat T2DM (Belenguer et al., 2006). Previous experiments using FMT to interfere with metabolic syndrome showed that FMT improved insulin sensitivity, increased gut microbial diversity, and significantly increased butyric acid producing bacteria in patients with metabolic syndrome (Tolhurst et al., 2012; Yadav et al., 2013). Our results are consistent with previous findings on transient lean donor -9gut microbiota in patients with metabolic syndrome, which showed a significant improvement in peripheral insulin sensitivity at 6 weeks (Belenguer et al., 2006). The species with increased relative abundance 4 weeks after FMT mainly belong to *ruminis, D. welbiois* and *Xylanimicrobium* sp. *FW10M-9* were significantly negatively correlated with HOMA-IR, while *Chlorobium phaeovibrioides, B. adolescentis* and *Synechococcus* sp.*WH8103* were strongly negatively correlated with HOMA-IR. Previous studies have shown that long-term intake of fat rich diet is related to the increase of *Bacteroids*, and *vegetarians* are conducive to the proliferation of *Proctor bacilli. Proburella* was also found to be associated with improved glucose tolerance induced by dietary fiber (Su et al., 2022). Bifidobacterium, on the other hand, produces acetate and lactate during carbohydrate fermentation, which are converted into butyric acid by other gut microbiota (Li et al., 2016). Among them, butyric acid plays an important role in regulating insulin secretion (Turnbaugh et al., 2009; Giongo et al., 2011). Our results again show that *Plasmobacterium* and *bifidobacterium* may be the key organisms related to T2DM improvement, which is consistent with the previous research of others (Aggarwala et al., 2021).

A decent engraftment of donor-associated microbiota taxa in recipients during the FMT procedure is one of the prerequisites that guarantees the efficacy of FMT. It is generally believed that the diversity of gut microbiota is closely related to gut health, and the colonization rate of donor flora in the recipient is an important indicator to evaluate the success rate of transplantation. In our study, about 2/3 of the FMT group and the FMT plus metformin group reached the target of ≥ 20% donor derived microbial species, which was significantly increased by 23 compared with other previous studies

(Herfarth et al., 2019). This is considered to be effective colonization, which is closely related to our strict donor selection before transplantation. Previous studies have shown that the number of Firmicutes in diabetes patients is lower than that in non-diabetes patients (Mocanu et al., 2021). The progression of diabetes is associated with a decrease in the number of Firmicutes and Bacteroidetes (Mayo and Sinderen, 2010). Firmicutes usually participate in the transport of nutrients and promote the absorption and fermentation of SCFA in indigestible carbohydrates (Pinzone et al., 2012). In our clinical experiment, at the 4th week after FMT, Microbial richness (observed taxa and Chao1) and Shannon diversity were significantly improved compared with the baseline. After FMT and FMT plus 8 metformin groups, the relative abundance of Bacteroidetes decreased, while Firmicutes increased, which indirectly proved that the health of receptor gut microecology was improved after FMT. Aggarwala et al. observed that bacterial strain engraftment in Clostridium difficile infection (CDI) recipients independently explained (precision 100%, recall 95%) the clinical outcomes (relapse or success) after initial and repeat FMT (Pinzone et al., 2012). Low donor FMT engraftment resulted in low clinical efficacy of FMT in patients with Antibiotic-dependent pouchitis (ADP) (Bordalo Tonucci et al., 2017). Siew et al. demonstrated that FMT repeated at scheduled intervals led to increased and sustainable engraftment of microbiota from lean donors in obese recipients with T2DM that persisted for at least 6months (Ng et al., 2022). Mocanu et al. Also found that engraftment of specific taxa in the FMT plus low-fermentable fiber group at 6 weeks was donor mediated, and the FMT serves as fiber degrader, as well as short-chain fatty acid (SCFA)-producers and suppressors of tumor growth (Vindigni and Surawicz, 2017). Our FMT procedure led to a quick clinical response within 4 weeks and we found Bifidobacterium was successfully reconstituted in FMT treatment patients. Bifidobacterium plays an important role in human health, including regulating gut microbiota homeostasis, regulating local and systemic immune responses, inhibiting pathogens and harmful bacteria colonizing or infecting gut mucosa (Al-Salami et al., 2008), improving gut mucosal barrier and reducing gut lipopolysaccharide level (Ren et al., 2017). In addition, *Bifidobacteria* improves mucosal barrier function, and T2DM patients' intake of probiotic yogurt containing Lactobacillus acidophilus La5 and Bifidobacterium lactis Bb12 for 6 weeks improved FBG and HbA1c (He et al., 2021).

Side effects of FMT include mild and self-limiting abdominal discomfort, cramps, abdominal distension, diarrhea or constipation, and very few diseases that cannot pass screening tests (Lu and Salzberg, 2020). In our study, T2DM patients displayed no adverse reactions after FMT, and the fasting and postprandial blood glucose, HbA1c, and insulin resistance decreased significantly without causing hypoglycemia or dyslipidemia after FMT treatment. In terms of metabolic research, previous studies tended to use FMT to intervene in metabolic syndrome, while few studies used FMT to intervene in T2DM. Our research is innovative. In a previous animal experiment, it was found that probiotics consumption in diabetes rats can increase the bioavailability of gliclazide (an oral sulfonylurea anti diabetes drug) (Segata et al., 2011). Our research found that FMT combined with metformin is better than metformin alone to achieve the improvement of blood glucose control and insulin resistance, which provides a new direction for FMT to intervene in T2DM and FMT combined with hypoglycemic drugs to intervene in T2DM.

Our study has several limitations. First, the relatively small sample size was not sufficient to evaluate the subtle differences or mechanisms associated with metformin treatment with or without FMT therapies. Second, the study period was limited to 4 weeks, restricting our understanding of the relationship between long-term clinical efficacy and engraftment of donor-associated microbiota. Third, due to scarcity of the donors, we used multi-donor FMT at different FMT times to enhance the microbial diversity transferred to recipients. However, a fixed donor choice based on donor-recipients matching for patients would help eliminate any confounding factors.

5 Conclusion

In conclusion, In conclusion, our study showed that FMT improved the BMI, PBG, HbA1c, FBG, HOMA-HBCI, and HOMA-IR of T2DM patients in 4 weeks and also promoted the engraftment of donor-associated microbiota in participants. Results from our trial will serve as a basis for the long-term intervention of FMT in T2DM patients and the further development of novel biotherapeutic strategies aimed at combatting T2DM through the safe, effective, and affordable bacterial formulations.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

The studies involving human participants were reviewed and approved by Longhu Hospital, The First Affiliated Hospital of Shantou University Medical College Ethics Committee in Shantou, China(Ethics number:LHLL2019001), and was registered at

Chinses Clinical Trial Registry. (Registration number: ChiCTR1900024636).(http://www.chictr.org.cn/showprojen.aspx?proj=41166), and written informed consent was provided from all the patients. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

ZW and BZ, conceptualization, investigation, methodology, and writing-original draft. RX and FC, conceptualization, investigation, methodology, writing-review and editing. DZ and BC, conceptualization, investigation, writing-review and editing. AL and CZ, conceptualization, supervision, writing-review and editing. DH, XL, and SZ, conceptualization, investigation, writing-review and editing. KH and YC, conceptualization, formal analysis, investigation, visualization, supervision, writing-review and editing, project administration, and funding acquisition. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by grants from National Health and Health Commission of the People's Republic of China-NHC Key Laboratory of Health Economics and Policy Research (No.NHC-HEPR2019003), Shantou Science and Technology Plan medical and health category project (No.190923115260372 and 180905124014368), Guangdong science and technology special fund (No.210629086900260) and Special Funds for Science and Technology of Guangdong Province (No. 2021-88).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2022.1089991/full#supplementary-material

SUPPLEMENTARY TABLE 1
Basic information and clinical data of participants.

References

Aggarwala, V., Mogno, I., Li, Z., Yang, C., Britton, G. J., Chen-Liaw, A., et al. (2021). Precise quantification of bacterial strains after fecal microbiota transplantation delineates long-term engraftment and explains outcomes. *Nat. Microbiol.* 6 (10), 1309–1318. doi: 10.1038/s41564-021-00966-0

Aguayo-Mazzucato, C., Andle, J., Lee, T. B.Jr., Midha, A., Talemal, L., Chipashvili, V., et al. (2019). Acceleration of beta cell aging determines diabetes and senolysis improves disease outcomes. *Cell Metab.* 30 (1), 129–42.e4. doi: 10.1016/j.cmet.2019.05.006

Al-Salami, H., Butt, G., Fawcett, J. P., Tucker, I. G., Golocorbin-Kon, S., and Mikov, M. (2008). Probiotic treatment reduces blood glucose levels and increases systemic absorption of gliclazide in diabetic rats. *Eur. J. Drug Metab. Pharmacokinet.* 33 (2), 101–106. doi: 10.1007/BF03191026

Aron-Wisnewsky, J., Clement, K., and Nieuwdorp, M. (2019). Fecal microbiota transplantation: a future therapeutic option for Obesity/Diabetes? *Curr. Diabetes Rep.* 19 (8), 51. doi: 10.1007/s11892-019-1180-z

Belenguer, A., Duncan, S. H., Calder, A. G., Holtrop, G., Louis, P., Lobley, G. E., et al. (2006). Two routes of metabolic cross-feeding between bifidobacterium adolescentis and butyrate-producing anaerobes from the human gut. *Appl. Environ. Microbiol.* 72 (5), 3593–3599. doi: 10.1128/AEM.72.5.3593-3599.2006

Bordalo Tonucci, L., Dos Santos, K. M., De Luces Fortes Ferreira, C. L., Ribeiro, S. M., De Oliveira, L. L., and Martino, H. S. (2017). Gut microbiota and probiotics: Focus on diabetes mellitus. *Crit. Rev. Food Sci. Nutr.* 57 (11), 2296–2309. doi: 10.1080/10408398.2014.934438

Buse, J. B., DeFronzo, R. A., Rosenstock, J., Kim, T., Burns, C., Skare, S., et al. (2016). The primary glucose-lowering effect of metformin resides in the gut, not the circulation: Results from short-term pharmacokinetic and 12-week dose-ranging studies. *Diabetes Care* 39 (2), 198–205. doi: 10.2337/dc15-0488

Carvalho, B. M., and Saad, M. J. (2013). Influence of gut microbiota on subclinical inflammation and insulin resistance. *Mediators Inflamm* 2013, 986734. doi: 10.1155/2013/986734

Chen, P. C., Chien, Y. W., and Yang, S. C. (2019). The alteration of gut microbiota in newly diagnosed type 2 diabetic patients. *Nutr. (Burbank Los Angeles County Calif)* 63-64, 51–56. doi: 10.1016/j.nut.2018.11.019

Chudnovskiy, A., Mortha, A., Kana, V., Kennard, A., Ramirez, J. D., Rahman, A., et al. (2016). Host-protozoan interactions protect from mucosal infections through activation of the inflammasome. *Cell* 167 (2), 444–456.e14. doi: 10.1016/j.cell.2016.08.076

DeFronzo, R. A., Buse, J. B., Kim, T., Burns, C., Skare, S., Baron, A., et al. (2016). Once-daily delayed-release metformin lowers plasma glucose and enhances fasting and postprandial GLP-1 and PYY: results from two randomised trials. *Diabetologia* 59 (8), 1645–1654. doi: 10.1007/s00125-016-3992-6

de Groot, P., Nikolic, T., Pellegrini, S., Sordi, V., Imangaliyev, S., Rampanelli, E., et al. (2021). Faecal microbiota transplantation halts progression of human new-onset type 1 diabetes in a randomised controlled trial. *Gut* 70 (1), 92–105. doi: 10.1136/gutjnl-2020-322630

de la Cuesta-Zuluaga, J., Mueller, N. T., Corrales-Agudelo, V., Velasquez-Mejia, E. P., Carmona, J. A., Abad, J. M., et al. (2017). Metformin is associated with higher relative abundance of mucin-degrading akkermansia muciniphila and several short-chain fatty acid-producing microbiota in the gut. *Diabetes Care* 40 (1), 54–62. doi: 10.2337/dc16-1324

Depommier, C., Van Hul, M., Everard, A., Delzenne, N. M., De Vos, W. M., and Cani, P. D. (2020). Pasteurized akkermansia muciniphila increases whole-body energy expenditure and fecal energy excretion in diet-induced obese mice. *Gut Microbes* 11 (5), 1231–1245. doi: 10.1080/19490976.2020.1737307

Duparc, T., Plovier, H., Marrachelli, V. G., Van Hul, M., Essaghir, A., Stahlman, M., et al. (2017). Hepatocyte MyD88 affects bile acids, gut microbiota and metabolome contributing to regulate glucose and lipid metabolism. *Gut* 66 (4), 620–632. doi: 10.1136/gutjnl-2015-310904

Erem, C., Ozbas, H. M., Nuhoglu, I., Deger, O., Civan, N., and Ersoz, H. O. (2014). Comparison of effects of gliclazide, metformin and pioglitazone monotherapies on glycemic control and cardiovascular risk factors in patients with newly diagnosed uncontrolled type 2 diabetes mellitus. *Exp. Clin. Endocrinol. Diabetes* 122 (5), 295–302. doi: 10.1055/s-0034-1370989

Foretz, M., Guigas, B., and Viollet, B. (2019). Understanding the glucoregulatory mechanisms of metformin in type 2 diabetes mellitus. *Nat. Rev. Endocrinol.* 15 (10), 569–589. doi: 10.1038/s41574-019-0242-2

Giongo, A., Gano, K. A., Crabb, D. B., Mukherjee, N., Novelo, L. L., Casella, G., et al. (2011). Toward defining the autoimmune microbiome for type 1 diabetes. *ISME J.* 5 (1), 82–91. doi: 10.1038/ismej.2010.92

Hartstra, A. V., Bouter, K. E., Backhed, F., and Nieuwdorp, M. (2015). Insights into the role of the microbiome in obesity and type 2 diabetes. *Diabetes Care* 38 (1), 159–165. doi: 10.2337/dc14-0769

He, J., He, X., Ma, Y., Yang, L., Fang, H., Shang, S., et al. (2021). A comprehensive approach to stool donor screening for faecal microbiota transplantation in China. *Microb. Cell Fact.* 20 (1), 216. doi: 10.1186/s12934-021-01705-0

Herfarth, H., Barnes, E. L., Long, M. D., Isaacs, K. L., Leith, T., Silverstein, M., et al. (2019). Combined endoscopic and oral fecal microbiota transplantation in patients with antibiotic-dependent pouchitis: Low clinical efficacy due to low donor microbial engraftment. *Inflamm. Gut. Dis.* 4 (1), 1–6. doi: 10.1159/000497042

Hou, K., Wu, Z. X., Chen, X. Y., Wang, J. Q., Zhang, D., Xiao, C., et al. (2022). Microbiota in health and diseases. *Signal Transduct. Target. Ther.* 7 (1), 135. doi: 10.1038/s41392-022-00974-4

Hou, K., Zhang, S., Wu, Z., Zhu, D., Chen, F., Lei, Z. N., et al. (2022). Reconstruction of gut microecology of type 2 diabetes by fecal microbiota transplantation: Why and how. *Bosnian J. Basic Med. Sci.* 22 (3), 315–325. doi: 10.17305/bjbms.2021.6323

Ingle, H., Lee, S., Ai, T., Orvedahl, A., Rodgers, R., Zhao, G., et al. (2019). Viral complementation of immunodeficiency confers protection against enteric pathogens *via* interferon-lambda. *Nat. Microbiol.* 4 (7), 1120–1128. doi: 10.1038/s41564-019-0416-7

Karlsson, F. H., Tremaroli, V., Nookaew, I., Bergstrom, G., Behre, C. J., Fagerberg, B., et al. (2013). Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature* 498 (7452), 99–103. doi: 10.1038/nature12198

Kootte, R. S., Levin, E., Salojarvi, J., Smits, L. P., Hartstra, A. V., Udayappan, S. D., et al. (2017). Improvement of insulin sensitivity after lean donor feces in metabolic syndrome is driven by baseline gut microbiota composition. *Cell Metab.* 26 (4), 611–9.e6. doi: 10.1016/j.cmet.2017.09.008

Krentz, A. J., and Bailey, C. J. (2005). Oral antidiabetic agents: current role in type 2 diabetes mellitus. *Drugs* 65 (3), 385–411. doi: 10.2165/00003495-200565030-00005

Lee, P., Yacyshyn, B. R., and Yacyshyn, M. B. (2019). Gut microbiota and obesity: An opportunity to alter obesity through faecal microbiota transplant (FMT). *Diabetes Obes. Metab.* 21 (3), 479–490. doi: 10.1111/dom.13561

Li, X. V., Leonardi, I., and Iliev, I. D. (2019). Gut mycobiota in immunity and inflammatory disease. *Immunity* 50 (6), 1365–1379. doi: 10.1016/j.immuni.2019.05.023

Li, X., Watanabe, K., and Kimura, I. (2017). Gut microbiota dysbiosis drives and implies novel therapeutic strategies for diabetes mellitus and related metabolic diseases. *Front. Immunol.* 8, 1882. doi: 10.3389/fimmu.2017.01882

Li, S. S., Zhu, A., Benes, V., Costea, P. I., Hercog, R., Hildebrand, F., et al. (2016). Durable coexistence of donor and recipient strains after fecal microbiota transplantation. *Sci. (New York NY)* 352 (6285), 586–589. doi: 10.1126/science.aad8852

Lu, J., and Salzberg, S. L. (2020). Ultrafast and accurate 16S rRNA microbial community analysis using kraken 2. *Microbiome* 8 (1), 124. doi: 10.1186/s40168-020-00900-2

Ma, Q., Li, Y., Li, P., Wang, M., Wang, J., Tang, Z., et al. (2019). Research progress in the relationship between type 2 diabetes mellitus and gut microbiota. *Biomed. pharmacother. = Biomed. pharmacother.* 117, 109138. doi: 10.1016/j.biopha.2019.109138

Marchesi, J. R., Adams, D. H., Fava, F., Hermes, G. D., Hirschfield, G. M., Hold, G., et al. (2016). The gut microbiota and host health: a new clinical frontier. *Gut* 65 (2), 330–339. doi: 10.1136/gutjnl-2015-309990

Mayo, B., and Sinderen, D. V. (2010). Bifidobacteria genomics and molecular aspects.

Mocanu, V., Zhang, Z., Deehan, E. C., Kao, D. H., Hotte, N., Karmali, S., et al. (2021). Fecal microbial transplantation and fiber supplementation in patients with severe obesity and metabolic syndrome: a randomized double-blind, placebo-controlled phase 2 trial. *Nat. Med.* 27 (7), 1272–1279. doi: 10.1038/s41591-021-01399-2

Musso, G., Gambino, R., and Cassader, M. (2011). Interactions between gut microbiota and host metabolism predisposing to obesity and diabetes. *Annu. Rev. Med.* 62, 361–380. doi: 10.1146/annurev-med-012510-175505

Ng, S. C., Xu, Z., Mak, J. W. Y., Yang, K., Liu, Q., Zuo, T., et al. (2022). Microbiota engraftment after faecal microbiota transplantation in obese subjects with type 2 diabetes: a 24-week, double-blind, randomised controlled trial. *Gut* 71 (4), 716–723. doi: 10.1136/gutjnl-2020-323617

Pinzone, M. R., Celesia, B. M., Di Rosa, M., Cacopardo, B., and Nunnari, G. (2012). Microbial translocation in chronic liver diseases. *Int. J. Microbiol.* 2012, 694629. doi: 10.1155/2012/694629

Pollak, M. (2017). The effects of metformin on gut microbiota and the immune system as research frontiers. *Diabetologia* 60 (9), 1662–1667. doi: 10.1007/s00125-017-4352-x

Que, Y., Cao, M., He, J., Zhang, Q., Chen, Q., Yan, C., et al. (2021). Gut bacterial characteristics of patients with type 2 diabetes mellitus and the application potential. *Front. Immunol.* 12, 722206. doi: 10.3389/fimmu.2021.722206

Ren, Y. D., Ye, Z. S., Yang, L. Z., Jin, L. X., Wei, W. J., Deng, Y. Y., et al. (2017). Fecal microbiota transplantation induces hepatitis b virus e-antigen (HBeAg) clearance in patients with positive HBeAg after long-term antiviral therapy. *Hepatol. (Baltimore Md)* 65 (5), 1765–1768. doi: 10.1002/hep.29008

Rinott, E., Youngster, I., Yaskolka Meir, A., Tsaban, G., Zelicha, H., Kaplan, A., et al. (2021). Effects of diet-modulated autologous fecal microbiota transplantation on weight regain. *Gastroenterology* 160 (1), 158–73.e10. doi: 10.1053/j.gastro.2020.08.041

Rodriguez, J., Hiel, S., and Delzenne, N. M. (2018). Metformin: old friend, new ways of action-implication of the gut microbiome? *Curr. Opin. Clin. Nutr. Metab. Care* 21 (4), 294–301. doi: 10.1097/MCO.0000000000000468

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., et al. (2011). Metagenomic biomarker discovery and explanation. *Genome Biol.* 12 (6), R60. doi: 10.1186/gb-2011-12-6-r60

Shin, N. R., Lee, J. C., Lee, H. Y., Kim, M. S., Whon, T. W., Lee, M. S., et al. (2014). An increase in the akkermansia spp. population induced by metformin treatment improves glucose homeostasis in diet-induced obese mice. *Gut* 63 (5), 727–735. doi: 10.1136/gutjnl-2012-303839

Skelly, A. N., Sato, Y., Kearney, S., and Honda, K. (2019). Mining the microbiota for microbial and metabolite-based immunotherapies. *Nat. Rev. Immunol.* 19 (5), 305–323. doi: 10.1038/s41577-019-0144-5

Su, L., Hong, Z., Zhou, T., Jian, Y., Xu, M., Zhang, X., et al. (2022). Health improvements of type 2 diabetic patients through diet and diet plus fecal microbiota transplantation. *Sci. Rep.* 12 (1), 1152. doi: 10.1038/s41598-022-05127-9

Su, B., Liu, H., Li, J., Sunli, Y., Liu, B., Liu, D., et al. (2015). Acarbose treatment affects the serum levels of inflammatory cytokines and the gut content of bifidobacteria in Chinese patients with type 2 diabetes mellitus. *J. Diabetes* 7 (5), 729–739. doi: 10.1111/1753-0407.12232

Sun, L., Xie, C., Wang, G., Wu, Y., Wu, Q., Wang, X., et al. (2018). Gut microbiota and gut FXR mediate the clinical benefits of metformin. *Nat. Med.* 24 (12), 1919–1929. doi: 10.1038/s41591-018-0222-4

Thingholm, L. B., Ruhlemann, M. C., Koch, M., Fuqua, B., Laucke, G., Boehm, R., et al. (2019). Obese individuals with and without type 2 diabetes show different gut microbial functional capacity and composition. *Cell Host Microbe* 26 (2), 252–64.e10. doi: 10.1016/j.chom.2019.07.004

Tolhurst, G., Heffron, H., Lam, Y. S., Parker, H. E., Habib, A. M., Diakogiannaki, E., et al. (2012). Short-chain fatty acids stimulate glucagon-like peptide-1 secretion *via* the G-protein-coupled receptor FFAR2. *Diabetes* 61 (2), 364–371. doi: 10.2337/db11-1019

Turnbaugh, P. J., Hamady, M., Yatsunenko, T., Cantarel, B. L., Duncan, A., Ley, R. E., et al. (2009). A core gut microbiome in obese and lean twins. *Nature* 457 (7228), 480–484. doi: 10.1038/nature07540

Vindigni, S. M., and Surawicz, C. M. (2017). Fecal microbiota transplantation. *Gastroenterol. Clinics North Am* 46 (1), 171–185. doi: 10.1016/j.gtc.2016.09.012

Vrieze, A., Van Nood, E., Holleman, F., Salojarvi, J., Kootte, R. S., Bartelsman, J. F., et al. (2012). Transfer of gut microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* 143 (4), 913–6.e7. doi: 10.1053/j.gastro.2012.06.031

Wallace, H. J., Holmes, L., Ennis, C. N., Cardwell, C. R., Woodside, J. V., Young, I. S., et al. (2019). Effect of vitamin D3 supplementation on insulin resistance and beta-cell function in prediabetes: a double-blind, randomized, placebo-controlled trial. *Am. J. Clin. Nutr.* 110 (5), 1138–1147. doi: 10.1093/ajcn/nqz171

frontiers | Frontiers in Cellular and Infection Microbiology

TYPE Original Research
PUBLISHED 05 January 2023
DOI 10.3389/fcimb.2022.1086885

OPEN ACCESS

EDITED BY
Zhe-Sheng Chen,
St. John's University, United States

REVIEWED BY
Xiaolan Lu,
Pudong Medical Center Fudan Univerity, China
Juan Francisco Burgueño,
Independent researcher, Sweden
Yanling Wei,
Army Medical University, China

*CORRESPONDENCE
Jianlin Ren
renjianl@xmu.edu.cn
Hongzhi Xu
Xuhongzhi@xmu.edu.cn

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Intestinal Microbiome, a section of the journal Frontiers in Cellular and Infection Microbiology

RECEIVED 01 November 2022
ACCEPTED 08 December 2022
PUBLISHED 05 January 2023

CITATION
Chen Q, Fan Y, Zhang B, Yan C, Chen Z, Wang L, Hu Y, Huang Q, Su J, Ren J and Xu H (2023) Specific fungi associated with response to capsulized fecal microbiota transplantation in patients with active ulcerative colitis. *Front. Cell. Infect. Microbiol.* 12:1086885.
doi: 10.3389/fcimb.2022.1086885

Specific fungi associated with response to capsulized fecal microbiota transplantation in patients with active ulcerative colitis

Qiongyun Chen[1,2†], Yanyun Fan[1†], Bangzhou Zhang[1,2†], Changsheng Yan[1,2], Zhangran Chen[1,2], Lin Wang[1], Yiqun Hu[1], Qingwen Huang[1], Jingling Su[1], Jianlin Ren[1,2,3,4*] and Hongzhi Xu[1,2,3,4*]

[1]Department of Gastroenterology, Zhongshan Hospital of Xiamen University, School of Medicine, Xiamen University, Xiamen, China, [2]Institute for Microbial Ecology, School of Medicine, Xiamen University, Xiamen, China, [3]Xiamen Key Laboratory of Intestinal Microbiome and Human Health, Zhongshan Hospital of Xiamen University, Xiamen, China, [4]Department of Digestive Disease, School of Medicine, Xiamen University, Xiamen, China

Objective: Fecal microbiota transplantation (FMT) is a novel microbial treatment for patients with ulcerative colitis (UC). In this study, we performed a clinical trial of capsulized FMT in UC patients to determine the association between the gut fungal community and capsulized FMT outcomes.

Design: This study recruited patients with active UC (N = 22) and healthy individuals (donor, N = 9) according to the criteria. The patients received capsulized FMT three times a week. Patient stool samples were collected before (week 0) and after FMT follow-up visits at weeks 1, 4, and 12. Fungal communities were analysed using shotgun metagenomic sequencing.

Results: According to metagenomic analysis, fungal community evenness index was greater in samples collected from patients, and the overall fungal community was clustered among the samples collected from donors. The dominant fungi in fecal samples collected from donors and patients were *Ascomycota* and *Basidiomycota*. However, capsulized FMT ameliorated microbial fungal diversity and altered fungal composition, based on metagenomic analysis of fecal samples collected before and during follow-up visits after capsulized FMT. Fungal diversity decreased in samples collected from patients who achieved remission after capsulized FMT, similar to samples collected from donors. Patients achieving remission after capsulized FMT had specific enrichment of *Kazachstania naganishii*, *Pyricularia grisea*, *Lachancea thermotolerans*, and *Schizosaccharomyces pombe* compared with patients who did not achieve remission. In addition, the relative abundance of *P. grisea* was higher in remission fecal samples during the follow-up visit. Meanwhile, decreased levels of pathobionts, such as *Candida* and *Debaryomyces hansenii*, were associated with remission in patients receiving capsulized FMT.

Conclusion: In the metagenomic analysis of fecal samples from donors and patients with UC receiving capsulized FMT, shifts in gut fungal diversity and composition were associated with capsulized FMT and validated in patients with active UC. We also identified the specific fungi associated with the induction of remission. ClinicalTrails.gov (NCT03426683).

KEYWORDS

fecal microbiota transplantation, capsule administration, ulcerative colitis, mycobiota, metagenomics

Introduction

Ulcerative colitis (UC) is a prototypical autoimmune disease with chronic intestinal inflammation (Loftus, 2004; Kaplan and Windsor, 2021). The hallmarks of functional dysregulation in UC are an abnormal mucosal barrier, immune dysregulation, and gut microbiota dysbiosis, which result in inflammation-mediated intestinal destruction (Guan, 2019). Currently, therapeutic regimens for UC aim to regulate immune system disorders and intestinal inflammation, including 5-aminosalicylic acid (5-ASA), sulfasalazine (SASP), corticosteroids, immunosuppressants, and biological agents (Ordas et al., 2012; Khanna et al., 2013). However, these therapeutic regimens are not universally effective and can produce adverse effects. Hormone dependence, long-term maintenance, and relapse are the most common problems that can influence the response to therapy (Ordas et al., 2012). Therefore, it is important to explore practical and safe novel clinical therapies for patients with UC.

Dysbiosis of gut microbiota, including fungi, is associated with many diseases (Ni et al., 2017; Liu et al., 2020; Fan et al., 2021; Liu et al., 2021). Gut fungal communities are often ignored in clinical practice studies because of their low abundance in the intestinal tract (approximately 0.1% of the total microorganisms); however, they are critical components of the human gut microbiota that are essential for human health (Arumugam et al., 2011). Growing evidence has revealed that dysbiosis of intestinal fungi is involved in UC. The biodiversity and composition of fungi are different between healthy individuals and patients with UC, as well as those in remission (Nishikawa et al., 2009; Sokol et al., 2017). These changes also exist between inflamed and non-inflamed intestinal mucosa (Li et al., 2014). Fungi-specific 18S rRNA sequencing analysis of colonic biopsies and fecal samples between UC patients and healthy individuals showed that the fungal community diversity in patients tended to increase, and *Candida albicans* more heavily colonized the patient's intestinal tract (Ott et al., 2008), causing worse mucosal injury and higher production of anti-*Saccharomyces cerevisiae* antibodies (McKenzie et al., 1990; Colombel et al., 2013). An increased abundance of pathogenic fungi, such as *Trichosporon*, and a lower abundance of *Saccharomyces* were found in the colonic mucosa in severe dextran sodium sulfate (DSS)-induced colitis in mice (Qiu et al., 2015), also demonstrating a close relationship between fungal dysbiosis and UC. Another studies showed that Dectin-1-deficient mice were more susceptible to DSS-induced colitis because of the aberrant immune response to the host fungus (Goodridge et al., 2011; Iliev et al., 2012). Furthermore, UC patients with severe intestinal damage are associated with a polymorphism in the gene encoding Dectin-1 (CLEC7A) (Iliev et al., 2012). Although fungal dysbiosis has been found in patients with UC and some fungi can regulate the intestinal inflammatory response (Calich et al., 2008; Ott et al., 2008; Sonoyama et al., 2011; Sokol et al., 2017; Wang et al., 2019), it is unclear whether the change in fungal composition is a result or cause of UC. Moreover, the functional roles of fungi remain largely unknown.

Fecal microbiota transplantation (FMT) is a strategy to reconstruct gut microbiota by administering a new microbiome from healthy individuals to patients (Ooijevaar et al., 2019). It has been proven that FMT partly works in patients with UC (Moayyedi et al., 2015; Costello et al., 2019; Crothers et al., 2021; Haifer et al., 2022). In contrast to the great success of FMT in treating recurrent *Clostridium difficile* infection (CDI), heterogeneous responses to FMT have been reported in patients with UC (Borody et al., 2003; Smits et al., 2013; Paramsothy et al., 2017). A systemic study including 122 patients with IBD found that approximately 22% of patients with UC achieved clinical remission after FMT during a follow-up visit (Colman and Rubin, 2014). However, compared with numerous studies on the influence of FMT on bacteria (Rossen et al., 2015; Li et al., 2016; Mintz et al., 2018; Paramsothy et al., 2019; Huang et al., 2022), little is known about the alterations in fungal communities after FMT. Siew et al. recently reported durable engraftment of donor-derived fungi after FMT in graft-versus-host disease (Zhang et al., 2021). Another study also revealed that fungal species were associated with the efficacy of FMT in recurrent CDI (Zuo et al., 2018). In 2020, Irina et al. found that *Candida* colonization may be

associated with the clinical outcomes of FMT in UC patients (Leonardi et al., 2020). In addition, experimental studies have revealed the effects of gut *Candida* on intestinal inflammation (Jawhara et al., 2008; Leonardi et al., 2018; Sovran et al., 2018; Li et al., 2019). These changes imply that alterations in gut fungi after FMT may be important for clinical outcomes. However, more evidence is needed to elucidate the function of fungi associated with the response to FMT in UC.

In this study, we used shotgun metagenomics to analyze the longitudinal dynamic change of gut fungal communities in active UC patients after capsulized FMT treatment. We demonstrated significant alterations in the fungal community in patients who received capsulized FMT and identified specific fungi associated with clinical remission after FMT.

Material and methods

Study design

We recruited volunteers as healthy donors from Xiamen University. After screening questionnaires and medical examinations to exclude disease risks, we finally obtained nine volunteers to provide healthy fecal microbiota. After applying the inclusion and exclusion criteria, 22 patients with active UC were enrolled. Before capsulized FMT, all patients underwent endoscopies to assess the severity of GI mucosal to calculate the Mayo scores. Capsulized FMT was administered three times and followed up for 12 weeks. We collected stool samples at weeks 0, 1, 4, and 12 for fungal analysis. Figure S1 showed the flow diagram of the study. This study was approved by Zhongshan Hospital of Xiamen University and registered in Clinical Trials.gov (NCT03426683).

Donor management

Donors were recruited by advertisement from Xiamen University. Screening questionnaires, including lifestyle and medical history interviews, were undertaken before blood and fecal tests. According to the European consensus conference on FMT (Cammarota et al., 2017), the donor's medical history screened for the following criteria with subsequent excluded from donating if present: personal history of cigarette smoking, drinking; drug use in the last 1 month including antibiotics, probiotics, probiotics, and any other drugs; personal history of any infection, such as hepatitis, HIV; gastrointestinal diseases, such as functional gastrointestinal disorders, infections, polyps; autoimmune disease, metabolic syndrome, depression, and other system diseases. Blood screening: full blood count, urine routine, liver function tests, urea and creatinine, CRP, ESR, HIV, Hepatitis A, B and C, Epstein-Barr virus (IgG, IgM), tumor markers, TORCH, cytomegalovirus (IgG, IgM), H. pylori (IgG). Stool screening: fecal occult blood test, routine enteric pathogens test, C. difficile toxin B.

Participant eligibility

Eligible patients were aged 18–70 years with active UC (total Mayo score of 4 - 12) and had a poor response to their current medications (including 5-ASA, SASP, and prednisone). Key exclusion criteria were as follows (Costello et al., 2019; Paramsothy et al., 2019): indeterminate colitis or proctitis alone; other causes of diarrhea; antibiotics or probiotics use within 4 weeks of the enrollment; contraindications for gastrointestinal endoscopy; other diseases such as respiratory failure, heart failure, and severe immunodeficiency; gastrointestinal infection; and other diseases associated with microbiota, such as hypertension, diabetes, systemic lupus erythematosus (SLE).

FMT capsule preparation

Fresh stool samples from donors were collected in sterile plastic containers. Fresh fecal samples (25%) were mixed with saline (60%) and pharmaceutical-grade glycerol (15%) and filtered using an automatic extraction instrument (TG-01, Treatgut Corporation, China). Microbial suspensions were further centrifuged to obtain the fecal microbiota precipitates. The precipitates were thoroughly mixed and filled into capsules (DrCaps, 19504907), and the capsules were immediately frozen at −80°C. The mean stool per capsule was 0.9 grams.

Capsulized FMT

All patients provided written informed consent. Authorized physicians administered FMT in a monitored clinical setting. Patients should fast for at least 8 hours prior to FMT. The frozen capsules were removed to room temperature before use. After administration, patients were onwards observed for adverse effects. Enrolled patients received three FMT treatments (once every two days) in a single dose of 30 capsules. The donors were randomly assigned to enrolled patients. Each patient received two donors flora at least. Patients were followed up for 12 weeks after FMT treatment, and their stool samples were collected at weeks 0, 1, 4, and 12. At baseline (W0, before taking FMT) and W12, coloscopies were taken to assess patients' response to FMT. Maintenance therapy was required in this study according to their usage before enrollment: 1) 5-ASA or SASP- kept using the stable dosage of 5-ASA or SASP; 2) prednisone- with a mandatory taper of 5 mg (>10mg/d) or 2.5mg (≤10mg/d) per week. The weekly time points were abbreviated as W0 (baseline, week 0), W1 (week 1), W4 (week 4), and W12 (week 12).

Assessment

The efficacy of capsulized FMT was assessed using the Mayo scores (Paramsothy et al., 2017) at W0 and W12. The total Mayo score was 0 to 12, including stool frequency (0 to 3), rectal bleeding (0 to 3), findings on endoscopy (0 to 3), and physician's global assessment (0 to 3). The primary outcome was patients' remission (Rm) at W12, with the total Mayo score ≤ 2. Patients could withdraw from the study at any time. To ensure the reliability of data, patients need to contact clinicians before withdrawal and provide their reasons if possible.

Metagenomic sequencing

Fresh fecal samples were collected and immediately frozen at −80°C. Total fecal DNA was extracted using QIAamp Fast DNA Stool Mini Kit (Qiagen, Germany). After the detection of concentrations of DNA samples by Qubit 3.0 (Thermo Fisher Scientific, USA), the quality was further checked on a 1.5% agarose gel. Next, 100 ng DNA was cut into fragments with an average size of 350 bp using the NEBNext® Ultra™ II DNA Library Prep Kit for Illumina (New England Biolabs, USA). We diluted the library to 1 ng/μL and checked the insert size with Agilent 2100 (Agilent, USA). The effective concentration of the library was accurately quantified using an ABI 7300 Plus (Thermo Fisher Scientific, USA) fluorescent quantitative PCR instrument. Finally, the library was sequenced on an Illumina Novaseq 6000 (Illumina, Inc., San Diego, CA, USA) using PE150 reagents. On the average, 84,594,918 ± 19,431,972 raw paired-end reads per sample were obtained in this study.

Bioinformatic and statistical analysis

We used Trimmomatic software (Bolger et al., 2014) to delete unqualified reads and sequencing adaptors from raw sequencing reads. The data was also filtered using knead data (https://huttenhower.sph.harvard.edu/kneaddata) to remove the human host's contaminants. Consequently, 56,668,575 ± 28,151,279 high-quality reads were obtained and subjected to Kraken (Wood et al., 2019) for fungal classification, resulting 21,946 ± 14,082 fungal reads per sample. We utilized the vegan package to analyze fungal α-diversity metrics, Bray-Curtis distances by the vegdist function, and PERMANOVA with 999 permutations by the adonis function (Oksanen et al., 2015). We further used the agricolae package to analyze the diversity indices and taxa among groups by a nonparametric Kruskal-Wallis rank-sum test and Benjamini-Hochberg corrections (Mendiburu, 2015). Figures were plotted using ggplot2-v3.0.0 (Wickham et al., 2017) and GraphPad Prism 9. Statistical significance is represented by p value (≤ 0.05). $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, and $^{***}p \leq 0.001$.

Results

Gut fungal communities of donors

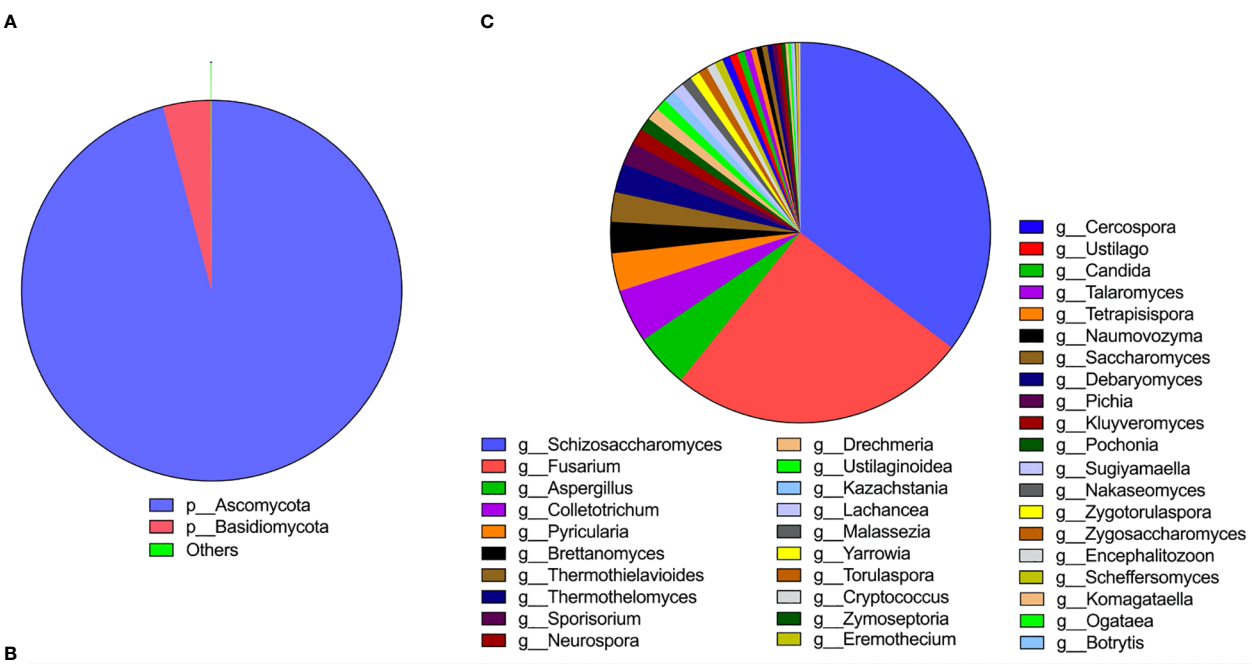
Gut fungal communities of the donor samples were profiled at different classification levels. We found that at the phylum level, *Ascomycota* and *Basidiomycota* were the predominant fungi in fecal samples, especially *Ascomycota*. The relative abundance of *Ascomycota* was as high as 95% (Figure 1A). Ten classes were detected (Figure 1B) as follows: *Sordariomycetes* (42.40%), *Schizosaccharomycetes* (33.98%), *Saccharomycetes* (12.00%), *Eurotiomycetes* (5.15%), *Ustilaginomycetes* (2.53%), *Dothideomycetes* (2.20%), *Malasseziomycetes* (0.85%), *Tremellomycetes* (0.72%), and *Leotiomycetes* (0.17%). We had identified 40 fungal genera in donor samples, including *Schizosaccharomyces*, *Fusarium*, *Aspergillus*, *and Colletotrichum*. Among them, the relative abundance of 13 genera was above 1%, and *Schizosaccharomyces* (35.35%) and *Fusarium* (25.52%) were the main genera (Figure 1C).

Gut fungal dysbiosis in patients with UC

To reveal gut fungal dysbiosis in patients, α-diversity was analyzed between the donor and UC patients, including observed richness, Chao 1, and Shannon and Pielou's evenness indices. The results showed that Shannon and Pielou's evenness indices were significantly increased in samples from UC patients. But Chao 1 richness indice showed a slight decrease in samples from UC patients without a p-value ($p = 0.16$) (Figure 2A). The p-values for the different indices are shown in Table 1. β-diversity analyzed using principal component analysis (PCA) revealed that the overall fungal community in patients with UC was significantly clustered from the donor samples (PERMANOVA, $F = 2.413$, $p < 0.05$). This change was significantly associated with the abundance of *Thermothielavioides terrestris*, *Fusarium fujikuroi*, *Thermothelomyces thermophilus*, *Sporisorium graminicola*, *Brettanomyces nanus*, *F. pseudograminearum*, and *Schizosaccharomyces pombe* (Figure 2B).

Changes in fungi in patients with UC

Fungal taxa at different classification levels were compared between the donor and patient fecal samples to determine the specific changes in gut fungal communities. As expected, *Ascomycota* was the dominant fungal phylotype in patients with UC at the phylum level (Figure S2). To further explore differences in the composition of the two groups of fungi, the top 15 dominant genera, namely, *Schizosaccharomyces*, *Fusarium*, *Aspergillus*, *Thermothielavioides*, *Colletotrichum*, *Pyricularia*, *Thermothelomyces*, *Candida*, *Sporisorium*, *Brettanomyces*,



B

Taxonomy (Class)	Sordariomycetes	Schizosaccharomycetes	Saccharomycetes	Eurotiomycetes	Ustilaginomycetes	Dothideomycetes	Others
Percentage (%)	42.40	33.98	12.00	5.15	2.53	2.20	1.74

FIGURE 1
Gut fungal community in the donors. **(A)** Sector diagram showed the overall fungal community structure at the phylum level. **(B)** The percentage of different fungi at the class level. **(C)** Sector diagram showed the composition of the fungal genera.

Zymoseptoria, *Ustilaginoidea*, *Drechmeria*, *Neurospora*, and *Cryptococcus*, were analyzed at the genus level (Figure 3A; Table 2). However, compared with the donor samples, only *Thermothielavioide* was significantly increased in patients with UC, whereas *Cryptococcus*, *Nakaseomyces*, and *Encephalitozoon* were significantly decreased (Figure 3C). The shift in the fungal community of patients was more remarkable at the species level. Consistent with the phylum level, *S. pombe* was the dominant species in both the donor and patient fecal samples (Figure 3B). As Figure 3D showed five species showed significant changes, including *Thermothielavioide terrestris*, *F. pseudograminearum*, *F. oxysporum, C. glabrata*, and *Encephalitozoon hellem*. Notably, the relative abundance of *F. pseudograminearum* decreased from 23.76% in donor samples to 2.65% in patient samples (Table 2). Furthermore, the relative abundance of *T. terrestris* and *F. oxysporum* markedly increased in UC patients, while the relative abundance of *C. glabrata* and *Encephalitozoon hellem* significantly decreased. Unlike the predominant fungi, the change of fungi with low abundance was more remarkable (Figures 3B, D). These findings suggested that fungi with low abundance might be of concern in patients with UC.

Changes of gut fungal communities in patients after FMT

To explore the effect of capsulized FMT on gut fungal communities, we profiled the fungal community structures at all time points (W0, W1, W4, and W12) during follow-up visits after capsulized FMT. We analyzed the fungal α-diversity before and after capsulized FMT. There were no obvious changes in the fungal richness of fecal samples after FMT. However, compared with W0, the Shannon and Evenness diversities significantly decreased in samples from UC patients at weeks 1, 4, and 12, which was comparable to that in samples from donor (Figures 4A, S3). PCA results showed that the overall microbial communities of the patient samples changed after FMT compared to W0 (Figure 4B). Notably, the dysbiosis of fungal profiles of patients was restored after FMT, which was more similar to that in donor samples (Figure 4C). *Kazachstania and Lachancea* were significantly enriched after FMT, while the relative abundance of *Ustilaginoidea* was decreased at the genus level (Figure 4E). In addition, we found more remarkable shifts in microbial profiles at the species level (Figure 4D). It was worth

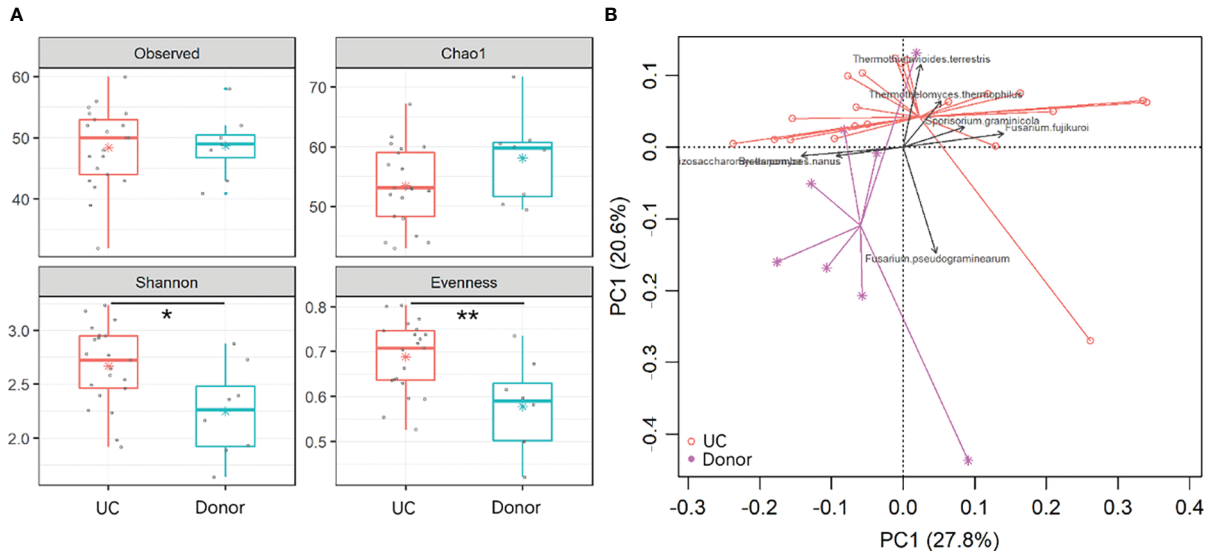


FIGURE 2
Dysbiosis of gut fungal communities in UC patients. **(A)** α-diversity indices in UC patients and donors were analyzed, including observed OTUs, Chao 1, and Shannon and Pielou's evenness indices. The asterisk indicated statistical differences between the two groups, $*p \leq 0.05$,$**p \leq 0.01$,. **(B)** Clusters of gut fungal communities were analyzed using PCA of the fungal species by the Euclidean distance. The top 7 species were fitted to PCA with a significance cutoff at $p < 0.05$.

TABLE 1 The analysis of α-diversity.

Parameters	P value
Chao 1	0.16
Observed	0.86
Shannon	0.02
Evenness	0.01
Simpson	0.02

noting that the relative abundance of some pathogens (*F. fujikuroi* and *C. dubliniensis*) significantly decreased after FMT (Figure 4F). Simultaneously, the relative abundance of *B. nanus*, *Kazachstania naganishii*, *Pyricularia grisea*, and *Lachancea thermotolerans* increased during follow-up visits.

Decreased pathobiont levels associated with the clinical response to FMT

In this study, 22 patients with active UC underwent capsulized FMT, and we assessed the primary outcome at W12 after capsulized FMT. During the follow-up visit, one patient withdrew because of a failed communication. Patients with a total Mayo score ≤ 2 at W12 were defined as clinical Rm (Figure S4). Twelve patients received clinical remission at week 12 (12 out of 21). Shotgun sequencing revealed differences in the fungal community in patients who achieved Rm and those who did not (NRm), especially at W4. Compared to W0, the α-diversity analysis showed that the diversity of the fungal community decreased in remission patient samples (Rm) at weeks 1, 4, and 12, similar to that in donor samples (Figure 5A). However, compared to W0, Shannon and Pilou's evenness indices for non-remission patient samples (NRm) decreased at weeks 1 and 12 and increased at week 4. Moreover, Pilou's evenness index showed a significant difference between Rm and NRm patients at W4 after capsulized FMT (Figure 5A).

Fungal communities visualized using PCA exhibited differences between Rm and NRm samples after capsulized FMT (Figure 5B). Rm patients clustered tightly since W1, unlike NRm patients. Rm patients who received capsulized FMT also tended to have a steady situation on the left side of the donor cluster and were closer to it. Interestingly, the cluster of NRm patients became tighter and moved closer to the cluster of Rm patients who received capsulized FMT.

To identify specific fungi associated with clinical remission, paired rank sum tests were used to analyze the change in abundance of each species between W0 and other time points. The most distinguished taxa are shown in Figures 5C–E. The relative abundance of *K. naganishii*, *P. grisea*, *L. thermotolerans*, and *S. pombe* was increased at W1 after capsulized FMT, whereas *F. oxysporum*, *Aspergillus oryzae*, *F. fujikuroi*, *C. dubliniensis*, and *Ustilaginoidea virens* were decreased (Figure 5C). The relative abundance of *P. grisea* remained high abundance at weeks 1, 4, and 12 (Figures 5C–E). Interestingly,

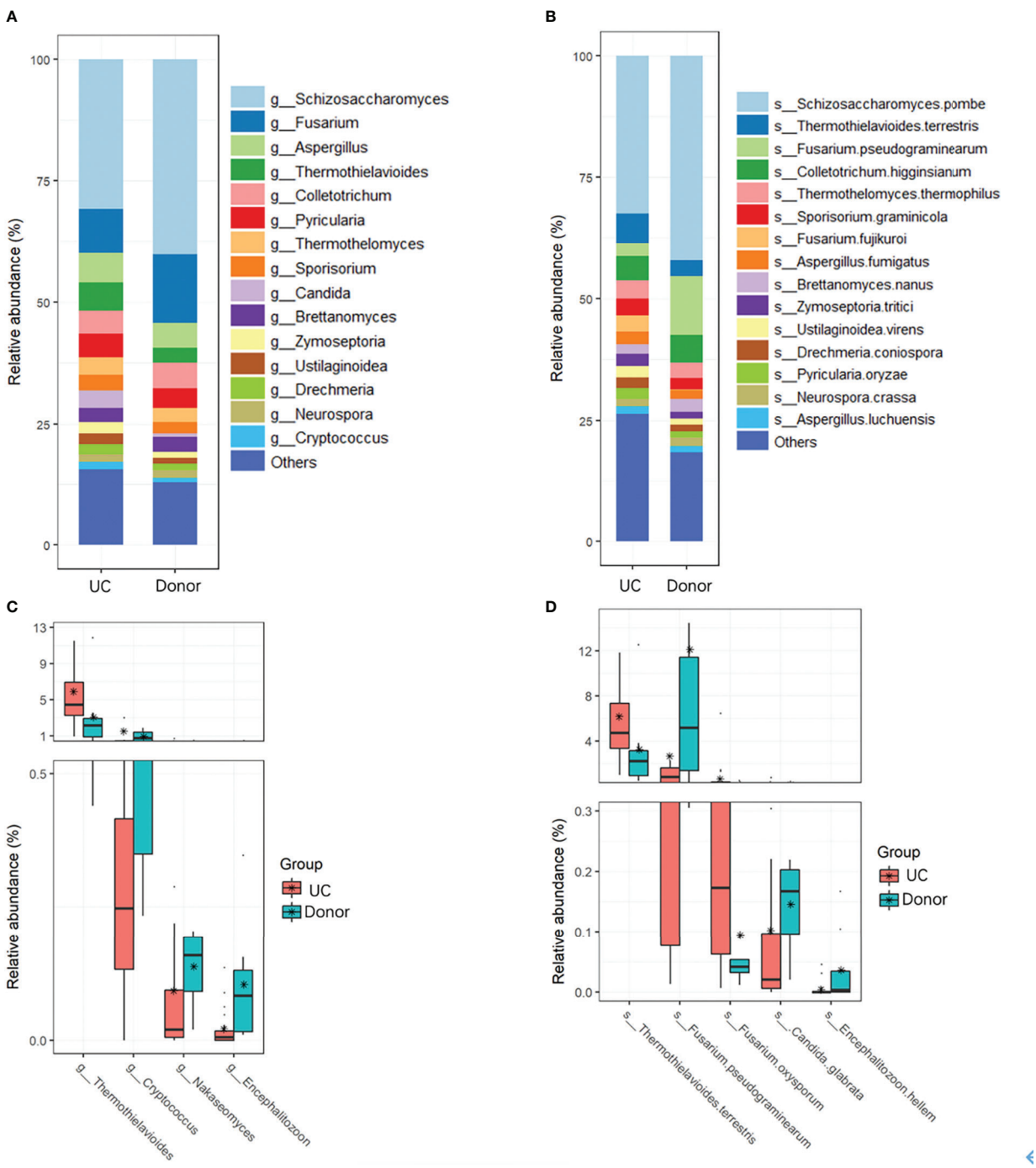


FIGURE 3
Changes in fungi in UC patients. Overall fungal community structures at the genera **(A)** and species **(B)** levels. **(C)** Relative abundances of genera **(C)** and species **(D)** were significantly different between UC patients and donors.

the relative abundance of *Debaryomyces hansenii*, which could injure intestinal mucosal, was significantly decreased in samples that achieved Rm after capsulized FMT at weeks 4 and 12 (Figures 5C, D). We also found that the relative abundance of *Candida dubliniensis* and *Candida glabrata* was decreased at weeks 1 and 12 (Figures 5C, D). Furthermore, the relative abundance of most of the specific taxa decreased at W12, except for the increased level in *P. grisea* (Figure 5E). The

TABLE 2 Comparison of the main taxonomy of fungi in donor and UC patients.

Taxonomy (Genus)	Donor	UC	P value	Taxonomy (Species)	Donor	UC	P value
g Schizosaccharomyces	35.35	30.75	0.4164	S Schizosaccharomyces pombe	36.86	32.41	0.8193
g Fusarium	25.52	9.12	0.0721	S Thermothielavioides terrestris	2.64	6.16	0.0031
g Aspergillus	4.62	5.99	0.1725	S Colletotrichum higginsianum	4.81	4.99	0.6930
g Thermothielavioides	2.51	5.90	0.0031	S Thermothelomyces thermophilus	2.60	3.77	0.0654
g Colletotrichum	3.22	4.85	0.6930	S Sporisorium graminicola	1.98	3.40	0.1594
g Pyricularia	4.57	4.76	0.2011	S Fusarium fujikuroi	0.16	3.32	0.0194
g Thermothelomyces	0.62	3.69	0.0793	S Fusarium pseudograminearum	23.76	2.65	0.0013
g Candida	2.47	3.60	0.0592	S Aspergillus fumigatus	1.66	2.63	0.1594
g Sporisorium	1.90	3.11	0.1725	S Zymoseptoria tritici	1.14	2.43	0.1470
g Brettanomyces	2.67	2.84	0.9503	S Ustilaginoidea virens	1.05	2.34	0.0311
g Zymoseptoria	1.09	2.33	0.1864	S Pyricularia oryzae	1.02	2.24	0.0101
g Ustilaginoidea	1.01	2.24	0.0348	S Drechmeria coniospora	1.14	2.21	0.0247
g Drechmeria	1.08	2.11	0.0247	S Brettanomyces nanus	2.41	2.00	0.5189
g Neurospora	0.75	1.50	0.1244	S Neurospora crassa	1.43	1.55	0.0955
g Cryptococcus	1.35	1.49	0.2166	S Aspergillus luchuensis	1.00	1.54	0.2866

changes of these specific fungi at different time points were shown in Figure 5F. These changes might be a sign of rebalancing gut dysbiosis.

Discussion

In this study, we focused on the gut dysbiosis of fungal communities in patients with UC and conducted capsulized FMT to explore the effect of capsulized FMT on gut fungal diversity and composition. Capsulized FMT reconstructed gut fungal communities in patients with UC. Meanwhile, specific fungal changes from capsulized FMT in UC have been identified and associated with the primary clinical outcome. Improvements in microbial richness, depletion of purported pathobionts (*Candida* and *D. hanseni*), and enrichment of fungal microbes (*K. naganishii*, *P. grisea*, *L. thermotolerans*, and *S. pombe*) were associated with the therapeutic effects of capsulized FMT.

There is growing evidence that gut fungal dysbiosis is associated with UC, even varying in composition in the inflamed or non-inflamed intestinal tract. As reported, the diversity of the fungal community was inconsistent in different types of samples from patients with UC (Ott et al., 2008; Hoffmann et al., 2013; Sokol et al., 2017). Our results showed increased diversity in patient fecal samples based on shotgun sequencing, mainly in microbiota evenness (Shannon and Pilou's evenness). For the Chao 1 index, fungal biodiversity in UC nominally decreased compared with donor samples, without reaching statistical significance. After capsulized FMT, the fungal microbiota evenness index in patients with UC decreased significantly and was closer to that of donors. Notably, these changes were more remarkable in Rm patients after capsulized FMT than in NRm patients. The borderline differences may be due to limitations related to the sample size of this study.

Following published reports, *Ascomycota* and *Basidiomycota* were the dominant fungal fecal microbiota in both donors and UC patients (Hoffmann et al., 2013; Chehoud et al., 2015; Mukherjee et al., 2015; Richard et al., 2015). We also identified specific fungal community dysbiosis in patients with UC belonging to the dominant phyla *Ascomycota* and *Basidiomycota*. In addition, capsulized FMT reversed the imbalance between *Ascomycota* and *Basidiomycota*. The abundance of *Ascomycota* and *Basidiomycota* was strongly associated with the disease status and also different in biopsies from the inflamed or non-inflamed intestinal tract. Sokol et al. reported that *Ascomycota* and *Basidiomycota*, especially the Basidiomycota-to-Ascomycota ratio, were two of the most distinguishing features between healthy individuals and UC microbiota, as well as the inflamed or non-inflamed intestinal tract in UC (Sokol et al., 2017). *Ascomycota* and *Basidiomycota* may be involved in the intestinal tract inflammatory process or driven by disease. Interestingly, we also found significant changes in taxa at genera (such as *Cryptococcus*, *Nakaseomyces*, and *Encephalitozoon*) and species (such as *Fusarium oxysporum*, *Candida glabrata*, and *Encephalitozoon hellem*) levels with low abundances. These low-abundance taxa might be associated with the disease and deserve

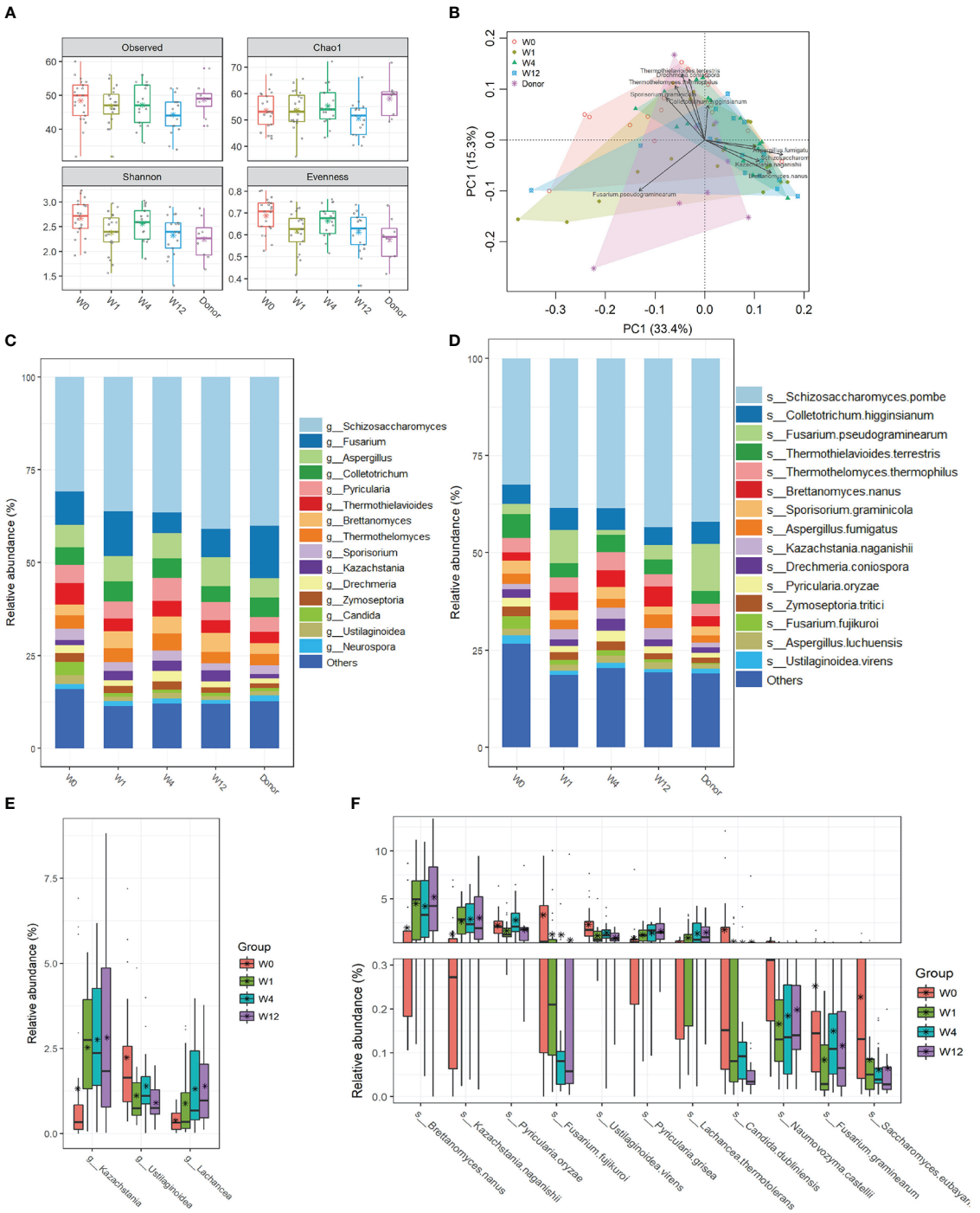


FIGURE 4
Changes of gut fungal communities of UC patients after FMT. **(A)** α-diversity indices in UC patients were analyzed during the follow-up visits, including observed OTUs, Chao 1, and Shannon and Pielou's evenness indices. **(B)** Clusters of gut fungal community were analyzed using PCA of the Euclidean distance of OTU abundance. The top ten genera were fitted to PCA with a statistical significance of $p < 0.05$. Relative abundances of the top 15 genus **(C)** and species **(D)** in samples from donors and patients before and after FMT. **(E)** The significant change in relative abundances of genera in samples from patients between week 0 and other time points after FMT. **(F)** The significant change in relative abundances of species in samples from patients between week 0 and other time points after FMT.

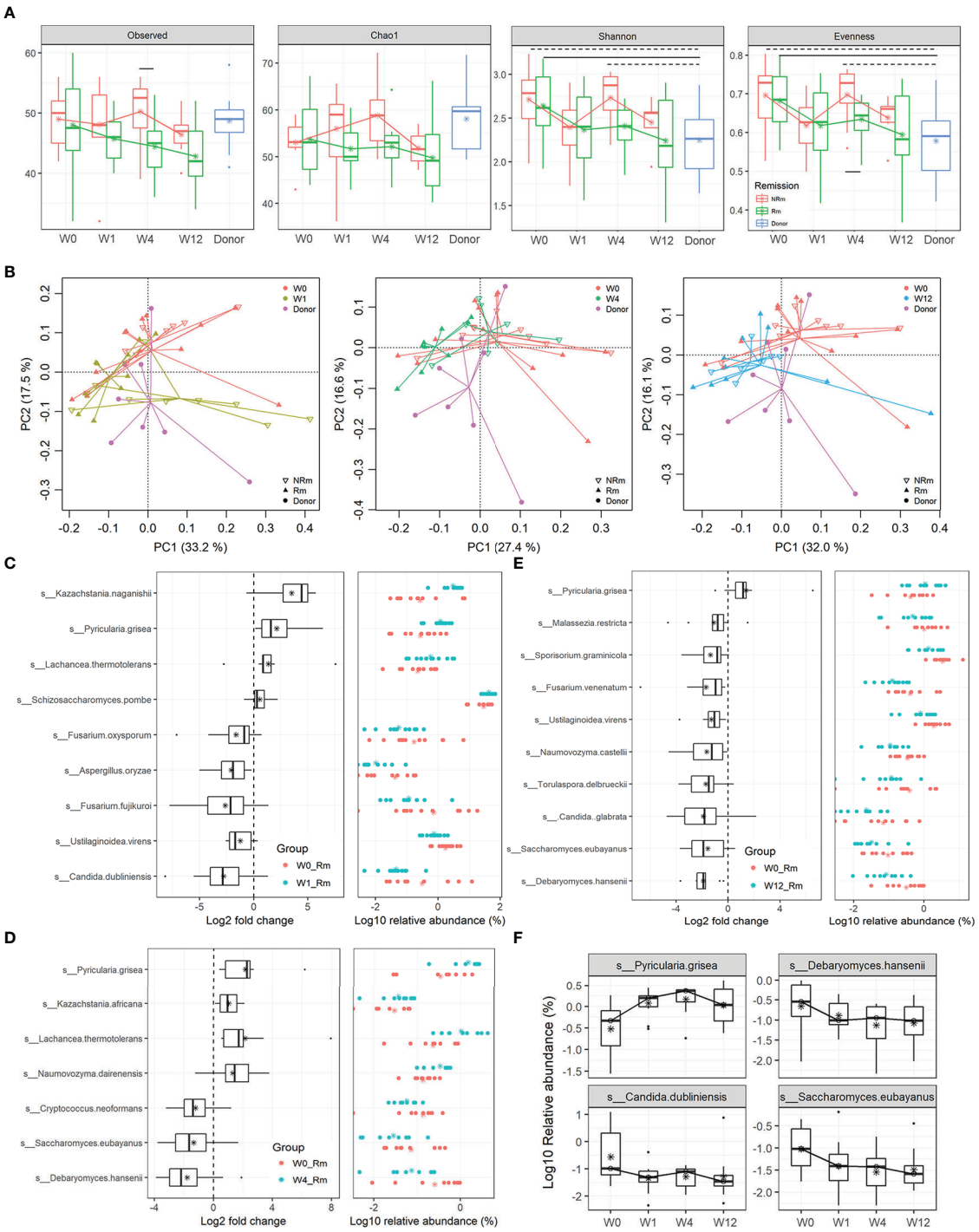


FIGURE 5
Alterations in gut fungal taxa associated with Rm after FMT. **(A)** α-diversity indices in samples from donors and patients who received remission (Rm) or not (NRm) were analyzed, including Observed species, Chao 1, Shannon, and Pielou's evenness. **(B)** Clusters of gut fungal community were analyzed using PCA of the Euclidean distance of OTU abundance. The circle represented the donor, the triangle represented patients who received remission (Rm), and the inverted triangle represented patients who did not receive remission (NRm). **(C–E)** The changes in relative abundances of fungal species were assessed by the paired rank-sum test between week 0 and other time points (weeks 1, 4, and 12) ($p < 0.05$). Box plots showed the species significantly changed in the Rm group at different time points. **(F)** The relative abundance of fungal species in remission patients at different time points.

further exploration. However, since the total fungal reads per sample obtained in this study were relatively limited, future studies with deeper fungal sequencing depth and bigger sample sizes are needed to confirm this result of significant taxa with low abundances, as well as to study the mycobiome.

Different fungal fecal flora was found in donor individuals and UC patients before and after capsule FMT. Most fungal species have been isolated from the intestinal contents, including *Candida*, *Saccharomyces*, *Trichosporon Cryptococcus*, *Galactomyces*, *Penicillium*, *Yarrowia*, and *Debaryomyces* (Haupt et al., 1983; Galan-Sanchez et al., 1999; Khatib et al., 2001; Silva et al., 2004; Jain et al., 2021). Among these, *Cryptococcus*, *Saccharomyces, Debaryomyces*, and *Candida* were significantly altered in patients who achieved Rm after capsulized FMT. Other species were originally cultured from other habitats, including the skin and respiratory tract (Maesaki et al., 1993; Timm et al., 2020). Some fungal species, such as *Candida*, *Fusarium*, and *Debaryomyces*, have been reported as opportunistic pathogenic fungal colonic microbiota in the disease and are related to disease status.

Candida can thrive in the gut, respiratory and urogenital tract, vagina, oral mucosa, and skin of humans (Vautier et al., 2015). A high abundance of *Candida* species is observed in Crohn's disease (CD) and UC (Li et al., 2014; Hoarau et al., 2016; Liguori et al., 2016; Sokol et al., 2017). Moreover, different *Candida* species have been involved in UC pathogenesis, including *C. albicans* (Chehoud et al., 2015; Mukherjee et al., 2015; Richard et al., 2015) and *C. glabrate* (Gouba and Drancourt, 2015; Sierra-Diaz et al., 2019). In this study, we did not find significant changes in *C. albicans* which might be due to the heterogeneity of *C. albicans* in patients using our shotgun sequencing approach. However, we found a decreased abundance of *C. dubliniensis* and *C. glabrate* was associated with clinical Rm after capsulized FMT. *C. dubliniensis* is phenotypically similar to *C. albicans* and is increasingly recognized as an opportunistic pathogenic fungus in immunocompromised hosts (Gutierrez et al., 2002; Jewtuchowicz et al., 2008; Yamahiro et al., 2016; Tahir et al., 2020). *C. albicans* can break through the mucosal barrier and induce an intestinal immune response related to UC pathogenesis (Naglik et al., 2011; Romani, 2011). *C. dubliniensis* also has the potential to break through the mucosal barrier. A genome-wide inventory of peptide transport (PTR) transporters identified two PTR transporters in *C. dubliniensis (*Khatoon et al., 2022). Madiha (Tahir et al., 2020) also cultured *C. dubliniensis* in the cerebrospinal fluid from a 27-year-old patient with chronic meningitis and chronic hepatitis C. Unlike *C. albicans* or *C. dubliniensis*, *C. glabrate* grows only as blastoconidia *(yeast)* and has been reported to be overrepresented in CD (Liguori et al., 2016). Notably, a major group of adhesions in *C. glabrate* is encoded by the epithelial adhesin gene family, which enables *C. glabrate* to adhere to epithelial cells, causing mucosal infections (Cormack et al., 1999; De Las Penas et al., 2003).

In addition, we observed a significant decrease in *Fusarium* spp. in patients who achieved Rm after capsulized FMT. *Fusarium* spp. can produce beauvericin and moniliformin. These secondary metabolites are mycotoxins found in cereal samples (Kamyar et al., 2006). *In vitro*, beauvericin can increase the intracellular calcium concentrations leading to eryptosis. Fumonisins is also one of mycotoxins produced by *Fusarium* spp. that are considered potentially carcinogenic mycotoxins in humans (Woloshuk and Shim, 2013; Munkvold, 2017). Notably, the relative abundance of *D. hansenii* was persistently decreased in patients who achieved clinical Rm after capsulized FMT from W4 to W12. In a previous study (Jain et al., 2021), *D. hansenii* was localized in intestinal inflammatory wounds and impaired colonic healing in CD and colitis mice.

In conclusion, this study identified fungal dysbiosis in patients with UC and the specific fungal taxa associated with therapeutic outcomes in patients with UC receiving capsulized FMT. Our findings, such as fungal community diversity and removal of *Candida*, *Fusarium*, and *Debaryomyces* species, may provide new insights into FMT in UC and provide reference information about therapeutic microbial manipulation of FMT to enhance its effects.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, PRJNA672846.

Ethics statement

The studies involving human participants were reviewed and approved by ClinicalTrails.gov (NCT03426683). The patients/participants provided their written informed consent to participate in this study.

Author contributions

QC, YF, BZ, JR and HX designed this study. QC, YF, BZ underwent the FMT treatment and analyzed the data. LW, YH, QH, JS were responsible for patients' management. ZC and CY were responsible for donors' management. QC and BZ wrote the manuscript.

Funding

This project was sponsored by the Xiamen Key Programs of Medical Health (3502Z20204007), the Xiamen Priority Programs of Medical Health (3502Z20199172 and 3502Z20209026) and the Fujian Provincial Natural Science Foundation (2021J011329).

Acknowledgments

The authors thank the clinicians and laboratory members for their help in the volunteers' management and their comments on this manuscript. The authors also thank TopEdit (www.topeditsci.com) for its linguistic assistance during the preparation of this manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2022.1086885/full#supplementary-material

References

Arumugam, M., Raes, J., Pelletier, E., Paslier, D. L., Yamada, T., Mende, D. R., et al. (2011). Enterotypes of the human gut microbiome. *Nature* 473 (7346), 174–180. doi: 10.1038/nature09944

Bolger, A. M., Lohse, M., and Usadel, B. (2014). Trimmomatic: A flexible trimmer for illumina sequence data. *Bioinformatics* 30 (15), 2114–2120. doi: 10.1093/bioinformatics/btu170

Borody, T. J., Warren, E. F., Leis, S., Surace, R., and Ashman, O. (2003). Treatment of ulcerative colitis using fecal bacteriotherapy. *J. Clin. Gastroenterol.* 37 (1), 42–47. doi: 10.1097/00004836-200307000-00012

Calich, V. L., Pina, A., Felonato, M., Bernardino, S., Costa, T. A., and Loures, F. V. (2008). Toll-like receptors and fungal infections: the role of TLR2, TLR4 and MyD88 in paracoccidioidomycosis. *FEMS Immunol. Med. Microbiol.* 53 (1), 1–7. doi: 10.1111/j.1574-695X.2008.00378.x

Cammarota, G., Ianiro, G., Tilg, H., Stojanović, M. R., Kump, P., Satokari, R., et al. (2017). European Consensus conference on faecal microbiota transplantation in clinical practice. *Gut* 66 (4), 569–580. doi: 10.1136/gutjnl-2016-313017

Chehoud, C., Albenberg, L. G., Judge, C., Hoffmann, C., Grunberg, S., Bittinger, K., et al. (2015). Fungal signature in the gut microbiota of pediatric patients with inflammatory bowel disease. *Inflammation Bowel Dis.* 21 (8), 1948–1956. doi: 10.1097/MIB.0000000000000454

Colman, R. J., and Rubin, D. T. (2014). Fecal microbiota transplantation as therapy for inflammatory bowel disease: a systematic review and meta-analysis. *J. Crohns Colitis* 8 (12), 1569–1581. doi: 10.1016/j.crohns.2014.08.006

Colombel, J. F., et al. (2013). Secukinumab failure in crohn's disease: the yeast connection? *Gut* 62 (5), 800–801. doi: 10.1136/gutjnl-2012-304154

Cormack, B. P., Ghori, N., and Falkow, S. (1999). An adhesin of the yeast pathogen candida glabrata mediating adherence to human epithelial cells. *Science* 285 (5427), 578–582. doi: 10.1126/science.285.5427.578

Costello, S. P., et al. (2019). Effect of fecal microbiota transplantation on 8-week remission in patients with ulcerative colitis: A randomized clinical trial. *JAMA* 321 (2), 156–164. doi: 10.1001/jama.2018.20046

Crothers, J. W., et al. (2021). Daily, oral FMT for long-term maintenance therapy in ulcerative colitis: results of a single-center, prospective, randomized pilot study. *BMC Gastroenterol.* 21 (1), 281. doi: 10.1186/s12876-021-01856-9

De Las Penas, A., Pan, S.J., Castaño, I., Alder, J., Cregg, R., and Cormack, B. P. (2003). Virulence-related surface glycoproteins in the yeast pathogen candida glabrata are encoded in subtelomeric clusters and subject to RAP1- and SIR-dependent transcriptional silencing. *Genes Dev.* 17 (18), 2245–2258. doi: 10.1101/gad.1121003

Fan, X., Jin, Y., Chen, G., Ma, X., and Zhang, L. (2021). Gut microbiota dysbiosis drives the development of colorectal cancer. *Digestion* 102 (4), 508–515. doi: 10.1159/000508328

Galan-Sanchez, F., García-Martos, P., Rodríguez-Ramos, C., Marín-Casanova, P., and Mira-Gutiérrez, J. (1999). Microbiological characteristics and susceptibility patterns of strains of rhodotorula isolated from clinical samples. *Mycopathologia* 145 (3), 109–112. doi: 10.1023/A:1007059005753

Goodridge, H. S., Reyes, C. N., Becker, C. A., Katsumoto, T. R., Ma, J., Wolf, A. J., et al. (2011). Activation of the innate immune receptor dectin-1 upon formation of a 'phagocytic synapse'. *Nature* 472 (7344), 471–475. doi: 10.1038/nature10071

Gouba, N., and Drancourt, M. (2015). Digestive tract mycobiota: a source of infection. *Med. Mal Infect.* 45 (1-2), 9–16. doi: 10.1016/j.medmal.2015.01.007

Guan, Q. (2019). A comprehensive review and update on the pathogenesis of inflammatory bowel disease. *J. Immunol. Res.* 2019, 7247238. doi: 10.1155/2019/7247238

Gutierrez, J., Morales, P., González, M. A., and Quindós, G.. (2002). Candida dubliniensis, a new fungal pathogen. *J. Basic Microbiol.* 42 (3), 207–227. doi: 10.1002/1521-4028(200206)42:3<207::AID-JOBM207>3.0.CO;2-C

Haifer, C., Paramsothy, S., Kaakoush, N. O., Saikal, A., Ghaly, S., Yang, T., et al. (2022). Lyophilised oral faecal microbiota transplantation for ulcerative colitis (LOTUS): A randomised, double-blind, placebo-controlled trial. *Lancet Gastroenterol. Hepatol.* 7 (2), 141–151. doi: 10.1016/S2468-1253(21)00400-3

Haupt, H. M., Merz, W. G., Beschorner, W. E., Vaughan, W. P., and Saral, R.. (1983). Colonization and infection with trichosporon species in the immunosuppressed host. *J. Infect. Dis.* 147 (2), 199–203. doi: 10.1093/infdis/147.2.199

Hoarau, G., Mukherjee, P. K., Gower-Rousseau, C., Hager, C., Chandra, J., Retuerto, M. A., et al. (2016). Bacteriome and mycobiome interactions underscore microbial dysbiosis in familial crohn's disease. *mBio* 7 (5), e01250–16. doi: 10.1128/mBio.01250-16

Hoffmann, C., Dollive, S., Grunberg, S., Chen, J., Li, H., Wu, G. D., et al. (2013). Archaea and fungi of the human gut microbiome: correlations with diet and bacterial residents. *PloS One* 8 (6), e66019. doi: 10.1371/journal.pone.0066019

Huang, C., Mei, Q., Lou, L., Zehua, H., Yan, F., Junjie, F., et al. (2022). Ulcerative colitis in response to fecal microbiota transplantation via modulation of gut microbiota and Th17/Treg cell balance. *Cells* 11 (11), 1851. doi: 10.3390/cells11111851

Iliev, I. D., Funari, V. A., Taylor, K. D., Nguyen, Q, Reyes, C. N., Strom, S. P., et al. (2012). Interactions between commensal fungi and the c-type lectin receptor dectin-1 influence colitis. *Science* 336 (6086), 1314–1317. doi: 10.1126/science.1221789

Jain, U., Heul, A. M, Shanshan, X., Gregory, M. H., Demers, E. G., Kern, J. T., et al. (2021). Debaryomyces is enriched in crohn's disease intestinal tissue and impairs healing in mice. *Science* 371 (6534), 1154–1159. doi: 10.1126/science.abd0919

Jawhara, S., Thuru, X., Standaert-Vitse, A., Jouault, T., Mordon, S., Sendid, B., et al. (2008). Colonization of mice by candida albicans is promoted by chemically induced colitis and augments inflammatory responses through galectin-3. *J. Infect. Dis.* 197 (7), 972–980. doi: 10.1086/528990

Jewtuchowicz, V. M., Mujica, M. T., Brusca, M. I., Sordelli, N., Malzone, M. C., Pola, S. J., et al. (2008). Phenotypic and genotypic identification of candida dubliniensis from subgingival sites in immunocompetent subjects in Argentina. *Oral. Microbiol. Immunol.* 23 (6), 505–509. doi: 10.1111/j.1399-302X.2008.00465.x

Kamyar, M. R., Kouri, K., Rawnduzi, P., Studenik, C., and Lemmens-Gruber, R.. (2006). Effects of moniliformin in presence of cyclohexadepsipeptides on isolated mammalian tissue and cells. *Toxicol. In Vitro* 20 (8), 1284–1291. doi: 10.1016/j.tiv.2006.03.001

Kaplan, G. G., and Windsor, J. W. (2021). The four epidemiological stages in the global evolution of inflammatory bowel disease. *Nat. Rev. Gastroenterol. Hepatol.* 18 (1), 56–66. doi: 10.1038/s41575-020-00360-x

Khanna, R., Sattin, B. D., Afif, W., Benchimol, E. I., Bernard, E-J., Bitton, A., et al. (2013). Review article: a clinician's guide for therapeutic drug monitoring of infliximab in inflammatory bowel disease. *Aliment Pharmacol. Ther.* 38 (5), 447–459. doi: 10.1111/apt.12407

Khatib, R., Riederer, K. M., Ramanathan, J., and Baran, J. Jr. (2001). Faecal fungal flora in healthy volunteers and inpatients. *Mycoses* 44 (5), 151–156. doi: 10.1046/j.1439-0507.2001.00639.x

Khatoon, R., Sharma, S., Prasad, R., Lynn, A. M., Prakash, A., and Banerjee, A.. (2022). Genome-wide analysis of PTR transporters in candida species and their functional characterization in candida auris. *Appl. Microbiol. Biotechnol.* 106 (11), 4223–4235. doi: 10.1007/s00253-022-11998-9

Leonardi, I., Xin, L., Alexa, S., Dalin, L., Itai, D., Gregory, P., et al. (2018). CX3CR1(+) mononuclear phagocytes control immunity to intestinal fungi. *Science* 359 (6372), 232–236. doi: 10.1126/science.aao1503

Leonardi, I., Paramsothy, S., Doron, I., Semon, A., Kaakoush, N. O., Clemente, J. C., et al. (2020). Fungal trans-kingdom dynamics linked to responsiveness to fecal microbiota transplantation (FMT) therapy in ulcerative colitis. *Cell Host Microbe* 27 (5), 823–829 e3. doi: 10.1016/j.chom.2020.03.006

Li, Q., Chenyang, W., Chun, T., Qin, H., Ning, L., and Jieshou, L.. (2014). Dysbiosis of gut fungal microbiota is associated with mucosal inflammation in crohn's disease. *J. Clin. Gastroenterol.* 48 (6), 513–523. doi: 10.1097/MCG.0000000000000035

Li, S. S., Ana, Z., Vladimir, B., Paul, I. C., Rajna, H., Falk, H., et al. (2016). Durable coexistence of donor and recipient strains after fecal microbiota transplantation. *Science* 352 (6285), 586–589. doi: 10.1126/science.aad8852

Liguori, G., Lamas, B., Richard, M. L., Brandi, G., Costa, G., Hoffmann, T. W., et al. (2016). Fungal dysbiosis in mucosa-associated microbiota of crohn's disease patients. *J. Crohns Colitis* 10 (3), 296–305. doi: 10.1093/ecco-jcc/jjv209

Li, X. V., Leonardi, I., and Iliev, I. D. (2019). Gut mycobiota in immunity and inflammatory disease. *Immunity* 50 (6), 1365–1379. doi: 10.1016/j.immuni.2019.05.023

Liu, S., Jiguo, G., Mingqin, Z., Kangding, L., and Hong-Liang, Z.. (2020). Gut microbiota and dysbiosis in alzheimer's disease: Implications for pathogenesis and treatment. *Mol. Neurobiol.* 57 (12), 5026–5043. doi: 10.1007/s12035-020-02073-3

Liu, B. N., XiaoTong, L., ZiHan, L., and JiHuI, W.. (2021). Gut microbiota in obesity. *World J. Gastroenterol.* 27 (25), 3837–3850. doi: 10.3748/wjg.v27.i25.3837

Loftus, E. V.Jr. (2004). Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. *Gastroenterology* 126 (6), 1504–1517. doi: 10.1053/j.gastro.2004.01.063

Maesaki, S., Kohno, S., Tanaka, K., Mitsutake, K., Matsuda, H., Yoshitomi, Y., et al. (1993). [Incidence of fungal isolation in clinical specimens from the respiratory tract]. *Nihon Kyobu Shikkan Gakkai Zasshi* 31 (2), 154–161.

McKenzie, H., Main, J., Pennington, C. R., and Parratt, D.. (1990). Antibody to selected strains of saccharomyces cerevisiae (baker's and brewer's yeast) and candida albicans in crohn's disease. *Gut* 31 (5), 536–538. doi: 10.1136/gut.31.5.536

Mendiburu, F.d. (2015). *Agricolae: Statistical procedures for agricultural research*. Available online at: http://CRAN.R-project.org/package=agricolae.

Mintz, M., Khair, S., Grewal, S., LaComb, J. F., Park, J., Channer, B., et al. (2018). Longitudinal microbiome analysis of single donor fecal microbiota transplantation in patients with recurrent clostridium difficile infection and/or ulcerative colitis. *PloS One* 13 (1), e0190997. doi: 10.1371/journal.pone.0190997

Moayyedi, P., Surette, M. G., Kim, P. T., Libertucci, J., Wolf, M., Onischi, C., et al. (2015). Fecal microbiota transplantation induces remission in patients with active ulcerative colitis in a randomized controlled trial. *Gastroenterology* 149 (1), 102–109 e6. doi: 10.1053/j.gastro.2015.04.001

Mukherjee, P. K., Sendid, B., Hoarau, G., Colombel, J. F., Poulain, D., and Ghannoum, M. A.. (2015). Mycobiota in gastrointestinal diseases. *Nat. Rev. Gastroenterol. Hepatol.* 12 (2), 77–87. doi: 10.1038/nrgastro.2014.188

Munkvold, G. P. (2017). Fusarium species and their associated mycotoxins. *Methods Mol. Biol.* 1542, 51–106. doi: 10.1007/978-1-4939-6707-0_4

Naglik, J. R., Moyes, D. L., Wächtler, B., and Hube, B.. (2011). Candida albicans interactions with epithelial cells and mucosal immunity. *Microbes Infect.* 13 (12-13), 963–976. doi: 10.1016/j.micinf.2011.06.009

Ni, J., Wu, G. D., Albenberg, L., and Tomov, V. T. (2017). Gut microbiota and IBD: causation or correlation? *Nat. Rev. Gastroenterol. Hepatol.* 14 (10), 573–584. doi: 10.1038/nrgastro.2017.88

Nishikawa, J., Kudo, T., Sakata, S., Benno, Y., and Sugiyama, T. (2009). Diversity of mucosa-associated microbiota in active and inactive ulcerative colitis. *Scand. J. Gastroenterol.* 44 (2), 180–186. doi: 10.1080/00365520802433231

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., and Wagner, H. (2015). Vegan: community ecology package. *R Package*. Available online at: https://CRAN.R-project.org/package=vegan.

Ooijevaar, R. E., Terveer, E. M., Verspaget, H. W., Kuijper, E. J., and Keller, J. J. (2019). Clinical application and potential of fecal microbiota transplantation. *Annu. Rev. Med.* 70, 335–351. doi: 10.1146/annurev-med-111717-122956

Ordas, I., Eckmann, L., Talamini, M., Baumgart, D. C., and Sandborn, W. J. (2012). Ulcerative colitis. *Lancet* 380 (9853), 1606–1619. doi: 10.1016/S0140-6736(12)60150-0

Ott, S. J., Kühbacher, O., Musfeldt, M., Rosenstiel, P., Hellmig, S., Rehman, A., et al. (2008). Fungi and inflammatory bowel diseases: Alterations of composition and diversity. *Scand. J. Gastroenterol.* 43 (7), 831–841. doi: 10.1080/00365520801935434

Paramsothy, S., Kühbacher, T., Musfeldt, M., Rosenstiel, P., Hellmig, S., Rehman, A., et al. (2017). Multidonor intensive faecal microbiota transplantation for active ulcerative colitis: a randomised placebo-controlled trial. *Lancet* 389 (10075), 1218–1228. doi: 10.1016/S0140-6736(17)30182-4

Paramsothy, S., Nielsen, S., Kamm, M. A., Deshpande, N. P., Faith, J. J., Clemente, J. C., et al. (2019). Specific bacteria and metabolites associated with response to fecal microbiota transplantation in patients with ulcerative colitis. *Gastroenterology* 156 (5), 1440–1454 e2. doi: 10.1053/j.gastro.2018.12.001

Qiu, X., Feng, Z., Xi, Y., Na, W., Weiwei, J., Xia, L., et al. (2015). Changes in the composition of intestinal fungi and their role in mice with dextran sulfate sodium-induced colitis. *Sci. Rep.* 5, 10416. doi: 10.1038/srep10416

Richard, M. L., Lamas, B., Liguori, G., Hoffmann, T. W., and Sokol, H. (2015). Gut fungal microbiota: the yin and yang of inflammatory bowel disease. *Inflammation Bowel Dis.* 21 (3), 656–665. doi: 10.1097/MIB.0000000000000261

Romani, L. (2011). Immunity to fungal infections. *Nat. Rev. Immunol.* 11 (4), 275–288. doi: 10.1038/nri2939

Rossen, N. G., Fuentes, S., Spek, M. J., Tijssen, J. G., Hartman, J. H., Duflou, A., et al. (2015). Findings from a randomized controlled trial of fecal transplantation for patients with ulcerative colitis. *Gastroenterology* 149 (1), 110–118 e4. doi: 10.1053/j.gastro.2015.03.045

Sierra-Diaz, E., Hernandez-Rios, C. J., and Bravo-Cuellar, A. (2019). Antibiotic resistance: Microbiological profile of urinary tract infections in Mexico. *Cir Cir* 87 (2), 176–182. doi: 10.24875/CIRU.18000494

Silva, J. O., Franceschini, S. A., Lavrador, M. A. S., and Candido, R. C. (2004). Performance of selective and differential media in the primary isolation of yeasts from different biological samples. *Mycopathologia* 157 (1), 29–36. doi: 10.1023/B:MYCO.0000012223.38967.7d

Smits, L. P., Bouter, K. E. C., Vos, W. M., Borody, T. J., and Nieuwdorp, M. (2013). Therapeutic potential of fecal microbiota transplantation. *Gastroenterology* 145 (5), 946–953. doi: 10.1053/j.gastro.2013.08.058

Sokol, H., Leducq, V., Aschard, H., Pham, H. P., Jegou, S., Landman, C., et al. (2017). Fungal microbiota dysbiosis in IBD. *Gut* 66 (6), 1039–1048. doi: 10.1136/gutjnl-2015-310746

Sonoyama, K., Miki, A., Sugita, R., Goto, H., Nakata, H., and Yamaguchi, N.. (2011). Gut colonization by candida albicans aggravates inflammation in the gut and extra-gut tissues in mice. *Med. Mycol* 49 (3), 237–247. doi: 10.3109/13693786.2010.511284

Sovran, B., Planchais, J., Jegou, S., Straube, M., Lamas, B., Natividad, J. M., et al. (2018). Enterobacteriaceae are essential for the modulation of colitis severity by fungi. *Microbiome* 6 (1), 152. doi: 10.1186/s40168-018-0538-9

Tahir, M., Peseski, A. M., and Jordan, S. J. (2020). Case report: Candida dubliniensis as a cause of chronic meningitis. *Front. Neurol.* 11, 601242. doi: 10.3389/fneur.2020.601242

Timm, C. M., Loomis, K., Stone, W., Mehoke, T., Brensinger, B., Pellicore, M., et al. (2020). Isolation and characterization of diverse microbial representatives from the human skin microbiome. *Microbiome* 8 (1), 58. doi: 10.1186/s40168-020-00831-y

Vautier, S., Drummond, R. A., Kong, C., Murray, G. I., Kadosh, D., Brown, A. J., et al. (2015). Candida albicans colonization and dissemination from the murine gastrointestinal tract: the influence of morphology and Th17 immunity. *Cell Microbiol.* 17 (4), 445–450. doi: 10.1111/cmi.12388

Wang, C., Wenbin, L., Hongying, W., Yiming, M., Xinhua, Z., Xudong, Z., et al. (2019). Saccharomyces boulardii alleviates ulcerative colitis carcinogenesis in mice by reducing TNF-alpha and IL-6 levels and functions and by rebalancing intestinal microbiota. *BMC Microbiol.* 19 (1), 246. doi: 10.1186/s12866-019-1610-8

Wickham, H., Chang, W.RStudio (2017). *Ggplot2: Create elegant data visualisations using the grammar of graphics* (New York, NY: Springer).

Woloshuk, C. P., and Shim, W. B. (2013). Aflatoxins, fumonisins, and trichothecenes: a convergence of knowledge. *FEMS Microbiol. Rev.* 37 (1), 94–109. doi: 10.1111/1574-6976.12009

Wood, D. E., Lu, J., and Langmead, B. (2019). Improved metagenomic analysis with kraken 2. *Genome Biol.* 20 (1), 257. doi: 10.1186/s13059-019-1891-0

Yamahiro, A., Lau, K. H. V., Peaper, D. R., and Villanueva, M. (2016). Meningitis caused by candida dubliniensis in a patient with cirrhosis: A case report and review of the literature. *Mycopathologia* 181 (7-8), 589–593. doi: 10.1007/s11046-016-0006-7

Zhang, F., Tao, Z., Yeoh, Y. K., Cheng, F. W., Qin, L., Tang, W., et al. (2021). Longitudinal dynamics of gut bacteriome, mycobiome and virome after fecal microbiota transplantation in graft-versus-host disease. *Nat. Commun.* 12 (1), 65. doi: 10.1038/s41467-020-20240-x

Zuo, T., Won, S. H., Cheung, C., Lam, K., Lui, R, Cheung, K., et al. (2018). Gut fungal dysbiosis correlates with reduced efficacy of fecal microbiota transplantation in clostridium difficile infection. *Nat. Commun.* 9 (1), 3663. doi: 10.1038/s41467-018-06103-6

frontiers | Frontiers in Cellular and Infection Microbiology

TYPE Original Research
PUBLISHED 11 January 2023
DOI 10.3389/fcimb.2022.1094737

OPEN ACCESS

EDITED BY
Xiangtian Yu,
Shanghai Jiao Tong University, China

REVIEWED BY
Marina Sabaté Brescó,
University Clinic of Navarra, Spain
Helioswilton Sales-Campos,
Universidade Federal de Goiás, Brazil

*CORRESPONDENCE
Zaixing Wang
wzx2370@163.com

SPECIALTY SECTION
This article was submitted to Intestinal Microbiome, a section of the journal Frontiers in Cellular and Infection Microbiology

RECEIVED 10 November 2022
ACCEPTED 28 December 2022
PUBLISHED 11 January 2023

CITATION
Luo Z, Jin Z, Tao X, Wang T, Wei P, Zhu C and Wang Z (2023) Combined microbiome and metabolome analysis of gut microbiota and metabolite interactions in chronic spontaneous urticaria.
Front. Cell. Infect. Microbiol. 12:1094737.
doi: 10.3389/fcimb.2022.1094737

Combined microbiome and metabolome analysis of gut microbiota and metabolite interactions in chronic spontaneous urticaria

Zhen Luo[1,2], Zhangsi Jin[1,2], Xiaoran Tao[1,2], Ting Wang[1,2], Panling Wei[1,2], Caihong Zhu[1,2] and Zaixing Wang[1,2]*

[1]Department of Dermatology, The First Affiliated Hospital of Anhui Medical University, Anhui, China, [2]Institute of Dermatology, Anhui Medical University, Anhui, China

Background: The pathogenesis of chronic spontaneous urticaria (CSU) is unclear, and it turned out to be involved in biological processes, such as autoimmunity, autoallergy, inflammation, and coagulation. The gut microbiota plays an important role in immune and inflammatory diseases. However, the relationship between chronic spontaneous urticaria and the gut microbiota remains unknown.

Methods: The stool and serum samples were taken from 15 CSU patients and 15 normal controls. Changes in the composition of gut microbiota and serum metabolism in CSU patients and normal controls were analyzed by 16S ribosomal RNA (rRNA) gene sequencing and untargeted metabolomics.

Results: The results of 16S rRNA gene sequencing showed that compared with normal controls, CSU patients had increased α-diversity of gut microbiota and significant differences in β-diversity. At the phylum level, the relative abundance of *Firmicutes* increased and the relative abundance of *Bacteroidetes* and *Proteobacteria* decreased in CSU patients compared with healthy controls. At the genus level, six kinds of bacteria were significantly enriched in CSU patients and five in normal controls. Metabolomic analysis revealed altered levels of metabolites such as unsaturated fatty acids and purines. Correlation analysis of gut microbiota and metabolites showed that *Lachnospira* was negatively correlated with arachidonic acid, and *Gemmiger* was also negatively correlated with (±)8-HETE.

Conclusion: This study suggests that changes in gut microbiota and metabolites may play a role in immune and inflammatory pathways in the pathogenesis of CSU patients.

KEYWORDS

chronic spontaneous urticaria, allergy, pathogenesis, gut microbiota, serum metabolites, correlation analysis

1 Introduction

Chronic Spontaneous urticaria (CSU) is defined as wheals, angioedema, or both for more than 6 weeks without a clear predisposing factor (Zuberbier et al., 2022). The incidence of CSU is 0.1%-1.5%, which seriously affects both adults and children, with the former being more prevalent in women (Fricke et al., 2020). Approximately 80% of patients recover within 1 year, while more than 10% have illnesses that last for up to five years or more (Stepaniuk et al., 2020), and may relapse within months or years after remission. The cost on patients and society is considerably increased by the recurring course of CSU.

The pathogenesis of urticaria is primarily due to the activation of mast cells and basophils to degranulate and release histamine and other proinflammatory mediators that induce stimulation of sensory nerves, vasodilation and plasma extravasation, and inflammatory cell recruitment. However, the reasons for the activation of mast cells and basophils in CSU patients are complicated. There is increasing evidence that different physiological and pathological reactions including autoimmunity (Saini, 2014; Kolkhir et al., 2022), autoallergy (Hatada et al., 2013; Cugno et al., 2018), inflammation (Tedeschi et al., 2010; Kasperska-Zajac et al., 2015; Kolkhir et al., 2018) and coagulation (Tedeschi et al., 2014) are involved in the above-mentioned cell activation process leading to the formation of wheals.

The gut microbiota is considered to be a new metabolic organ that plays a crucial role in immunity and metabolism, and its metabolites have induction and intervention effects on host immunity and inflammation. In the past, researchers recognized the significance of gut microbiota in immune-mediated diseases such as inflammatory bowel disease (Glassner et al., 2020), type 2 diabetes (Karlsson et al., 2013), systemic lupus erythematosus (Katz-Agranov and Zandman-Goddard, 2017), atopic dermatitis (Paller et al., 2019). In 2017, Nabizadeh et al, for the first time, found that *Akkermansia muciniphila*, *Clostridium leptum* and *Faecalibacterium prausnitzii* levels were different relative to healthy controls, suggesting changes in the gut microbiota in chronic urticaria (Nabizadeh et al., 2017). In 2020, Wang et al. found that changes in gut microbiota were related to unsaturated fatty acid and butyrate metabolic pathways in CSU, providing a new research direction for the pathogenesis of CSU (Wang et al., 2020). However, the specific mechanism between CSU and gut microbiota remains unclear.

Here, we performed 16S gene sequencing and untargeted metabolomics on CSU and normal controls, and obtained the differences in gut microbiota and metabolism through joint analysis, which provided more data for the study of CSU gut microbiota.

2 Materials and methods

2.1 Patient information and sample collection

This study recruited 15 patients and 15 normal controls (NCs), aged 18-60 years, who were diagnosed with CSU in the First Affiliated Hospital of Anhui Medical University between March 2020 and July 2020. The diagnosis of CSU was established according to the EAACI/GA²LEN/EuroGuiDerm/APAAACI guideline (Zuberbier et al., 2022). Basic information such as age, gender, body mass index (BMI), place of residence and clinical symptoms were recorded. Exclusion criteria: use of antibiotics, probiotics, herbal medicines, steroidal drugs, immunosuppressive agents and biologics within 1 month; hemorrhoidal attacks, diarrhea and constipation within 1 month; allergies, diabetes, gastrointestinal disorders, autoimmune diseases, hypertension or systemic diseases known to seriously affect vital organs; pregnancy, lactation; induced urticaria (except dermographism). The patients were all on long-term oral antihistamines and had a recurring course of more than three months without clinical remission. All samples and clinical data were obtained with the informed consent of the research subjects. At the same time, this study was approved by the Ethics Review Committee of the First Affiliated Hospital of Anhui Medical University and in accordance with the Declaration of Helsinki.

Feces and serum from the same subjects were collected on the same day while fasting. The study subjects self-collected stool samples after defecation in the hospital and immediately transferred them to laboratory for cryopreservation. Blood samples were centrifuged at 3000 rpm for 10 minutes at room temperature, and the supernatant was collected and transferred to cryotubes. Serum samples with any hemolysis were excluded from the study. All fecal and serum samples were quickly frozen in liquid ammonia for 30s after collection, and then transferred to -80°C for storage.

2.2 Fecal DNA extraction and 16S sequencing

The genomic DNA of microbial samples in feces was extracted by CTAB/SDS method. The forward primer (5′-ACT CCT ACG GGA GGCAGC AG-3′) and reverse primer (5′-GGA CTA CHV GGG TWT CTA AT-3′) were used to amplify the 16S rRNA gene V3–V4 variable region from the bacterial DNA by PCR. The PCR products were purified with VAHTS™ DNA Clean magnetic beads (Vazyme Biotech, China). Finally, the library was sequenced on an Illumina MiSeq platform (Illumina,

California, USA), and 250 bp/300 bp paired-end reads were generated. The raw sequencing data is saved in FASTQ format, and each deduplicated sequence generated after quality filtering, denoising, merging and removing chimeras is called ASVs (amplicon sequence variants) by the DADA2 method. Using the QIIME2 (2019.4) software, select the Greengenes database (Release 13.8) to perform species annotation on the characteristic sequences of each ASVs, and analyze the Alpha diversity and Beta diversity of the samples. Principal coordinates analysis (PCoA) was performed with the R programming language (Version 3.5.1). Linear discriminant analysis effect size (LEfSe) method was used to identify the biomarkers with statistical difference between CSU patients and NCs (Segata et al., 2011).

2.3 Serum sample collection, metabolite extraction, and UHPLC-MS/MS analysis

After the serum samples were thawed, they were vortexed for 10s to mix well, 50μL of the samples were transferred into a centrifuge tube, 300μL of 20% acetonitrile methanol internal standard extraction solution was added, and vortexed for 3min, centrifuged at 12,000r/min for 10min at 4°C. After centrifugation, 200μL of the supernatant was removed and placed in a -20°C refrigerator for 30min, and then centrifuged again at 12000r/min for 3min at 4°C. Pipette 180μL of the supernatant and place it in a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system for metabolomic analysis.

Liquid chromatography–tandem mass spectrometry(LC-MS/MS) was performed using an ultra-high-performance liquid chromatography (UHPLC) system (1290 Infinity II LC System, Agilent Technologies, CA, USA) and an ultraperformance liquid chromatography (UPLC) high-strength silica (HSS) T3 column (1.8 mm, 2.1 mm × 100 mm) coupled to a quadrupole time-of-flight (Q-TOF) mass spectrometer (6545 LC/Q-TOF MS, Agilent Technologies). The samples were analyzed in both positive and negative ion modes. Mobile phase A used in positive ion mode was 0.1% formic acid in water, and in negative mode it was 5 mmol/L ammonium acetate in water. Mobile phase B was acetonitrile. The elution gradient was set as follows: 5% B at 0 min, 90% B at 11 min, 90% B at 12 min, and 5% B at 12.1 min, 5% B at 14 min. The flow rate was set to 0.4 mL/min. The source conditions of the electrospray ionization were set as follows: spray voltage was 2.5kV in positive mode and -1.5kV in negative mode; sheath gas flow rate was 11L/min; auxiliary gas flow rate was 8L/min; atomized gas voltage was 40V; sheath temperature was 325°C.

The acquired MS raw data were converted into mzML format using ProteoWizard software and processed by XCMS. Data preprocessing steps include peak identification, peak alignment, peak extraction, retention time correction, and peak integration. Peak areas were corrected using the " support vector regression (SVR)" method, and peaks with a missing rate > 50% in each group of samples were filtered. The remaining peaks were determined by comparing their retention time and mass-to-charge ratio (m/z) with databases including HMDB database (http://www.hmdb.ca), KEGG database (http://www.genome.jp/kegg) and an internal online database. Univariate analysis was performed using Student's t-test to detect the changes of metabolites in CSU patients. At the same time, we conducted multivariate statistical analysis, including principal component analysis (PCA) and orthogonal partial least squares-discriminant analysis (OPLS-DA), to understand the metabolite differences between CSU patients and NCs.

2.4 Statistical analysis

Microbial diversity was analyzed using QIIME2 software (Caporaso et al., 2010). α-diversity includes: Chao1 and Observed species indices to characterize richness, Shannon and Simpson indices to characterize diversity and Pielou's evenness index to characterize evenness. The significance of diversity was calculated using the Kruskal-Wallis test. β-diversity was determined by the Jaccard, Bray-Curtis distance matrix calculated by QIIME2, and its significance was determined by PerMANOVA (permutational multivariate analysis of variance). The larger the Jaccard and Bray-Curtis distances, the smaller the similarity between microbial communities. LEfSe analysis found a statistically significant difference in gut microbiota between the two groups, with LDA values>3 and p-values <0.05 considered significant differences. For metabolomics, the Variable Importance in Projection (VIP) value>1 in the OPLS-DA analysis, and P<0.05 in the univariate analysis were considered to be significantly changed metabolites. Spearman correlation coefficient analysis was performed between the differential gut microbiota obtained by 16S rRNA analysis and the differential metabolites obtained by metabolomic analysis.

3 Results

3.1 Characterization of participants

A total of 15 CSU patients and 15 normal controls were recruited in this study. No significant differences were found in age, sex ratio, and BMI between the two groups (p>0.05) (Supplementary Table S1). The subjects who participated at the same time all lived in the same city for a long time, and there was no obvious mobility. Some clinical features of CSU patients are provided in Supplementary Table S1.

3.2 Altered gut microbiota diversity in CSU patients

In this study, a total of 2,214,279 valid 16S sequencing markers (average 79,081) were obtained from 30 fecal samples, with a minimum of 65,594 per sample. A total of 7146 ASVs (Supplementary Table S2) were obtained after DADA2 processing, taxonomic annotation, and extraction with a minimum sample sequence size of 95% (Kemp and Aller, 2004), including 3787 in the CSU group, 2260 in the NCs group, and 1099 in the overlapping part of the two groups (Figure 1). The rarefaction curve showed that the number of ASVs increased with the deepening of sequencing depth and eventually leveled off, indicating that the current sequencing depth was sufficient for community identification (Figure 2).

To comprehensively assess the changes in gut microbial diversity between CSU patients and NCs, we used six indices to analyze the alpha-diversity of the samples, namely Chao1 and Observed species index, Shannon and Simpson index, Pielou's evenness index (Figure 3). Chao1 and Observed species were statistically different, Shannon was statistically different, but Simpson and Pielou's evenness were not. It can be seen that the microbial α-diversity of the CSU group is higher than NCs. For β-diversity, Jaccard and Bray-Curtis distance matrix PCoA analysis was performed, and according to PerMANOVA analysis we obtained a significant difference in Jaccard and Bray-Curtis distances (Figure 4) between CSU and NCs group (Supplementary Table S3), confirming that CSU patients changes in microbial community structure.

In the comparison of the abundance of microbiota in different taxonomy, it was found that the two groups of gut microbiota were mainly composed of *Firmicutes*, *Bacteroidetes*, *Proteobacteria* and *Actinobacteria*. *Firmicutes* was the predominant gut microbiota, accounting for 64.98% and 56.70% of CSU patients and NCs, respectively. At the phylum level, the relative abundance of *Firmicutes* increased and the relative abundances of *Bacteroidetes* and *Proteobacteria* decreased in CSU patients compared with NCs (Figure 5, Supplementary Table S4). At the genus level, we observed that the relative abundance of *Faecalibacterium*, *Roseburia*, *Prevotella*, *Dialister*, *Coprococcus*, *Gemmiger*, *Oscillospira* and *Lachnospira* was increased in patients with CSU, whereas the relative abundance of *Bacteroides*, *unidentified-Ruminococcus*, *Pseudomonas*, *Megamonas* and *Lactobacillus* was decreased in this group compared with NCs (Figure 5, Supplementary Table S5).

We performed LEfSe analysis to further discover differences in gut microbiota between CSU and NCs (Figure 7, Supplementary Table S6). In comparison to normal controls. At the phylum level, the abundance of *Proteobacteria* in the feces of CSU patients was decreased. At the family level, *Rikenellaceae* and *Peptostreptococcus* were increased in CSU patients, while *Bacteroidaceae*, *Enterobacteriaceae*, and *Pseudomonadaceae* were decreased. At the genus level, *Gemmiger*, *Dialister*, *Lachnospira*, *Holdemania*, *unidentified-Prevotella*, *Blvii28* were increased in CSU patients, while *Bacteroidaceae*, *Pseudomonas*, *unidentified-Ruminococcus*, *Megasphaera*, *Anaerofustis*, *Shigella* were decreased (Figure 6). According to the LEfSe analysis of the Cladogram (Figure 7), the differences between the two groups of *Proteobacteria* are mainly concentrated in *Enterobacteriales* (*Enterobacteriaceae*, *Shigella*) and *Pseudomonadales* (*Pseudomonadaceae*, *Pseudomonas*) are opportunistic pathogens. Meanwhile, we used the R language and PICRUSt2

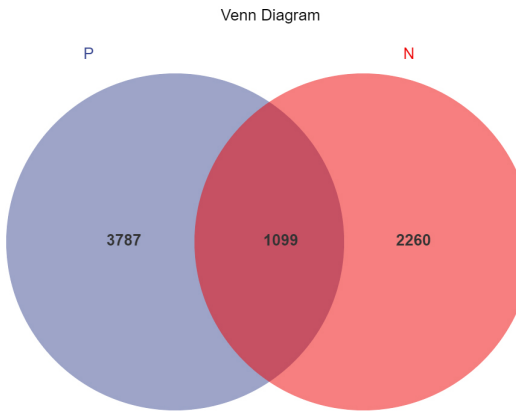


FIGURE 1
Venn diagram of ASVs (amplicon sequence variants) distribution in CSU group and normal controls group. A total of 7146 ASVs were obtained, including 3787 in the CSU group, 2260 in the normal controls group, and 1099 in the overlapping part of the two groups. P, CSU patients (blue); N, normal controls (red).

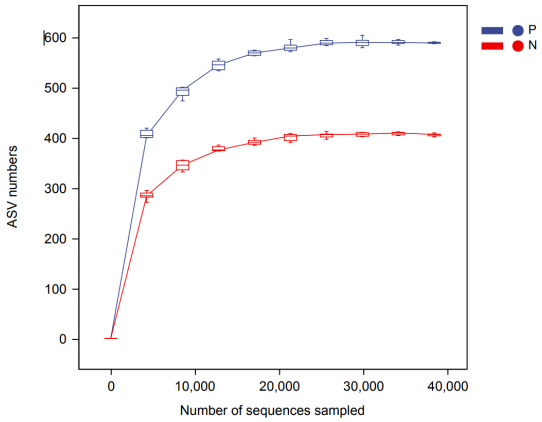


FIGURE 2
Rarefaction curve based on ASVs (amplicon sequence variants) count in normal controls and CSU patients. The number of ASVs increased with the deepening of sequencing depth and finally plateaued, indicating that the current sequencing depth was sufficient for community identification. P, CSU patients (blue); N, normal controls (red).

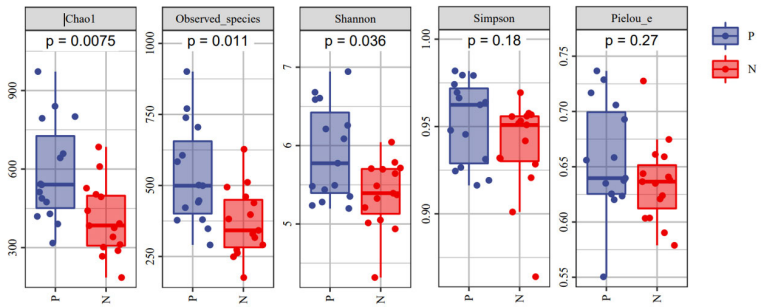


FIGURE 3
The α-diversity of CSU patients and normal controls was measured with the Chao1 and Observed species index, Shannon and Simpson index, Pielou's evenness index. Chao1 and Observed species indices to characterize richness, Shannon and Simpson indices to characterize diversity and Pielou's evenness index to characterize evenness. P, CSU patients (blue); N, normal controls (red).

platform to predict the metabolic function of the differential microbiota through the KEGG database, and no significant metabolic pathways were detected (Supplementary Table S7).

3.3 Changes of intestinal metabolites

Metabolites and fermentation products of gut microbiota can enter the bloodstream and affect human physiology. Therefore, we analyzed the serum metabolites of the CSU group and NCs by LC-MS/MS metabolomics to further discover the interaction between the various microbiota and the host. PCA shows clear separation of metabolic profiles between CSU patients and NCs. OPLS-DA showed that the CSU group and the NCs group exhibited different clustering (Figure 8), with R^2Y of 0.956 and Q^2 of 0.767, indicating that the model was valid, and the two groups exhibited different metabolic activity, demonstrating the presence of multiple metabolic pathways. According to the screening criteria of OPLS-DA model VIP>1 and t-test p<0.05, the differential metabolites with biological significance were mined. The bigger the value, the greater the contribution of the variable to the grouping. We hierarchically clustered the expression of differential metabolites, and drew a heatmap according to the relative metabolite content (Figure 9). We screened out a total of 50 differential metabolites. In the positive ion mode, there were 43 differential metabolites in the two groups, of which 18 were up-regulated and 25 were down-regulated; in the negative ion mode, there were 7 differential metabolites in the two groups, of which 1 was up-regulated and 6 were down-regulated (Figure 10). These metabolites include unsaturated fatty acids ((±)8-HETE, gamma-Linolenic acid, arachidonic acid), amino acids (leucine, phenylalanine), purine and other nucleotide metabolites (adenine, adenosine, inosine), etc. Unsaturated fatty acids were all down-regulated, while purine and other nucleotide metabolites were up-regulated (Supplementary Table S8).

The enriched metabolic pathways were screened by KEGG pathway analysis. The top 20 metabolic pathways in positive and negative ion mode are shown in Figures 11. These pathways mainly include purine metabolism, nucleotide metabolism, unsaturated fatty acid biosynthesis, linoleic acid metabolism, renin secretion, vascular smooth muscle contraction, cGMP-PKG signaling pathway, etc. The abundance analysis showed that the KEGG pathway associated with the two fatty acids presented a negative differential score.

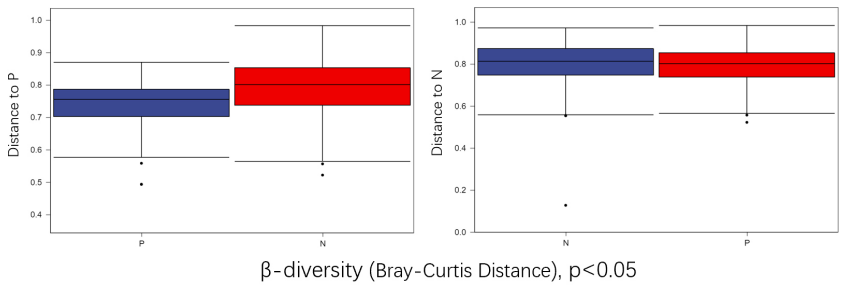


FIGURE 4
Group differences in β-diversity (Bray-Curtis distance index) with respect to CSU patients and normal controls. Statistical analyses were performed using PerMANOVA (permutational multivariate analysis of variance) (p < 0.05). P, CSU patients (blue); N, normal controls (red).

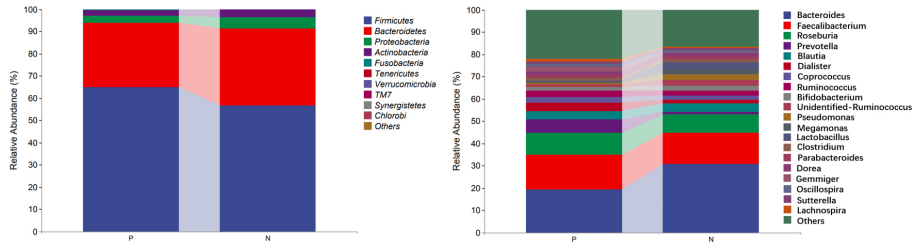


FIGURE 5
Profiles of the relative abundance at the phylum level (left) and the genus level (right) between the two groups. Only the top 10 phyla and the top 20 genera are shown. P, CSU patients; N, normal controls.

3.4 Conjoint analysis

Correlation analysis was performed to better understand the relationship between the microbiome and the plasma metabolome. The altered metabolites screened by serum metabolomics analysis and the genus-level differential gut microbiota screened by 16S rRNA gene sequencing analysis were subjected to Spearman correlation analysis, and the criterion for statistical significance was the correlation coefficient $|r|>0.60$, $p<0.05$. We only found that *Lachnospira* was negatively correlated with arachidonic acid, and *Gemmiger* was negatively correlated with (±) 8-HETE. (Figure 12).

4 Discussion

Our study confirmed changes in gut microbiota and serum metabolic profiles in CSU patients. We attempted to search for possible pathogenesis and potential biomarkers of CSU by combining 16s gene sequencing and untargeted metabolomics.

This study explored the microbial diversity of the gut microbiota in CSU and NCs. α-diversity was used to compare the diversity within a sample, and we found that the two groups of microorganisms were statistically different in richness (Chao1 and Observed species) and diversity (Shannon), but not different in evenness (Pielou's evenness). The α-diversity of the CSU was generally higher than that of the control group, which contradicted earlier research (Lu et al., 2019; Wang et al., 2020; Wang et al., 2021; Liu et al., 2021; Yüksekal et al., 2022). Some studies reported that there were significant differences in α-diversity, and the species diversity of chronic urticaria group decreased (Lu et al., 2019; Wang et al., 2020); other studies found no significant difference in α-diversity between CSU patients and controls (Zhang et al., 2021; Wang et al., 2021). This result may be controversial, and we think that it may be related to factors such as sample size, the geographical location of the subjects, eating habits, and research methods. While β-diversity was used to assess diversity across various samples, there was a significant difference between the CSU and NCs group, which is consistent with previous studies (Lu et al., 2019; Wang et al.,

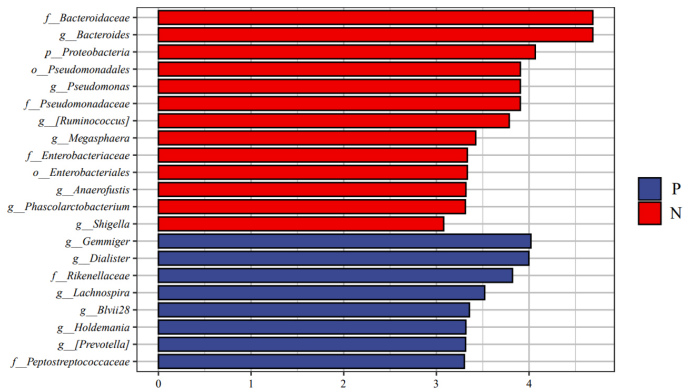


FIGURE 6
Linear discriminant analysis effect size (LEfSe) analysis was performed on the bacterial taxa relative abundance values between the two groups. Bacterial with linear discriminant analysis (LDA) score >3.0 and P < 0.05 were considered to be significantly differences. P, CSU patients (blue); N, normal controls (red).

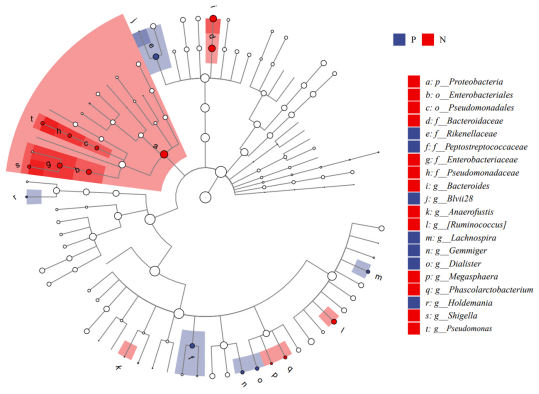


FIGURE 7
Cladogram of Linear discriminant analysis effect size (LEfSe) analysis of microbiome from 16S rDNA sequencing of normal controls and CSU patients. P, CSU patients (blue); N, normal controls (red).

2020; Wang et al., 2021; Liu et al., 2021; Zhang et al., 2021; Yüksekal et al., 2022). Composition analysis of the two groups of samples showed that the relative abundance of *Firmicutes* increased in the CSU group, while the relative abundance of *Bacteroidetes* and *Proteobacteria* decreased, which was similar to the study by Wang et al (Wang et al., 2021), but different from the decreased abundance of *Firmicutes* in the study by Wang et al (Wang et al., 2020). Through LEfSe analysis, we further found that the variations in gut microbiota between two groups were mainly concentrated at the genus level, so we speculated that the changes in the microbiota associated with CSU may be mainly at the lower taxonomical level.

Our study found that *Bacteroides*, *unidentified-Ruminococcus*, *Megasphaera*, and *Anaerofustis* in the CSU group decreased compared with the control group. The decline of *Bacteroides* is consistent with some studies (Lu et al., 2019; Wang et al., 2020). *Bacteroides*, as the main component of the gut microbiota, is the main producer of short-chain fatty acids (SCFAs), mainly acetic and propionic acids. It has been demonstrated that *Bacteroides* has a regulatory effect on human immunity, mainly through its production of capsular polysaccharide A and SCFAs. Capsular polysaccharide A can maintain and regulate immune system homeostasis and prevents bacterial and viral infections (Zafar and Saier, 2021). Both acetate and propionate are effective anti-inflammatory mediators, which can inhibit the release of pro-inflammatory cytokines from neutrophils and macrophages, while SCFAs play an important role in various immune regulatory pathways such as inducing regulatory T cell differentiation, enhancing IL-10 production and inhibiting Th17 cells (Smith et al., 2013). Decreased abundance of *unidentified-Ruminococcus*, consistent with previous study (Wang et al., 2020). Some studies also found that the abundance of *Ruminococcaceae* decreased in infants with eczema, and inflammatory cytokines such as IL-6 and TNF-α increased, and a decrease in the number of *Ruminococcus* can induce a toll-like receptor inflammatory response (Arshi et al., 2014; West et al., 2015). These may confirm that inflammation is associated with CSU. In addition, *Ruminococcus* can also metabolize to produce SCFAs. *Megasphaera* are normal bacteria in the mouth, intestines and vagina that can metabolize to produce valerate. Valerates are also SCFAs, some studies have found that it may increase the cytotoxic activity of $CD8^+$T cells and enhance the immune activity of the body (Luu et al., 2021). *Anaerofustis* is a gram-positive anaerobic bacteria that can produce acetate and butyrate from glucose (Finegold et al., 2004). Growing research proves that CSU is an autoimmune disease, and SCFAs play an important role in regulating and maintaining human immune function. Therefore, the reduction of some SCFAs-producing bacteria can lead to the occurrence of CSU.

We also found that *Gemmiger*, *Dialister*, *Lachnospira*, *Holdemania*, *unidentified-Prevotella* in the CSU group were

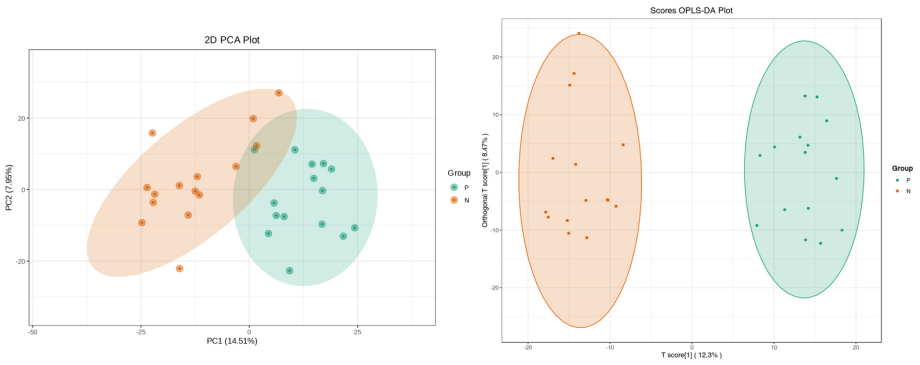


FIGURE 8
Principal component analysis (PCA) of fecal metabolomic data from CSU patients and normal controls. PCA shows clear separation of metabolic profiles between two groups. Orthogonal partial least squares discrimination analysis (OPLS-DA) of fecal metabolomic data from CSU patients and normal controls, the CSU group and the NCs group exhibited different clustering with R^2Y of 0.956 and Q^2 of 0.767. P, CSU patients (green); N, normal controls (yellow).

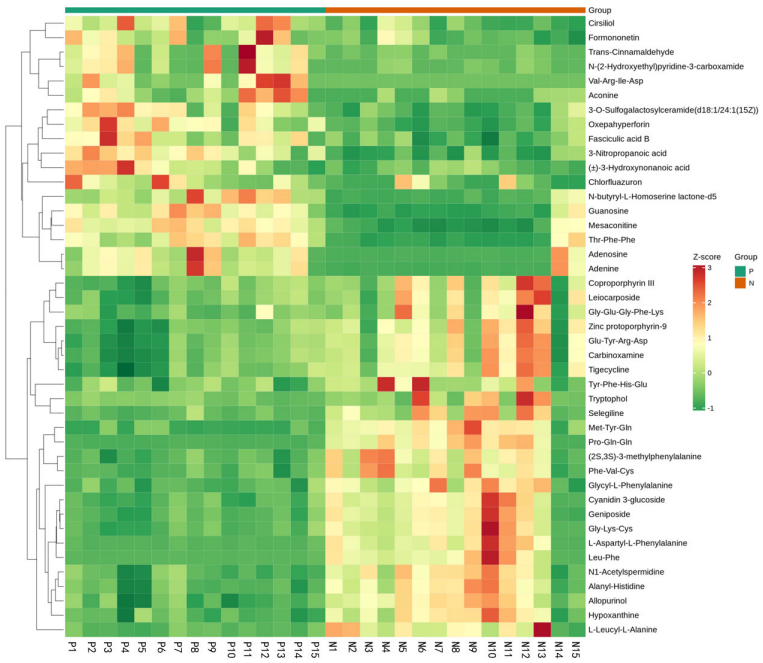


FIGURE 9
Fecal metabolic patterns in CSU patients and normal controls shown as a heatmap. Rows represent data for metabolites and columns represent the subjects. Red and green colors represent increased and decreased levels, respectively, of metabolites in patients with CSU compared to those in normal controls.

higher than those in the control group. One study found that *Gemmiger* was consistently abundant in children with eczema across different modes of delivery (vaginal or caesarean section) or feeding type (infant formula or breastfeeding) (Zheng et al., 2016), consistent with our findings of increased abundance in the gut of CSU patients, suggesting that *Gemmiger* may have a facilitating role in allergic reactions. Our finding of increased abundance of *Dialister* in CSU patients contradicts a study of the gut microbiota of infants with food allergies, which found that children with allergic symptoms had lower numbers of *Dialister* than controls (Savage et al., 2018), and we speculate that this may be due to age differences in our study subjects. However, another study found that *Dialister*, as a microbial marker, was positively correlated with ankylosing spondylitis disease activity score in ileal and colon biopsies, and was more abundant in the acute inflammatory phase (Tito et al., 2017), suggesting that *Dialister* is closely related to the inflammatory reaction. As CSU is an inflammatory disease and *Dialister* abundance increases, there may be a certain correlation between the two. *Lachnospira* belongs to *Clostridiales* and has been found to be a microorganism that produces SCFAs. One study found that *Lachnospiraceae* and its subgroups were the major differences in gut microbiota between antihistamine responders and non-responders in CSU patients, with *Lachnospira* more abundant

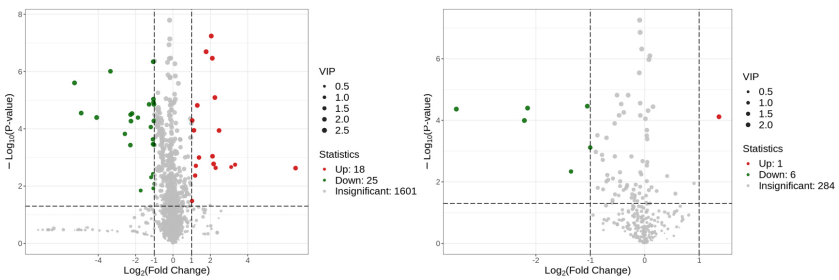


FIGURE 10
Volcano plot of the identified metabolites in positive ion mode (left) and negative ion mode (right). There were 43 differential metabolites in positive ion mode and 7 differential metabolites in negative ion mode.

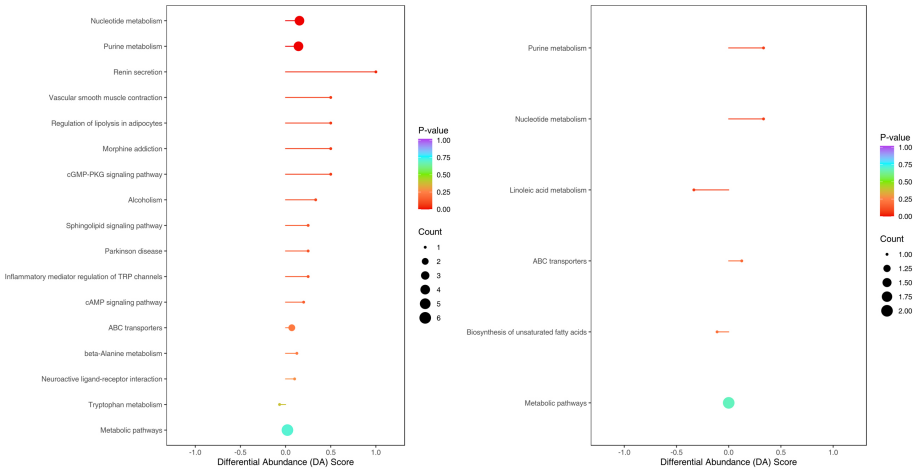


FIGURE 11
Metabolites were annotated into different metabolic pathways in positive ion mode and negative ion mode by KEGG enriched bubble chart based on KEGG. The abundance analysis showed that the KEGG pathway associated with the two fatty acids presented a negative differential score.

in antihistamine responders than non-responders (Liu et al., 2022). Our study did not distinguish between the efficacy of antihistamines in patients, so further studies are needed to address the increased abundance of *Lachnospira* in our study. Some studies have found that *Holdemania* is related to the occurrence of inflammatory response (Jang et al., 2020; Barandouzi et al., 2020), and CRP, IL-6, TNF-α and other inflammatory factors are elevated in CSU patients (Kasperska-Zajac et al., 2015; Kolkhir et al., 2018), so there may be a certain relationship between the two. Similarly, studies in mice have demonstrated that *Prevotella* can promote inflammatory diseases, and can promote Th17 immune response and neutrophil recruitment to induce chronic inflammation, so *Prevotella* may also have a role in promoting inflammation in CSU. In conclusion, changes in gut microbiota may promote or induce the occurrence of CSU in terms of inflammation and immunity.

Metabolites of gut microbiota are normally secreted in the gut and enter the circulatory system *via* the intestinal barrier, and are very important regulators of host metabolism (Canfora et al., 2019), so analyzing metabolite differences helps us discover the underlying pathological mechanisms of CSU. Through metabolomics analysis, we found that the metabolite differences between the CSU group and the control group were mainly concentrated in unsaturated fatty acids ((±)8-HETE, gamma-Linolenic acid, arachidonic acid), and they were all in a downward trend. At the same time, KEGG pathway analysis also showed that the unsaturated fatty acid metabolism pathway was down-regulated. This finding is consistent with the study by Wang et al, who also found that down-regulation of docosahexaenoic acid and arachidonic acid was positively associated with decreased abundance of *Bacteroides* (Wang et al., 2020). However, our study did not find a significant correlation between *Bacteroides* and unsaturated fatty acids,

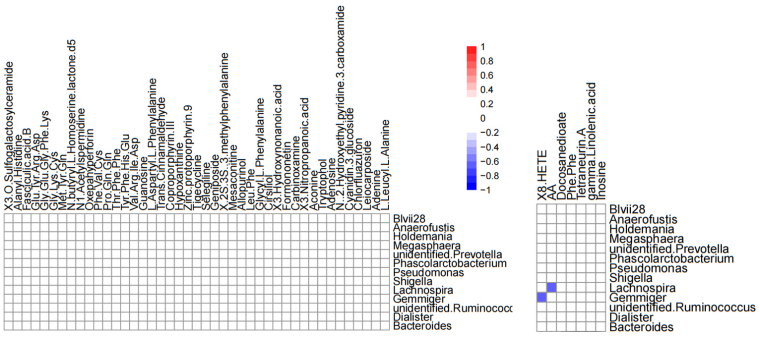


FIGURE 12
Heatmap of differential gut microbiota correlations between serum metabolites and genus levels in positive ion mode (left) and negative ion mode (right). Only significant correlations ($p \leq 0.05$) are colored. Positive correlations are indicated in red and negative correlations in blue.

but suggested that *Lachnospira* was negatively correlated with arachidonic acid. Recent studies have shown that infants receiving formula plus long-chain unsaturated fatty acids such as arachidonic acid have a lower risk of allergic disease and respiratory disease than those receiving formula alone, and have better immune maturation (Birch et al., 2010; Lapillonne et al., 2014), suggesting that unsaturated fatty acids may have a protective effect on allergy. Unsaturated fatty acids such as arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid are also present in the cell membrane of immune system cells and affect immune function through various interaction mechanisms (Miles et al., 2021). Meanwhile, unsaturated fatty acids were found to have anti-inflammatory effects (Calder and Grimble, 2002). Therefore, the reduction of unsaturated fatty acids may aggravate or induce inflammation and allergic reactions. In addition, arachidonic acid can produce inflammatory mediators (prostaglandins, leukotrienes and related metabolites), which regulate the activity of inflammatory cells, the production of cytokines and the balance of Th1 and Th2, which is crucial to the occurrence and resolution of inflammation (Calder and Grimble, 2002; Das, 2021). Our study suggests that arachidonic acid and *Lachnospira*, (±) 8-HETE and *Gemmiger* are all negatively correlated, (±)8-HETE is one of the six monohydroxy fatty acids produced by non-enzymatic oxidation of arachidonic acid. 16s rRNA sequencing revealed that *Lachnospira* and *Gemmiger* abundances were elevated in the CSU group. Therefore, we speculate that the increase in the abundance of *Lachnospira* and *Gemmiger* may lead to the reduction of unsaturated fatty acids such as arachidonic acid, resulting in the imbalance of the immune and inflammatory systems, thereby promoting or inducing CSU. However, this may require further research and more data to verify. Our study also found that nucleotide metabolites such as adenine, adenosine, and inosine were up-regulated in CSU patients. Adenine, adenosine, and inosine are all key metabolites in purine metabolism. A study found that the level of inosine increased while the level of uric acid, the end product of purine metabolism, decreased in a mouse model of allergic asthma (Yu et al., 2016), which is similar to our study, but we did not find changes in uric acid. Another study showed that inflammatory cells in a mouse model of allergic asthma can induce the breakdown of ATP, resulting in increased levels of adenine and adenosine (Moon et al., 2010). Therefore, the above metabolite changes may be related to the inflammatory reaction, which in turn confirms that the inflammation is involved in the pathogenesis of CSU.

Due to the limited sample size and a single-center study, there are potential errors in the sample collection process, and confounding factors such as age, gender, BMI, ethnicity, region, and dietary habits cannot be controlled for matching. For example, CSU patients typically follow a low-protein diet, and changes in long-term eating habits may result in changes in microbiota. Also, metabolomics is impacted by factors such as age, sleep, circadian clock, and exercise, and almost half of the patients in our research had poor sleep quality, so these aspects must be considered. Therefore, this study has great limitations, and more research is needed to verify it. The above factors may also be the reason why our results differ from other studies. At the same time, due to the limitations of research methods, we cannot obtain information on fungi and viruses in the gut.

In conclusion, this study combined 16S gene sequencing and serum metabolomics to identify differences in gut microbiota and serum metabolites between CSU patients and normal controls, revealing that these differences may play a role in immune dysregulation and inflammation in the pathogenesis of CSU patients, providing more data for the study of CSU and gut microbiota. But more research is still needed to further define the exact microbiota that play a key role in CSU and its impact on the host.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI, The accession number: PRJNA901136.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Review Committee of the First Affiliated Hospital of Anhui Medical University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Design of the study: ZL, and ZXW. Methodology: ZL, TW and ZXW. Collected samples and made clinical records: ZL, PLW, TW and XRT. Data curation: ZL, CHZ and ZXJ. Writing—original draft preparation: ZL and ZXJ. Writing—review and editing: ZL, XRT, and ZXW. All authors contributed to the article and approved the submitted version.

Funding

Supporting expenses of the First Affiliated Hospital of Anhui Medical University, National Natural Science Foundation of China (No.3101005002311)

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2022.1094737/full#supplementary-material

References

Arshi, S., Babaie, D., Nabavi, M., Tebianian, M., Ghalehbaghi, B., Jalali, F., et al. (2014). Circulating level of CD4+ CD25+ FOXP3+ T cells in patients with chronic urticaria. *Int. J. Dermatol.* 53, e561–e566. doi: 10.1111/ijd.12630

Barandouzi, Z. A., Starkweather, A. R., Henderson, W. A., Gyamfi, A., and Cong, X. S. (2020). Altered composition of gut microbiota in depression: A systematic review. *Front. Psychiatry* 11, 541. doi: 10.3389/fpsyt.2020.00541

Birch, E. E., Khoury, J. C., Berseth, C. L., Castañeda, Y. S., Couch, J. M., Bean, J., et al. (2010). The impact of early nutrition on incidence of allergic manifestations and common respiratory illnesses in children. *J. Pediatr.* 156, 902–906.e1. doi: 10.1016/j.jpeds.2010.01.002

Calder, P. C., and Grimble, R. F. (2002). Polyunsaturated fatty acids, inflammation and immunity. *Eur. J. Clin. Nutr.* 56 Suppl 3, S14–S19. doi: 10.1038/sj.ejcn.1601478

Canfora, E. E., Meex, R. C. R., Venema, K., and Blaak, E. E. (2019). Gut microbial metabolites in obesity, NAFLD and T2DM. *Nat. Rev. Endocrinol.* 15, 261–273. doi: 10.1038/s41574-019-0156-z

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., et al. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* 7, 335–336. doi: 10.1038/nmeth.f.303

Cugno, M., Asero, R., Ferrucci, S., Lorini, M., Carbonelli, V., Tedeschi, A., et al. (2018). Elevated IgE to tissue factor and thyroglobulin are abated by omalizumab in chronic spontaneous urticaria. *Allergy* 73, 2408–2411. doi: 10.1111/all.13587

Das, U. N. (2021). Essential fatty acids and their metabolites in the pathobiology of inflammation and its resolution. *Biomolecules* 11(12), 1873. doi: 10.3390/biom11121873

Finegold, S. M., Lawson, P. A., Vaisanen, M. L., Molitoris, D. R., Song, Y., Liu, C., et al. (2004). Anaerofustis stercorihominis gen. nov., sp. nov., from human feces. *Anaerobe* 10, 41–45. doi: 10.1016/j.anaerobe.2003.10.002

Fricke, J., Ávila, G., Keller, T., Weller, K., Lau, S., Maurer, M., et al. (2020). Prevalence of chronic urticaria in children and adults across the globe: Systematic review with meta-analysis. *Allergy* 75, 423–432. doi: 10.1111/all.14037

Glassner, K. L., Abraham, B. P., and Quigley, E. M. M. (2020). The microbiome and inflammatory bowel disease. *J. Allergy Clin. Immunol.* 145, 16–27. doi: 10.1016/j.jaci.2019.11.003

Hatada, Y., Kashiwakura, J., Hayama, K., Fujisawa, D., Sasaki-Sakamoto, T., Terui, T., et al. (2013). Significantly high levels of anti-dsDNA immunoglobulin e in sera and the ability of dsDNA to induce the degranulation of basophils from chronic urticaria patients. *Int. Arch. Allergy Immunol.* 161 Suppl 2, 154–158. doi: 10.1159/000350388

Jang, J. H., Yeom, M. J., Ahn, S., Oh, J. Y., Ji, S., Kim, T. H., et al. (2020). Acupuncture inhibits neuroinflammation and gut microbial dysbiosis in a mouse model of parkinson's disease. *Brain Behav. Immun.* 89, 641–655. doi: 10.1016/j.bbi.2020.08.015

Karlsson, F. H., Tremaroli, V., Nookaew, I., Bergström, G., Behre, C. J., Fagerberg, B., et al. (2013). Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature* 498, 99–103. doi: 10.1038/nature12198

Kasperska-Zajac, A., Grzanka, A., and Damasiewicz-Bodzek, A. (2015). IL-6 transsignaling in patients with chronic spontaneous urticaria. *PloS One* 10, e0145751. doi: 10.1371/journal.pone.0145751

Katz-Agranov, N., and Zandman-Goddard, G. (2017). The microbiome and systemic lupus erythematosus. *Immunol. Res.* 65, 432–437. doi: 10.1007/s12026-017-8906-2

Kemp, P. F., and Aller, J. Y. (2004). Bacterial diversity in aquatic and other environments: what 16S rDNA libraries can tell us. *FEMS Microbiol. Ecol.* 47, 161–177. doi: 10.1016/S0168-6496(03)00257-5

Kolkhir, P., Altrichter, S., Hawro, T., and Maurer, M. (2018). C-reactive protein is linked to disease activity, impact, and response to treatment in patients with chronic spontaneous urticaria. *Allergy* 73, 940–948. doi: 10.1111/all.13352

Kolkhir, P., Muñoz, M., Asero, R., Ferrer, M., Kocatürk, E., Metz, M., et al. (2022). Autoimmune chronic spontaneous urticaria. *J. Allergy Clin. Immunol.* 149, 1819–1831. doi: 10.1016/j.jaci.2022.04.010

Lapillonne, A., Pastor, N., Zhuang, W., and Scalabrin, D. M. (2014). Infants fed formula with added long chain polyunsaturated fatty acids have reduced incidence of respiratory illnesses and diarrhea during the first year of life. *BMC Pediatr.* 14, 168. doi: 10.1186/1471-2431-14-168

Liu, R., Peng, C., Jing, D., Xiao, Y., Zhu, W., Zhao, S., et al. (2021). Biomarkers of gut microbiota in chronic spontaneous urticaria and symptomatic dermographism. *Front. Cell Infect. Microbiol.* 11, 703126. doi: 10.3389/fcimb.2021.703126

Liu, R., Peng, C., Jing, D., Xiao, Y., Zhu, W., Zhao, S., et al. (2022). Lachnospira is a signature of antihistamine efficacy in chronic spontaneous urticaria. *Exp. Dermatol.* 31, 242–247. doi: 10.1111/exd.14460

Lu, T., Chen, Y., Guo, Y., Sun, J., Shen, W., Yuan, M., et al. (2019). Altered gut microbiota diversity and composition in chronic urticaria. *Dis. Markers* 2019, 6417471. doi: 10.1155/2019/6417471

Luu, M., Riester, Z., Baldrich, A., Reichardt, N., Yuille, S., Busetti, A., et al. (2021). Microbial short-chain fatty acids modulate CD8(+) T cell responses and improve adoptive immunotherapy for cancer. *Nat. Commun.* 12, 4077. doi: 10.1038/s41467-021-24331-1

Miles, E. A., Childs, C. E., and Calder, P. C. (2021). Long-chain polyunsaturated fatty acids (LCPUFAs) and the developing immune system: A narrative review. *Nutrients* 13(1), 247. doi: 10.3390/nu13010247

Moon, H. G., Tae, Y. M., Kim, Y. S., Gyu Jeon, S., Oh, S. Y., Song Gho, Y., et al. (2010). Conversion of Th17-type into Th2-type inflammation by acetyl salicylic acid *via* the adenosine and uric acid pathway in the lung. *Allergy* 65, 1093–1103. doi: 10.1111/j.1398-9995.2010.02352.x

Nabizadeh, E., Jazani, N. H., Bagheri, M., and Shahabi, S. (2017). Association of altered gut microbiota composition with chronic urticaria. *Ann. Allergy Asthma Immunol.* 119, 48–53. doi: 10.1016/j.anai.2017.05.006

Paller, A. S., Kong, H. H., Seed, P., Naik, S., Scharschmidt, T. C., Gallo, R. L., et al. (2019). The microbiome in patients with atopic dermatitis. *J. Allergy Clin. Immunol.* 143, 26–35. doi: 10.1016/j.jaci.2018.11.015

Saini, S. S. (2014). Chronic spontaneous urticaria: etiology and pathogenesis. *Immunol. Allergy Clin. North Am.* 34, 33–52. doi: 10.1016/j.iac.2013.09.012

Savage, J. H., Lee-Sarwar, K. A., Sordillo, J., Bunyavanich, S., Zhou, Y., O'connor, G., et al. (2018). A prospective microbiome-wide association study of food sensitization and food allergy in early childhood. *Allergy* 73, 145–152. doi: 10.1111/all.13232

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., et al. (2011). Metagenomic biomarker discovery and explanation. *Genome Biol.* 12, R60. doi: 10.1186/gb-2011-12-6-r60

Smith, P. M., Howitt, M. R., Panikov, N., Michaud, M., Gallini, C. A., Bohlooly, Y. M., et al. (2013). The microbial metabolites, short-chain fatty acids, regulate colonic treg cell homeostasis. *Science* 341, 569–573. doi: 10.1126/science.1241165

Stepaniuk, P., Kan, M., and Kanani, A. (2020). Natural history, prognostic factors and patient perceived response to treatment in chronic spontaneous urticaria. *Allergy Asthma Clin. Immunol.* 16, 63. doi: 10.1186/s13223-020-00459-5

Tedeschi, A., Asero, R., Lorini, M., Marzano, A. V., and Cugno, M. (2010). Plasma levels of matrix metalloproteinase-9 in chronic urticaria patients correlate with disease severity and c-reactive protein but not with circulating histamine-releasing factors. *Clin. Exp. Allergy* 40, 875–881. doi: 10.1111/j.1365-2222.2010.03473.x

Tedeschi, A., Kolkhir, P., Asero, R., Pogorelov, D., Olisova, O., Kochergin, N., et al. (2014). Chronic urticaria and coagulation: pathophysiological and clinical aspects. *Allergy* 69, 683–691. doi: 10.1111/all.12389

Tito, R. Y., Cypers, H., Joossens, M., Varkas, G., Van Praet, L., Glorieus, E., et al. (2017). Brief report: Dialister as a microbial marker of disease activity in spondyloarthritis. *Arthritis Rheumatol* 69, 114–121. doi: 10.1002/art.39802

Wang, D., Guo, S., He, H., Gong, L., and Cui, H. (2020). Gut microbiome and serum metabolome analyses identify unsaturated fatty acids and butanoate metabolism induced by gut microbiota in patients with chronic spontaneous urticaria. *Front. Cell Infect. Microbiol.* 10, 24. doi: 10.3389/fcimb.2020.00024

Wang, X., Yi, W., He, L., Luo, S., Wang, J., Jiang, L., et al. (2021). Abnormalities in gut microbiota and metabolism in patients with chronic spontaneous urticaria. *Front. Immunol.* 12, 691304. doi: 10.3389/fimmu.2021.691304

West, C. E., Rydén, P., Lundin, D., Engstrand, L., Tulic, M. K., and Prescott, S. L. (2015). Gut microbiome and innate immune response patterns in IgE-associated eczema. *Clin. Exp. Allergy* 45, 1419–1429. doi: 10.1111/cea.12566

Yu, M., Cui, F. X., Jia, H. M., Zhou, C., Yang, Y., Zhang, H. W., et al. (2016). Aberrant purine metabolism in allergic asthma revealed by plasma metabolomics. *J. Pharm. BioMed. Anal.* 120, 181–189. doi: 10.1016/j.jpba.2015.12.018

Yüksekal, G., Sevimli Dikicier, B., Koku Aydın, B., Yılmaz, K., Altındiş, M., and Köroğlu, M. (2022). Investigation of intestinal microbiome in chronic spontaneous urticaria patients. *Int. J. Dermatol.* 61, 988–994. doi: 10.1111/ijd.16054

Zafar, H., and Saier, M. H.Jr. (2021). Gut bacteroides species in health and disease. *Gut Microbes* 13, 1–20. doi: 10.1080/19490976.2020.1848158

Zhang, X., Zhang, J., Chu, Z., Shi, L., Geng, S., and Guo, K. (2021). Gut microbiome alterations and functional prediction in chronic spontaneous urticaria patients. *J. Microbiol. Biotechnol.* 31, 747–755. doi: 10.4014/jmb.2012.12022

Zheng, H., Liang, H., Wang, Y., Miao, M., Shi, T., Yang, F., et al. (2016). Altered gut microbiota composition associated with eczema in infants. *PloS One* 11, e0166026. doi: 10.1371/journal.pone.0166026

Zuberbier, T., Abdul Latiff, A. H., Abuzakouk, M., Aquilina, S., Asero, R., Baker, D., et al. (2022). The international EAACI/GA²LEN/EuroGuiDerm/APAAACI guideline for the definition, classification, diagnosis, and management of urticaria. *Allergy* 77, 734–766. doi: 10.1111/all.15090

frontiers | Frontiers in Cellular and Infection Microbiology

TYPE Original Research
PUBLISHED 12 January 2023
DOI 10.3389/fcimb.2022.1095053

OPEN ACCESS

EDITED BY
Chuanxing Xiao,
Xiamen University, China

REVIEWED BY
Weijun Kong,
Capital Medical University, China
Jiyu Zhang,
Lanzhou Institute of Husbandry and Pharmaceutical Sciences, Chinese Academy of Agricultural Sciences, China
Li Yan Hua,
Shanxi Datong University, China

*CORRESPONDENCE
Jin-Fa Tang
a0519@163.com

SPECIALTY SECTION
This article was submitted to Intestinal Microbiome, a section of the journal Frontiers in Cellular and Infection Microbiology

RECEIVED 10 November 2022
ACCEPTED 20 December 2022
PUBLISHED 12 January 2023

CITATION
Zhang M-L, Li W-X, Wang X-Y, Wu Y-L, Chen X-F, Zhang H, Yang L-Q, Wu C-Z, Zhang S-Q, Chen Y-L, Feng K-R, Wang B, Niu L, Kong D-X and Tang J-F (2023) Oxymatrine ameliorates experimental autoimmune encephalomyelitis by rebalancing the homeostasis of gut microbiota and reducing blood-brain barrier disruption. *Front. Cell. Infect. Microbiol.* 12:1095053.
doi: 10.3389/fcimb.2022.1095053

Oxymatrine ameliorates experimental autoimmune encephalomyelitis by rebalancing the homeostasis of gut microbiota and reducing blood-brain barrier disruption

Ming-Liang Zhang[1,2], Wei-Xia Li[1,2], Xiao-Yan Wang[1,2], Ya-Li Wu[1], Xiao-Fei Chen[1], Hui Zhang[1], Liu-Qing Yang[1], Cheng-Zhao Wu[3], Shu-Qi Zhang[1], Yu-Long Chen[4], Ke-Ran Feng[3], Bin Wang[4], Lu Niu[1], De-Xin Kong[4] and Jin-Fa Tang[1,2,4]*

[1]Department of Pharmacy, the First Affiliated Hospital of Henan University of Chinese Medicine, Zhengzhou, China, [2]Henan Province Engineering Research Center of Clinical Application, Evaluation and Transformation of Traditional Chinese Medicine, Henan Provincial Key Laboratory for Clinical Pharmacy of Traditional Chinese Medicine, Zhengzhou, China, [3]Chengdu University of Chinese Medicine, Chengdu, China, [4]School of Pharmacy, Henan University of Chinese Medicine, Zhengzhou, China

Background: Increasing evidence suggests that gut dysbiosis can directly or indirectly affect the immune system through the brain-gut axis and play a role in the occurrence and development of Multiple sclerosis (MS). Oxymatrine (OMAT) has been shown to ameliorate the symptoms of MS in the classical experimental autoimmune encephalomyelitis (EAE) model of MS, but whether its therapeutic role is through the correction of gut dysbiosis, is unclear.

Methods: The effects of OMAT on intestinal flora and short-chain fatty acids in EAE model mice were evaluated by 16S rRNA sequencing and GC-MS/MS, respectively, and the function change of the blood-brain barrier and intestinal epithelial barrier was further tested by immunohistochemical staining, Evans Blue leakage detection, and RT-qPCR.

Results: The alpha and beta diversity in the feces of EAE mice were significantly different from that of the control group but recovered substantially after OMAT treatment. Besides, the OMAT treatment significantly affected the gut functional profiling and the abundance of genes associated with energy metabolism, amino acid metabolism, the immune system, infectious diseases, and the nervous system. OMAT also decreased the levels of isobutyric acid and isovaleric acid in EAE mice, which are significantly related to the abundance of certain gut microbes and were consistent with the reduced expression of TNF-a, IL-6, and IL-1b. Furthermore, OMAT treatment

significantly increased the expression of ZO-1 and occludin in the brains and colons of EAE mice and decreased blood-brain barrier permeability.

Conclusion: OMAT may alleviate the clinical and pathological symptoms of MS by correcting dysbiosis, restoring gut ecological and functional microenvironment, and inhibiting immune cell-mediated inflammation to remodel the brain-gut axis.

KEYWORDS

oxymatrine, experimental autoimmune encephalomyelitis, gut microbiota, brain-gut axis, short-chain fatty acids

1 Introduction

Multiple sclerosis (MS) as chronic neuroinflammation and demyelinating disease in the central nervous system (CNS), is associated with key pathological features: multifocal inflammation, disseminated demyelination, axonal loss, and neurological disability (Thompson et al., 2018; Xu et al., 2020). For the treatment of MS, recent studies have found that oral probiotics can significantly ameliorate the incidence of MS (Tankou et al., 2018), while transplantation of fecal bacteria from MS patients can aggravate the incidence of its classical animal model (experimental autoimmune encephalomyelitis, EAE) mice (Berer et al., 2017; Cekanaviciute et al., 2017), suggesting therapeutic targeting of the gut microbiota as a treatment for MS (Leprun and Clarke, 2019), which aroused great interest in the study of the brain-gut axis. With the growing evidence of gut microbiota disorder in MS patients, the mechanism of gut microbiota disorder exacerbating MS is gradually clear. The current main view is that the disordered gut microbiota mediates the inflammatory immune response of CNS by changing the permeability of the intestinal epithelial barrier (IEB) and blood-brain barrier (BBB) and affecting the expression of neurotransmitters related to the brain-gut axis in MS patients or EAE mice (Preziosi et al., 2013; Chu et al., 2018; Stanisavljevic et al., 2018). Therefore, researching and developing gut microbiota-modifying therapies, such as drugs with antibacterial and immunomodulatory effects may be extremely urgent for the treatment of MS.

Significant progress has been made in therapeutic strategies for MS, including cladribine, dimethyl fumarate, fingolimod, teriflunomide, alemtuzumab, and ocrelizumab. However, long-term use of MS medications is costly and may cause certain side effects, such as liver injury, lymphopenia, and infections (Antonazzo et al., 2019; Moiola et al., 2020). Importantly, recent studies have found that some types of traditional herbal medicine can ameliorate clinical severity and reduce the frequency of clinical exacerbations in relapsing MS with fewer side effects and minor financial burdens (Kou et al., 2014; Zhou and Fan, 2015; Dou et al., 2021; Zha et al., 2021). Therefore, natural products may be a promising treatment for MS.

Sophora flavescens Ait. (Leguminosae), as traditional herb medicine, contains an important component namely oxymatrine (OMAT), which has been reported to exhibit significant anti-tumor, anti-virus, hepatoprotective, and immunomodulatory effects (Rashid et al., 2019; Zhang et al., 2020; Li et al., 2021). OMAT has been tested in a clinical trial for the treatment of human hepatitis B, with significant therapeutic effects and without noticeable side effects (Zhang et al., 2016). Our previous studies have found that OMAT could delay the development of the EAE and modulate immune responses (Liu et al., 2014; Zhang et al., 2017). It is reported in the literature that OMAT has a certain regulatory effect on the gut microbiota and intestinal barrier (Li et al., 2019; Wu et al., 2021), whether it treats MS by remodeling gut microbiota homeostasis and IEB function is not clear. Herein, we investigated the mechanisms of OMAT on the function of the IEB and BBB, and the regulating effect on intestinal microecology in the EAE mice.

2 Materials and methods

2.1 Animals and EAE induction

C57BL/6 female mice, 8-10 weeks of age (18-20 g), were obtained from SPF Biotechnology Co., Ltd. (Beijing, China), and housed in specific pathogen-free conditions at the Fifth Medical Center of PLA General Hospital (animal ethics committee approval No. YFYDW2020017), Beijing, China. All efforts were made to reduce animal suffering, and the Institutional Committee on Care and Use of Research animals approved the procedures used in this study. EAE was induced as described previously (Dou et al., 2021). Inactivated Mycobacterium tuberculosis (#231141, Difco) was added with Freund's

incomplete adjuvant (#F5506, Sigma) to prepare a 10 mg/mL solution, which was fully mixed with an equal volume of myelin oligodendrocyte glycoprotein peptide (MOG35-55, MEVGWYRSPFS RVVHLYRNGK; GenScript) solution (2 mg/mL) dissolved in sterile phosphate-buffered saline (PBS) to prepare water in oil antigen emulsion. Mice anesthetized with pentobarbital at four points on both sides of the midline of the head and back, a total of 0.2 mL/mouse. On 0 h and 48 h post-immunization (p.i.), 200 ng/mouse pertussis toxin (#180, List Biological) was injected intraperitoneally.

2.2 MAT treatment and clinical evaluation

Immunized mice were randomly divided into two groups (n=9 each group), and starting from day 11 p.i., 40 mg/kg/day OMAT (Macklin Biochemical Co., Ltd, Shanghai, China) or equal volumes of normal saline were administered in immunized mice by oral served as the EAE_OMAT group or EAE group, respectively. Non-immunized naive mice (n=9 in each group) received the same volumes of normal saline or OMAT served as the CON group or CON_OMAT group, respectively.

The mice were monitored and weighed daily by two independent observers to evaluate the clinical scores of EAE. Neurological assessments were recorded with a five-point standardized rating scale (Dou et al., 2021): 0, no deficit; 1, tail paralysis; 2, incomplete hind limb paralysis; 3, complete hind limb paralysis; 4, complete hind limb paralysis and partial forelimb paralysis; 5, moribund state or death.

2.3 Histopathological evaluation

On day 20 post-immunization (p.i.), mice were sacrificed, colons and spinal cords were harvested after extensive perfusion with saline, embedded in paraffin, and processed for histological evaluation, and fecal samples were collected in a sterile operating table in a sterile cryopreservation tube and placed in a refrigerator at -80°C for standby. Inflammatory infiltration in lumbar enlargement of spinal cords and colons was determined by Hematoxylin and Eosin (HE) staining, and demyelination in lumbar enlargement of spinal cords was determined by Luxol Fast Blue (LFB) staining.

2.4 Evans Blue leakage detection

The BBB permeability was determined by Evans Blue (EB) dye (Jiao-Yan et al., 2021). Mice were given 2% EB (2 mL/kg) *via* tail vein injection 90 minutes before being sacrificed. After weighing the brain, 50% trichloroacetic acid was added in the ratio of 1:3 (W/V) to make homogenate and centrifuged (12000 g. 15 minutes). The supernatant was collected, and its absorbance at 620 nm was measured by a microporous plate spectrophotometer to calculate EB transmittance.

2.5 Microbiota sequencing

Total bacterial DNA was extracted from fresh stool samples by the E.Z.N.A.® Stool DNA Kit (Omega Bio-Tek, U.S). The DNA in stools was quantified through agarose gel electrophoresis and amplified V3-V4 variable regions of the 16S rRNA gene with specific primers (338F: 5'-ACTCCTACGGGAGGCAGCAG-3' and 806R: 5'-GGACTACHVGGGTWTCTAAT-3'). To evaluate alpha-diversity, the levels of OTUs for each group. and the alpha diversity indices, including Ace, Chao, Coverage, Shannon, Simpson, and sobs index, are usually used for evaluating the community richness and community diversity. To analyze beta-diversity, principal coordinates analysis (PCA) analysis which is a phylogenic tree-based metric was used (Calderón et al., 2017). Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) was further used for genome prediction of microbial communities in this study.

2.6 Quantification of fecal short-chain fatty acids

SCFAs, including acetic acid (AA), propionic acid (PA), isobutyric acid (IBA), butyric acid (BA), isovaleric acid (IVA), valeric acid (VA), and hexanoic acid (HA), were extracted and analyzed by gas chromatography-mass spectrometry/mass spectrometry (GC-MS/MS) according to the protocol described in Margareta Nyman (Zhao et al., 2006). Accurately weigh the sample, add 0.5% phosphoric acid solution, grind it evenly by ball mill, vortex and mix it evenly, ultrasonic in the ice bath for 5 minutes, 4°C, 12000 rpm, centrifuge for 10 minutes, and take 100 μL supernatant and 500 μL methyl tert-butyl ether (MTBE) solvent containing internal standard, vortex for 3 minutes, ultrasonic for 5 minutes under ice bath, centrifuge for 10 minutes at 12000 rpm at 4°C, take the supernatant and store it in the refrigerator at - 80°C for standby. The supernatant was collected and used for GC-MS/MS analysis. Agilent 7890B-7000D was employed for GC-MS/MS analysis of SCFAs.

2.7 Immunohistochemistry analysis

On day 20 p.i., mice were deeply anesthetized with 2% pentobarbital sodium and extensively perfused by normal saline. Then lumbar spinal cords, brain, and colon were

immediately removed and fixed in 4% paraformaldehyde and serial cryostat longitudinal sections were cut at a 5 μm thickness for immunohistochemistry analysis. After deparaffinization in xylol, sections were transferred to gradient ethanol and then incubated with 3% H_20_2 for 30 minutes at room temperature. After washing in PBS, non-specific binding was blocked with bovine serum for 30 minutes at 37°C and incubated with anti-occludin, anti-zonula occludens-1 (anti-ZO-1) (Santa Cruz Biotechnology, Dallas, TX, USA) at 4°C overnight. Sections were washed again and then incubated with corresponding secondary antibodies at 37°C for 30 minutes. The chromophore product was developed using Horseradish Peroxidase-Streptavidin (HRP-Streptavidin) and 3,3N-Diaminobenzidine Tertrahydrochloride (DAB) (Beijing Zhongsan Biotech Co, LTD, Beijing, China). Positive cells in a restricted area were determined to represent target protein expression.

2.8 Quantitative reverse transcription-polymerase chain reaction

The cervical parts of the spinal cord and colon were harvested and flash-frozen in liquid N2 on day 20 p.i., and then stored at -80°C before use. Total RNA was isolated by RNA-Quick Purification Kit according to the manufacturer's instructions (ES Science, RN001, China), and cDNA was synthesized using reverse transcriptase (RT) by Fast All-in-One RT Kit (ES Science, RT001, China) followed by RT-qPCR (Thermal Cycler Device Real-Time System, Takara) using 2×Super SYBR Green qPCR Master Mix (ES Science, QP002, China) with appropriate primers and probe (Table 1). The cycle threshold (Ct) values of TNF-α, IL-6, IL-1β, ZO-1, and occludin were obtained and normalized to that of GAPDH (all reagents from TIANYI HUIYUAN, Beijing, China). Based on the expression of target genes normalized to GAPDH, we calculated and presented the relative quantification to GAPDH as fold change compared to the control. The above gene sequence information can be queried in Supplementary Table 1 (SI Appendix, Table S1).

2.9 Statistical analysis

All statistical analyses were conducted with R version 3.3.1 or GraphPad Prism 8.0 (Inc., La Jolla, CA, USA). Significant differences were evaluated using the two-tailed Student's t-test or Wilcoxon test, except that multiple treatment groups were compared within individual experiments by the Wilcoxon rank-sum test or LSD-t test. The relevance between the abundance of microbial genera/OTUs and neurologic score or the levels SFCAs was performed by using spearman's rank correlation coefficient. The ranking regression of environmental factors was analyzed according to beta diversity. All data were presented as mean ± SD. $P<0.05$ was considered significant.

3 Results

3.1 OMAT treatment ameliorates the clinical signs of ongoing EAE

As shown in Figure 1A, EAE group mice had the first signs of EAE on day 11 p.i., EAE_OMAT group mice showed a significant decrease in the severity of EAE from the 15th-day p.i. as compared with EAE group mice (all $P<0.05$). On day 20 p.i., EAE group mice were observed unilateral hind limb paralysis or bilateral hind limb paralysis, while EAE_OMAT group mice were observed tail paralysis or incomplete hind limb paralysis (Figure 1B). Consistent with clinical scores, the histopathological analysis revealed less inflammation (Figure 1C) and demyelination (Figure 1D) in the lumbar spinal cords samples from EAE_OMAT group mice. Furthermore, histopathology revealed milder crypt structure change and less inflammatory infiltration in the colons of CON_OMAT and EAE group mice, the inflammatory and crypt structure change in the OMAT-treated EAE mice has been distinctly ameliorated than EAE group mice (Figure 1E).

3.2 OMAT administration altered the alpha- and beta-diversities of the gut microbiome in EAE mice

From the Venn diagram in OUT levels (Figure 2A), 591 species in the CON group, 584 species in the CON_OMAT group, 388 species in the EAE group, and 586 species in the EAE_OMAT group were observed. The alpha diversity indices, including Ace, Chao, Coverage, Shannon, Simpson, and Sobs index, are usually used for evaluating community richness and community diversity (Figures 2B–G). In this study, Ace, Chao, Shannon, and sobs values in the EAE_OMAT-treated mice were markedly higher than that in the EAE group (all $P<0.05$), while coverage and Simpson values in the EAE_OMAT-treated mice were markedly lower than that in the EAE group (all $P<0.05$). In addition, beta-diversity in the four groups was evaluated by Bray-Curtis analysis (Heatmap diagram) and Principal coordinates analysis (PCoA) using the 16S data. According to the distance and separation of each sample in each group in Figures 2H–M, it is evident that the CON, CON_OMAT, and EAE_OMAT are completely separated from the EAE group in OUT (Figures 2H, K), phylum (Figures 2I, L) and genus (Figures 2J, M) levels, and there are differences between the EAE_OMAT group and the CON group in genus and OUT levels (all $P<0.05$).

10.3389/fcimb.2022.1095053

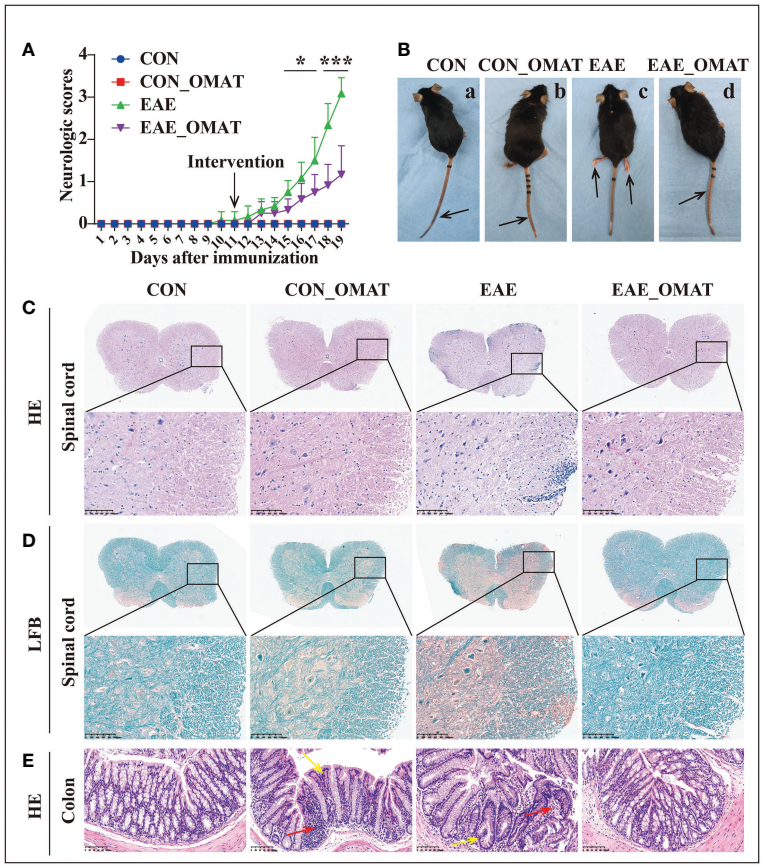


FIGURE 1
Results of the pathological and histological examination of the spinal cord and colon of the mice from the four groups (n = 6 in each group). **(A)** Clinical scores and **(B)** clinical phenotypes of mice from the different groups. The black arrow indicates the paralyzed area. a, CON group mice with no deficit; b, CON_EAE group mice with no deficit; c, EAE group mice showing paralysis of both hind limbs; d, EAE_OMAT group mice showing tail paralysis. **(C, D)** Representative images of the histology of the spinal cord were determined by HE **(C)** and LFB **(D)** staining. Original magnification, 4X; the partial enlarged picture of each group was magnified 20X. **(E)** Representative images of the histology of the colon determined by HE staining (20X). Red arrows indicate colon tissue infiltration by inflammatory cells, yellow arrows indicate the change in crypt structure. Data are expressed as the mean ± SD and statistical differences are represented by **, P < 0.05; ***, P < 0.001* based on Wilcoxon rank-sum test.

3.3 OMAT treatment influenced the gut microbial abundance and phenotypes in EAE mice

Microbial abundance at different taxonomic levels was further analyzed. The phylum-level assignment identified 10 phyla with an average relative abundance of ≥0.1% in at least one of the four groups. The comparison between all pairs of the four groups identified a total of 6 distinct phyla having significant changes in abundance between any two groups (SI Appendix, Table S2), and identified the abundance of Deferribacterota, Cyanobacteria, and Actinobacteriota significantly increased and Proteobacteria significantly decreased after OMAT-treated in EAE mice (all *P<0.05*) (Figure 3A).

The genus-level assignment identified 42 genera with an average relative abundance of ≥0.5% in at least one of the four groups, accounting for 93.03% of the total abundance. The comparison between all pairs of the four groups identified a total of 30 distinct genera having significant changes in abundance between any two groups (SI Appendix, Table S3). Sorted by fold changes (FCs, $|LOG_{10}$ (FCs)$|>1$) value and P-value (*P<0.05*), the abundance of Alistipes, Helicobacter, Dubosiella, norank_f:norank_o:Clostridia_UCG-014, Rikenellaceae_RC9_gut_group, Prevotellaceae_NK3B31_group were significantly higher in EAE_OMAT mice than EAE mice (all *P<0.05*), while the abundance of Clostridioides, Proteus, Clostridium_innocuum_group, Enterococcus, Escherichia-Shigella, Enterobacter, Lactococcus were significantly lower in EAE_OMAT group than in EAE group (Figure 3B).

To explore the possible association of microbial taxa with each patient group at the species level, the abundance of 38 species-equivalent clusters with an average relative abundance of ≥0.1% in at least one of the four groups were further compared, accounting for 17.79% (CON group), 19.40% (CON_OMAT

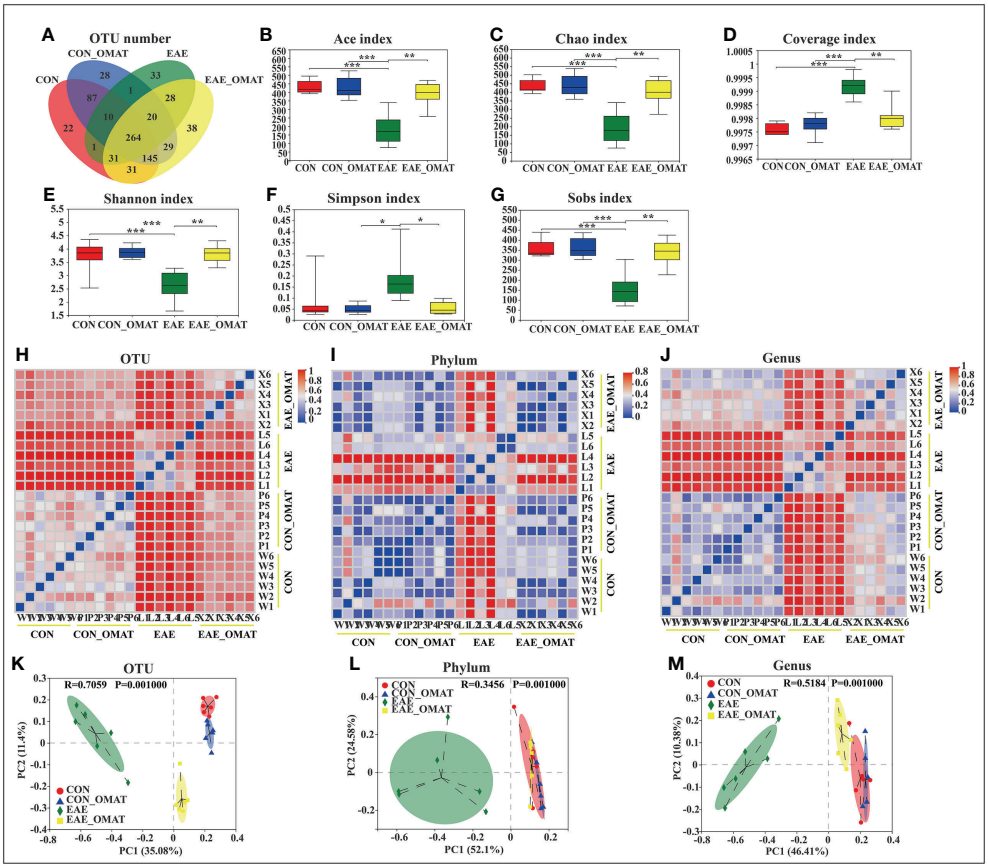


FIGURE 2
Gut microbiota alpha- and beta-diversities in the four mice groups (n = 6 in each group). **(A–D)** Alpha-diversity indices. **(A)** The number of species and **(B)** Ace-estimated OTUs, **(C)** the Chao index value **(D)** the Coverage index value **(E)** the Shannon index value **(F)** the Simpson index value, and **(G)** the Sobs index value of fecal microbiota from the four groups based on 16S rRNA analysis. Each box plot represents the median, interquartile range, minimum, and maximum values. **(H–M)** Beta-diversity indices. Sample-distance heatmap based on weighted UniFrac analysis at the level of OTUs **(H)**, phylum **(I)**, and genus **(J)**. Principal coordinates analysis (PCoA) was used to study the similarity or difference of the microbial community composition at the level of OTUs **(K)**, phylum **(L)**, and genus **(M)**. Data are expressed as the mean ± SD and statistical differences are represented by *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ based on the Wilcoxon rank-sum test with the Benjamini–Hochberg method for multiple group comparisons.

group), 68.30% (EAE group), 31.88% (EAE_OMAT group) of the total abundance, respectively. The comparison between all pairs of the four groups identified a total of 28 distinct species having significant changes in abundance between any two groups (SI Appendix, Table S4). Sorted by fold changes (FCs, $|LOG_{10}(FCs)|>1$) value and P-value ($P<0.05$), the abundance of Mucispirillum_schaedleri was significantly higher in the EAE_OMAT group than in the EAE group (all $P<0.05$), while the abundance of Clostridioides_difficile_g:Clostridioides, Clostridium_perfringens_g:Clostridium_sensu_stricto_1, Proteus_vulgaris_g:Proteus, Enterococcus_faecalis_g:Enterococcus, Enterococcus_casseliflavus_g:Enterococcus, Escherichia_coli_g:Escherichia-Shigella, Erysipelatoclostridium_ramosum were significantly lower in EAE_OMAT group than in EAE group (Figure 3C).

Considering various taxonomic levels of gut microflora have changed, the corresponding microflora phenotype will also change. The study identified 9 phenotypes in at least one of the four groups including 6 distinct phenotypes having significant changes in proportion between any two groups by BugBase analysis according to the data obtained from sequencing of 16S analysis (SI Appendix, Table S5). To investigate the therapeutic effect of OMAT on EAE mice, we focused on the functional difference between the EAE group and the EAE_OMAT group. The results indicated the proportion of Facultatively_Anaerobic, Stress_Tolerant, and Potentially_Pathogenic were significantly decreased after OMAT-treated in EAE mice (all $P<0.05$), while the proportion of Aerobic was significantly increased after OMAT-treated in EAE mice ($P<0.05$) (Figure 3D).

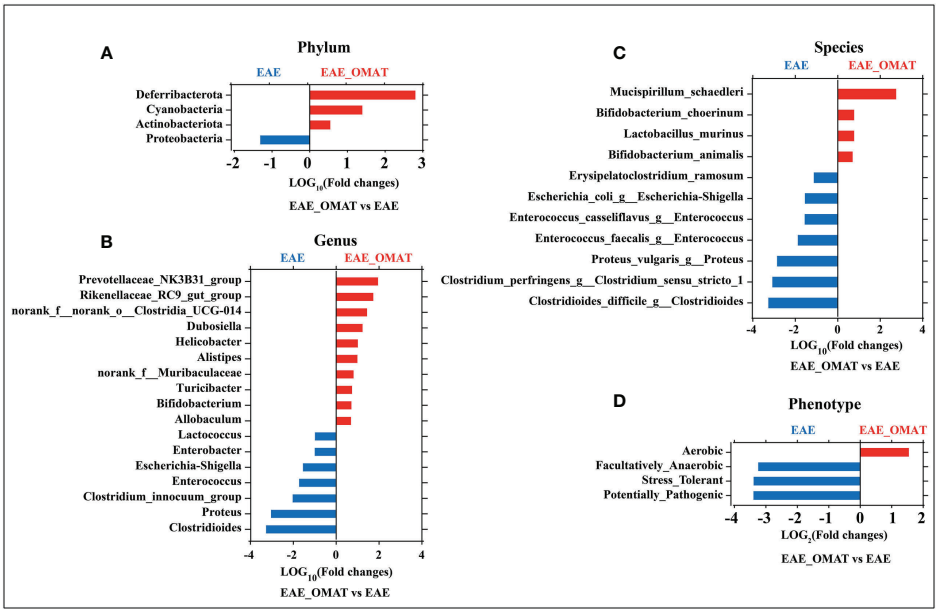


FIGURE 3
Effect of oxymatrine treatment on the gut microbial abundance and microbiome phenotypes in EAE mice. The fold-change (LOG_2 or LOG_{10}) of relative abundance at the level of phylum **(A)**, genus **(B)**, species and **(C)**, phenotype **(D)** between the EAE group and EAE_OMAT groups ($P < 0.05$). The Z-score based on the abundance of each KEGG ortholog is depicted from lowest (blue) to highest (red) according to the scale shown at the top. The P value was determined based on the Wilcoxon rank-sum test with the Benjamini–Hochberg method for multiple group comparisons.

3.4 Association between significantly increased or decreased microbial genera/OTUs in EAE-related groups and neurologic function scores

The possible association between the microbial species and disease activity (neurologic function scores) was further analyzed by comparing the abundance of the 30 genera/OTUs which existed significant differences between the EAE group and EAE_OMAT group. The linear relationship strength and direction are classified according to the correlation coefficient R-value (Rumsey, 2016). As Figure 4 showed, Clostridium_innocuum_group, Enterococcus, Proteus, Enterobacter, and Escherichia-Shigella had strong uphill linear relationships in abundance between the neurological function score and EAE-related samples (R=0.84, 0.83, 0.80, 0.77, 0.74, respectively), while Ileibacterium, Dubosiella, norank_f: Muribaculaceae had strong downhill linear relationships in abundance between the neurological function score and EAE-related samples (R=-0.78, -0.80, -0.85, respectively). Meanwhile, the abundance of Lactococcus, Clostridioides, Clostridium_sensu_stricto_1 and disease severity correlated positively (R=0.68, 0.65, 0.56, respectively), and Odoribacter, Helicobacter, Desulfovibrio, Enterorhabdus, Faecalibaculum, Rikenellaceae_RC9_gut_group, Muribaculum, norank_f: norank_o:Clostridia_UCG-014, Allobaculum and disease severity were negatively related to (R=-0.53, -0.55, -0.62, -0.63, -0.63, -0.65, -0.67, -0.67, -0.68, respectively). (SI Appendix, Table S6).

3.5 OMAT altered the functional profiling of the gut microbiomes

Based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Kanehisa et al., 2019), a total of 201 KEGG orthologs (KOs) in the four subject groups were identified by PICRUSt (SI Appendix, Table S7). Of them, 1 had significant changes in abundance between the CON group and CON_OMAT group, 95 had significant changes in abundance between the CON group and the EAE group, and 97 had significant changes in abundance between the EAE group and EAE_OMAT group. In the comparison of EAE and EAE_OMAT, we identified 54 significantly enriched and 43 significantly depleted KOs in EAE_OMAT with $P<0.05$ by Wilcoxon test, respectively (Figure 5A). Sorted by P-value ($P<0.05$) and FCs value ($|LOG_2$ (FCs)$|>1$), we picked out 21 KOs (Figure 5B). Among these pathways, 6 pathways including influenza A (KO05164), pertussis (KO05133), bacterial invasion of epithelial cells (KO05100), prion diseases (KO05020), viral myocarditis (KO05416), and flagellar assembly (KO02040) were involved in Bacterial or Viral Infection; 3 pathways including colorectal cancer (KO05210), small cell lung cancer (KO05222), and bladder cancer (KO05219) were involved in Cancer; 4 pathways including atrazine degradation (KO00791), drug metabolism-cytochrome P450 (KO00982), metabolism of xenobiotics by cytochrome P450 (KO00980), and caprolactam degradation

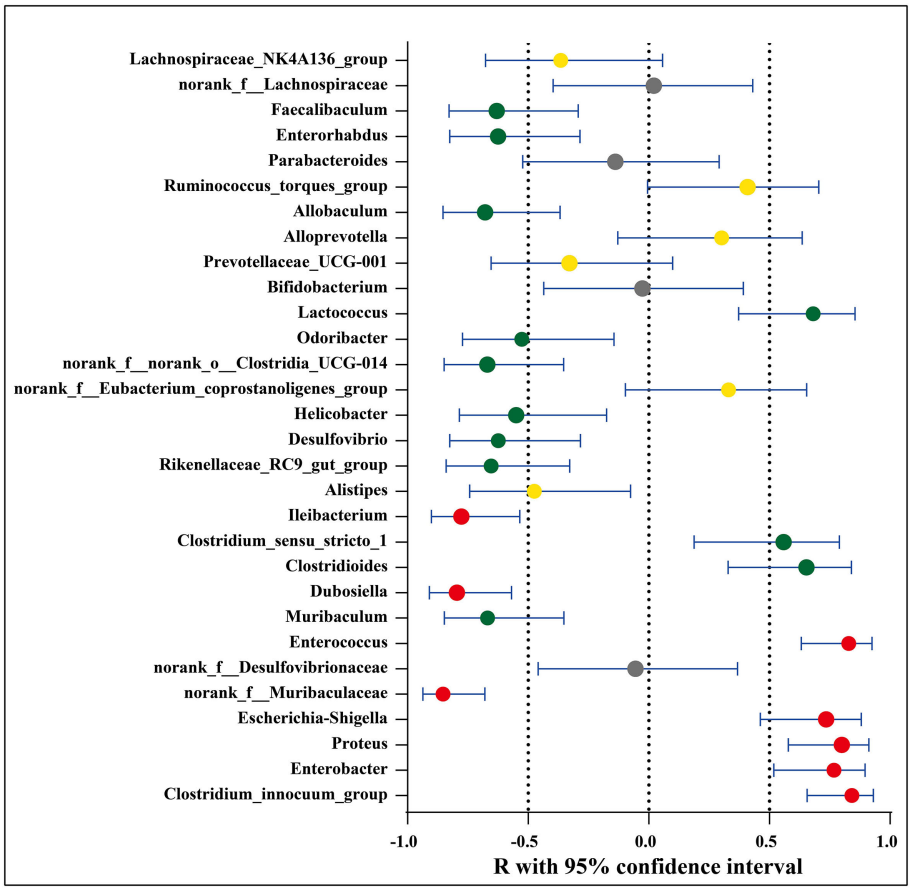


FIGURE 4
Association between significantly increased or decreased microbial genera/OTUs in EAE groups and corresponding neurologic function scores. Red dots represent a strong correlation (0.7 ≤ R < 1), green dots represent a moderate correlation (0.5 ≤ R < 0.7), yellow dots represent a weak correlation (0.3 ≤ R < 0.5), and gray dots represent a poor correlation (0 < R < 0.3). The relation between the abundance of microbial genera/OTUs and the neurologic function scores were performed using Spearman's rank correlation coefficient.

(KO00930) were involved in Xenobiotics Biodegradation and Metabolism; 2 pathways including ether lipid metabolism (KO00565), and Alpha-Linolenic Acid Metabolism (KO00592) were involved in Lipid Metabolism; 2 pathways including geraniol degradation (KO00281), and biosynthesis of siderophore group nonribosomal peptides (KO01053) were involved in Metabolism Of Terpenoids and Polyketides; 2 pathways including toxoplasmosis (KO05145), and amoebiasis (KO05146) were involved in Parasitic Infection; 1 pathways including N-Glycan biosynthesis (KO00510) was involved in Glycan Biosynthesis and Metabolism; 1 pathways including flavonoid biosynthesis (KO00941) was involved in Biosynthesis Of Other Secondary Metabolites. The study only showed the four metabolic pathways with large changes in relative abundances, such as pertussis (Figure 5C), bacterial invasion of epithelial cells (Figure 5D), α- Linolenic acid metabolism (Figure 5E), flavonoid biosynthesis (Figure 5F).

3.6 OMAT rebalanced the relative abundance of three major nutrients, the nervous system, immune system, and infectious diseases based on KEGG analysis

Metabolism pathways related to energy metabolism (including carbohydrates, proteins, and lipids), nervous system, immune system, and infectious diseases at the KEGG subcategory level to evaluate the possible effects of OMAT on the gut microbiome were also analyzed by comparing the abundance of genes in these metabolism pathways with EAE group. The results revealed that the KEGGs for the energy metabolism, amino acid, immune system, and nervous system metabolisms were significantly increased in EAE_OMAT compared to those in EAE (all $P<0.05$) (Figures 6A, C, E, F), while the genes-related with infectious diseases metabolisms were significantly decreased in EAE_OMAT compared to those in EAE (all

10.3389/fcimb.2022.1095053

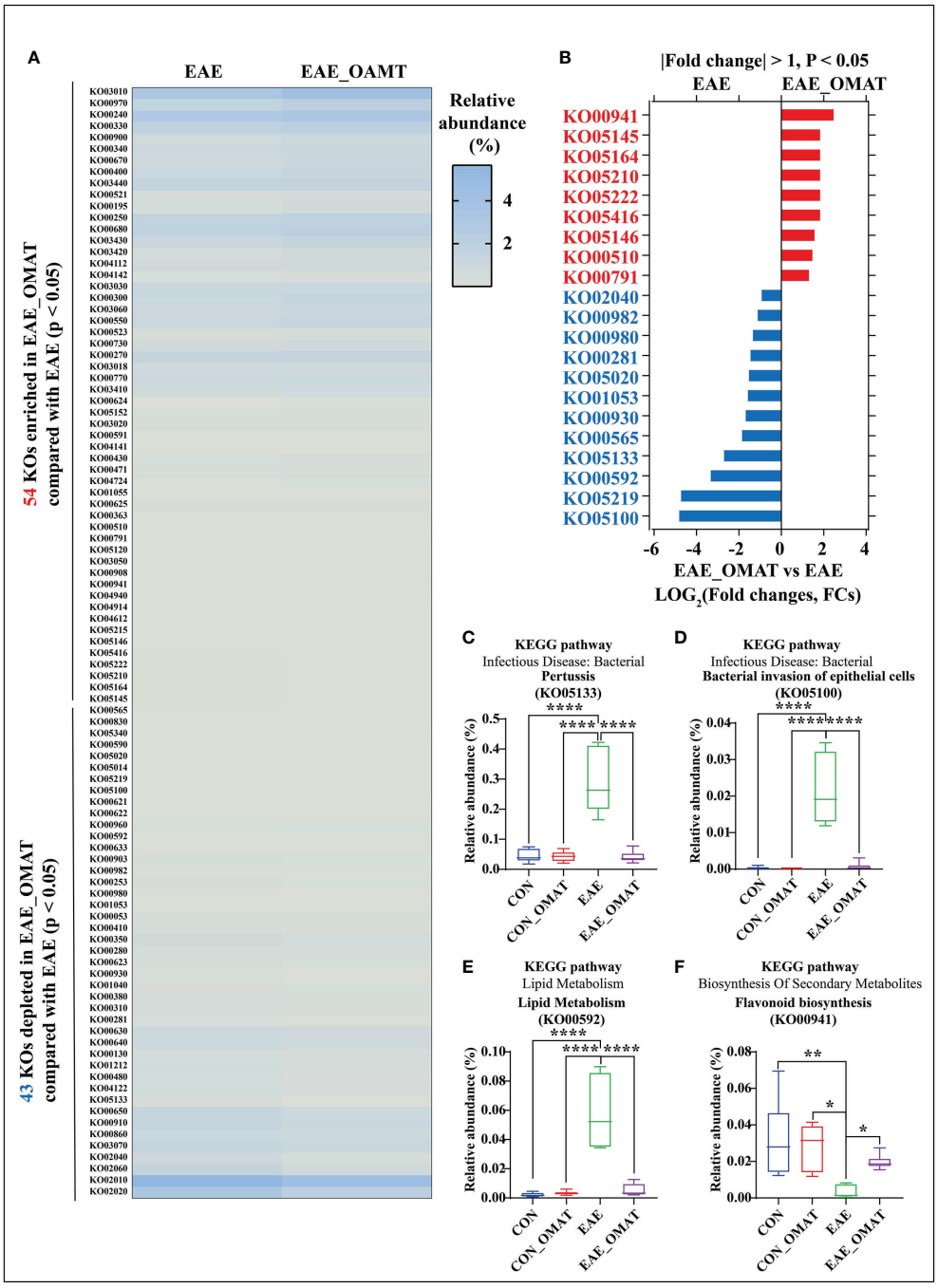


FIGURE 5
Effect of oxymatrine on the functional profiling of the gut microbiome. **(A)** The list of 54 significantly enriched and 43 significantly depleted KEGG ortholog (KOs) in the EAE_OMAT group than in the EAE group ($P < 0.05$). **(B)** Ranked based on the fold changes of relative abundance ($|LOG_2(FCs)| \geq 1$), these individual pathways include at least one of the 9 significantly enriched KOs (EAE_OMAT-enriched KOs) or one of the 12 significantly depleted KOs (EAE_OMAT-depleted KOs) in the comparisons between the EAE and EAE_OMAT groups. **(C–F)** Relative abundance of the 4 significantly enriched or depleted KOs in EAE compared with EAE_OMAT. The relative abundance of pertussis (KO05133; **(C)**, bacterial invasion of epithelial cells (KO05100; **(D)**, alpha-Linolenic acid metabolism (KO00592; **(E)**, flavonoid biosynthesis (KO00941; **(F)** between the four subject groups. Data are expressed as the mean ± SD and statistical differences are represented by *, $P < 0.05$, **, $P < 0.01$, and ****, $P < 0.0001$ based on Wilcoxon rank-sum test with the Benjamini–Hochberg method for multiple group comparisons.

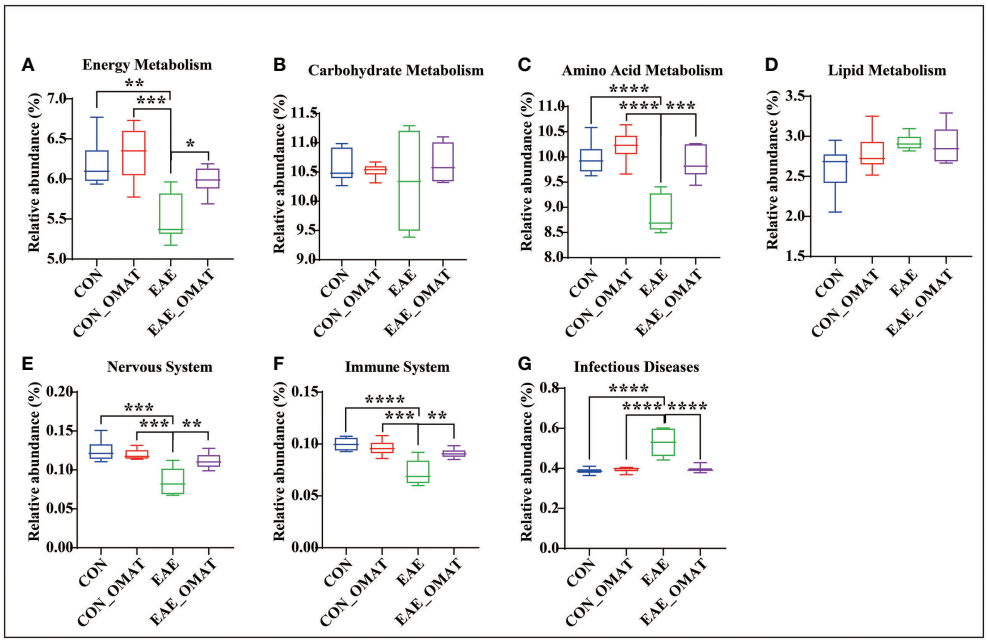


FIGURE 6
Comparison of the relative abundance of energy metabolism, nervous system, immune system, and infectious diseases based on KEGG analysis. Relative abundance of the KEGG orthologs belonging to Energy Metabolism **(A)**, Carbohydrate Metabolism **(B)**, Amino Acid Metabolism **(C)**, Lipid Metabolism **(D)**, Nervous System **(E)**, Immune System **(F)**, and Infectious Diseases **(G)** in the four mice groups. Data are expressed as the mean ± SD and statistical differences are represented by *, $P < 0.05$, **, $P < 0.01$, ***, and $P < 0.001$ based on Wilcoxon rank-sum test with the Benjamini–Hochberg method for multiple group comparisons.

P<0.05) (Figure 6G). However, Carbohydrate Metabolism and Lipid Metabolism in EAE mice have no significant changes under OMAT intervention (Figure 6B, D).

3.7 OMAT regulates the levels of SCFAs and related gut microbiota

Considering the regulatory effect of SCFAs on MS, the levels of SCFAs in fecal samples were analyzed. The result revealed were obvious potential reduction of the total levels of SCFAs in EAE group mice as compared to those from CON group mice, while this phenomenon was slightly reversed in EAE_OMAT group mice (Figure 7A). The levels of BA and PA were significantly lower in EAE group mice compared with CON group mice (both *P<0.05*), and the levels of IBA and IVA were significantly increased (both *P<0.05*). After OMAT treatment, the levels of IBA and IVA in EAE mice were significantly decreased (both *P<0.05*) (Figures 7B–H). Meanwhile, the metabolism pathways related to butyrate metabolism and propionate metabolism were significantly decreased in EAE_OMAT mice compared to EAE mice (all *P<0.05*) (Figures 7I, J).

The possible association between SCFAs expression and the top 50 kinds of microbial genus/OTUs according to the abundance was further analyzed by linear regression (Figure 7K). The results revealed the levels of total SCFAs, BA, and PA were markedly positively correlated with microbial abundance (R^2 = 0.2759, *P<0.01*; R^2 = 0.2735, *P<0.01*; R^2 = 0.2912, *P<0.01*; respectively), while the levels of IBA and IVA were dramatically negative correlation with microbial abundance (R^2 = 0.659, *P<0.0001*; R^2 = 0.6376, *P<0.0001*; respectively) (Figures 7L–S). Based on the above results, Clostridioides and Muribaculum screened out the significant correlation with BA, IBA, PA, and IVA (all *P<0.05*). The results showed the abundance of Clostridioides was found positively correlated with the expression of IBA, IVA (R=0.53; R=0.50; respectively), and negatively correlated with the expression of PA, BA (R=-0.60; R=-0.46; respectively). Meanwhile, the abundance of Muribaculum was positively correlated with the expression of PA, BA (R=0.75; R=0.46; respectively), and negatively correlated with the expression of IBA, IVA (R=-0.44; R=-0.55; respectively) (SI Appendix, Table S8). We reviewed the changes in the abundance of Clostridioides and Muribaculum in different groups and found Clostridioides was significantly reduced to the level of the normal group, and Muribaculum was increased

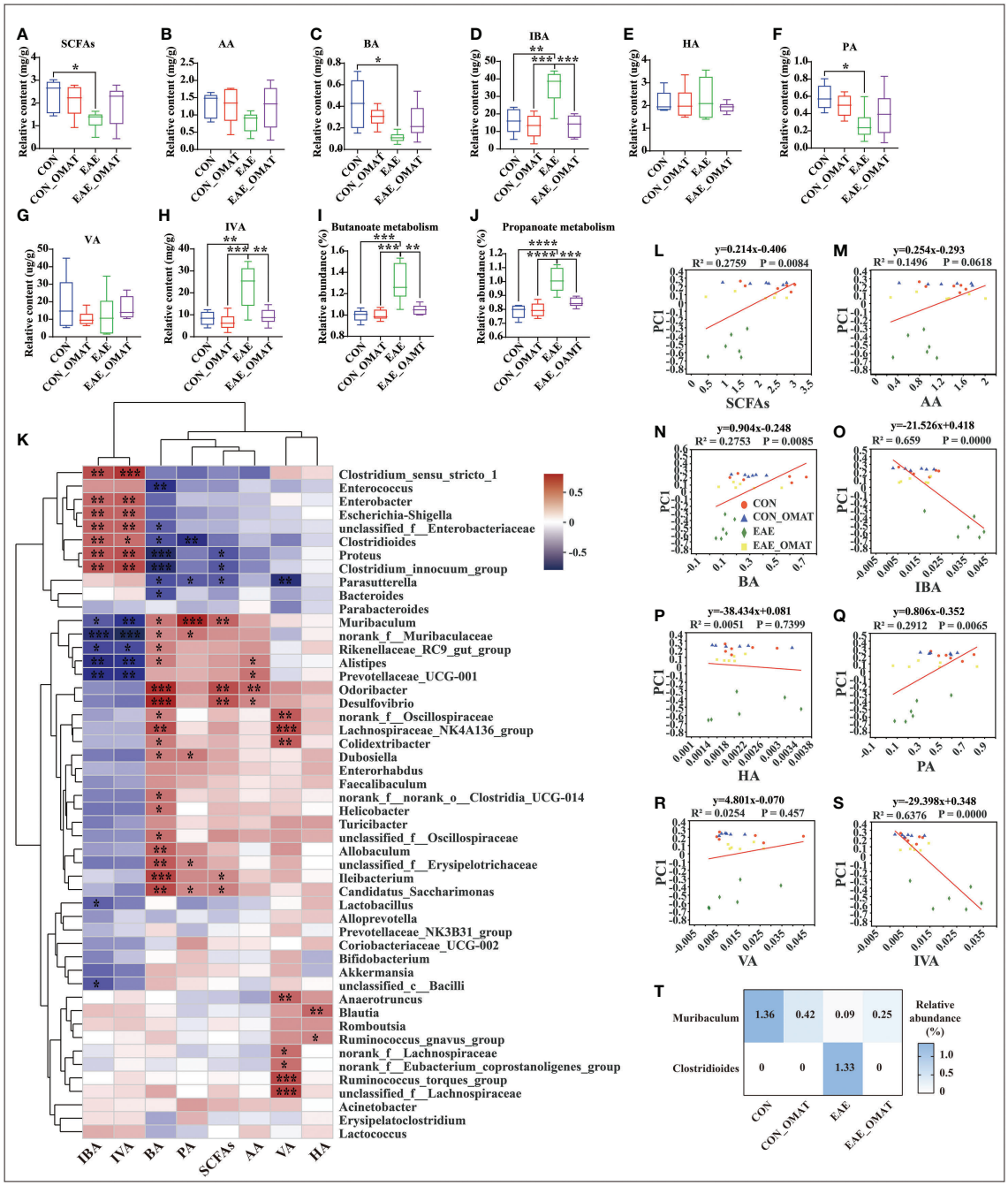


FIGURE 7
Effect of oxymatrine on the gut microbiota and the concentration of short-chain fatty acids (n = 6 in each group). **(A–I)** The concentration of SCFAs **(A)**, acetic acid (AA) **(B)**, butyrate acid (BA) **(C)**, isobutyrate acid (IBA) **(D)**, hexanoic acid (HA) **(E)**, propionic acid (PA) **(F)**, valeric acid (VA) **(G)**, and isovaleric acid (IVA) **(H)** in the feces of the four mice groups. **(I, J)** KEGG orthologs (KOs) are associated with Butyrate metabolism **(I)** and Propionate metabolism **(J)**. Correlation heatmap diagram of the concentration of short-chain fatty acids (SCFAs) and the relative abundance of gut microbes belonging to a different genus **(K)**. **(L–S)** Linear rank regression analysis of the relationship between the gut microbiome and the concentration of SCFAs **(M)**, acetic acid **(N)**, butyrate acid **(O)**, isobutyrate acid **(P)**, hexanoic acid **(Q)**, propionic acid **(R)**, valeric acid **(S)**, isovaleric acid **(T)** in the feces of the four mice groups. **(T)** Relative abundance of *Clostridioides* and *Muribaculum* in the four mice groups. The P values for **(A–H)** were derived from the LSD-t test. In **(I-J)** and **(T)**, the P value was determined based on Wilcoxon rank-sum test with the Benjamini–Hochberg method. In **(K-S)**, the relation between the abundance of microbial genera/OTUs and the concentration SFCAs was assessed using Spearman's rank correlation coefficient. Data are expressed as the mean ± SD and statistical differences are represented by *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$, and ****, $P < 0.0001$.

significantly in the OMAT group while compared with the EAE group (both $P<0.05$) (Figure 7T).

3.8 OMAT down-regulates the expression of pro-inflammatory cytokines in the brains and colons of EAE mice

Obvious trend changes of TNF-α, IL-6, and IL-1β between the EAE group and EAE_OMAT group were found in the brains and colons at the mRNA levels. We found the mRNA levels of IL-1β (Figure 8A), IL-6 (Figure 8B), and TNF-α (Figure 8C) in the brains of OMAT-treated EAE mice were significantly decreased compared with EAE group mice (all $P<0.05$). Meanwhile, similar phenomena were also observed in the colons with significantly decreased mRNA levels of IL-1β (Figure 8D), IL-6 (Figure 8E), TNF-α (Figure 8F) between the EAE group and EAE_OMAT group (all $P< 0.05$). However, markedly increased mRNA levels of IL-1β (Figure 8D), IL-6 (Figure 8E), and TNF-α (Figure 8F) in the colons were observed in the CON_OMAT group compared with CON group mice (all $P<0.05$), implied that OMAT has certain intestinal toxicity to normal mice.

3.9 OMAT improves the function of the BBB and IEB in EAE mice

Immunohistochemistry revealed an obvious decrease in the expression of Occludin and ZO-1 in the colons of EAE group mice, while the OMAT reversed this phenomenon (Figure 9A). Similar phenomenons for the mRNA levels of Occludin, ZO-1 also occurred in the colons of EAE mice, while reversed by OMAT treatment (all $P<0.05$) (Figures 9B, C). The significant change trend of Occludin, ZO-1 mRNA expression in brain tissue is the same as that in colon (all $P<0.05$) (Figures 9D, E). The decrease of Occludin and ZO-1 indicated the increased BBB permeability. Quantified the exosmosis of EB dye into the brain as an indicator of BBB permeability, and found EB content in the brain of EAE mice was significantly increased compared with CON mice and CON_OMAT mice, while the phenomenon was prominently reversed by OMAT treatment (all $P < 0.05$) (Figure 9F).

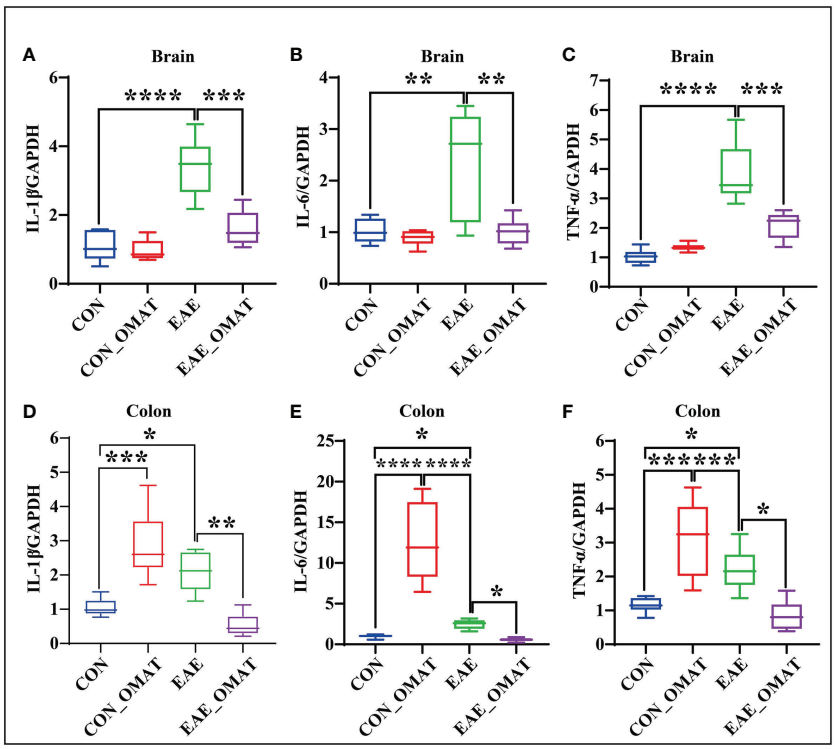


FIGURE 8
Effect of oxymatrine on the expression of pro-inflammatory cytokines in the brains and colons of EAE mice (n = 6 in each group). Results of RT-qPCR analysis showing a decrease in the mRNA expression of IL-1β **(A, D)**, IL-6 **(B, E)**, and TNF-α **(C, F)** in brain and colon samples of EAE mice after OMAT treatment. Data are expressed as the mean ± SD and statistical differences are represented by *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$, and ****, $P < 0.0001$ based on LSD-t test.

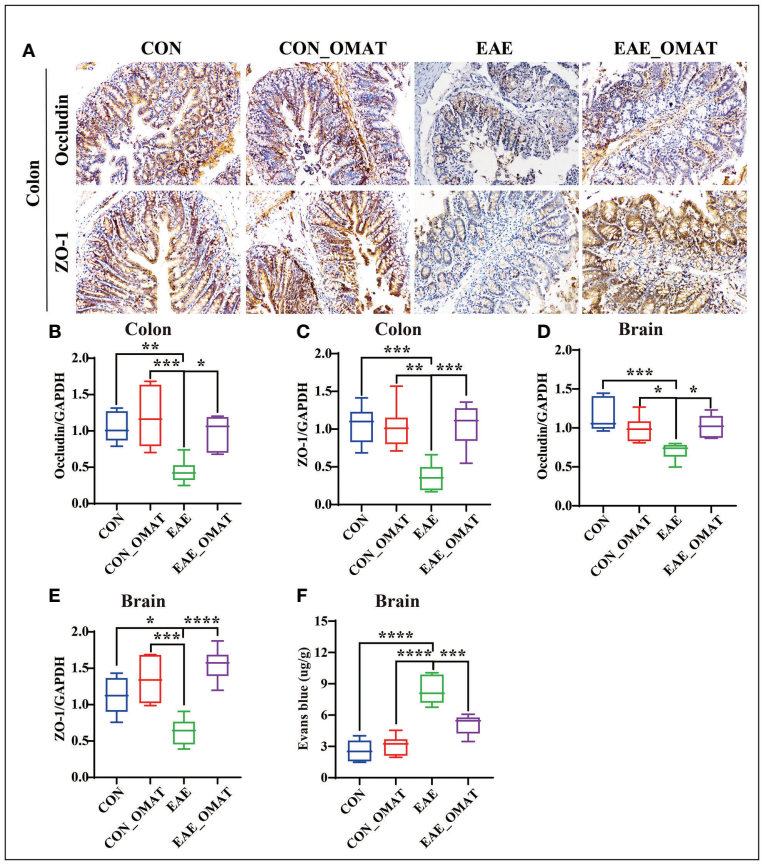


FIGURE 9
Effect of oxymatrine on the integrity of the blood-brain barrier and intestinal epithelial barrier in EAE mice. **(A)** Representative images of the hippocampus and colon tissue structure were detected by immunohistochemistry staining (n = 6 in each group). Original magnification 20X. **(B, C)** mRNA levels of occludin in the hippocampus **(B)** and colon **(C)**. **(D, E)** mRNA levels of ZO-1 in the hippocampus **(D)** and colon **(E)**. **(F)** Quantitative analysis of Evans blue extravasation in the brain of EAE mice (n = 3 in each group). All data were expressed as the mean ± SD and statistical differences are represented by *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$, and ****, $P < 0.0001$ based on the LSD-t test.

4 Discussion

The gut microbiome is the largest and most complex microbiome in the human body, which plays an important role in the stability of the intestinal environment and the regulation of the host immune system (Lynch and Pedersen, 2016), and the imbalance of intestinal microbiota structure and function are closely related to a variety of diseases, including MS and other neurodegenerative diseases (Sorboni et al., 2022). This study extends our previous research (Dou et al., 2021) and found OMAT improved the neurological function score in the EAE mice associated with altered alpha-diversity and beta-diversity indexes among the gut microbiomes, hinting that OMAT may play a therapeutic role by regulating the disorder of gut microflora in EAE mice.

Some studies have screened and identified special flora which may be harmful (Clostridium_perfringens, Clostridioides_difficile) to MS, and microbiome transplantation from MS patients has been proven to aggravate the incidence of EAE model mice (Berer et al., 2017; Cekanaviciute et al., 2017). Epsilon toxin from Clostridium perfringens can cross the intestinal wall and BBB by increasing cell permeability and accumulating in the brain, followed by binding the synaptosomal membrane, myelinated structure, glial cells, and oligodendrocytes to lead to demyelination (Linden et al., 2015; Bossu et al., 2020). In the present study, OMAT significantly reduced the content of Clostridium_perfringens in the feces of EAE mice from 3.40% to 0.0025%. The study found that OMAT also significantly reduced the clostridioides_difficile content in feces from 1.33% to 0.00059%. Clostridioides_difficile, possessing an encephalitogenic mimotope of myelin basic protein in the surface layer protein A, could activate autoreactive myelin-specific T cells to induce inflammation infiltration and demyelination in the CNS (Mindur et al., 2020). For common conditional pathogenic bacteria, especially for Escherichia_coli_g:Escherichia-Shigella, OMAT was also significantly reduced by 33.17%, respectively. These findings suggest that OMAT treatment, through modulation of the gut

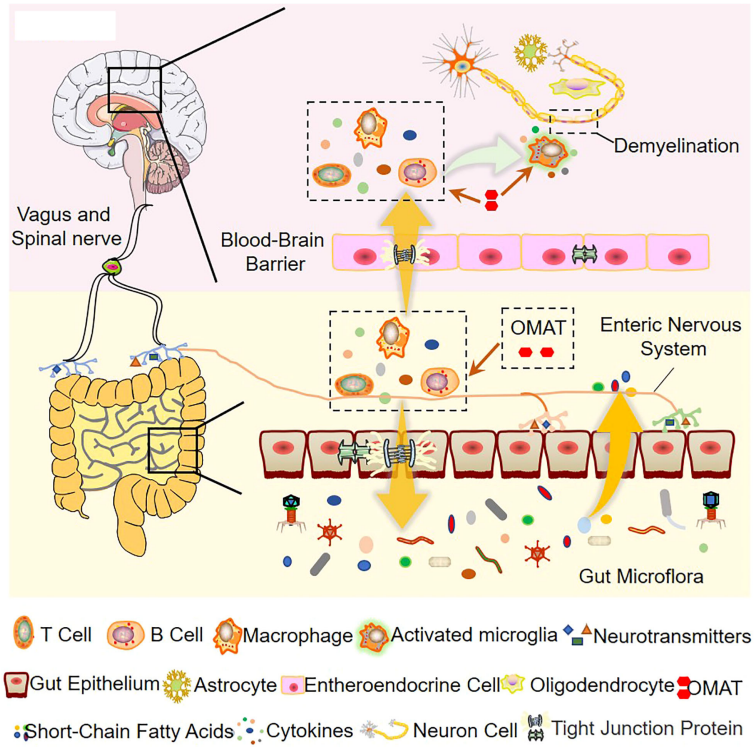


FIGURE 10
The role of OMAT on interfering with the progression of multiple sclerosis through the brain-gut axis.

microbiota, can prevent demyelination, which is characteristic of MS.

Studies have also reported that taking probiotics (Lactobacillus, Bifidobacterium, and Streptococcus) or transplanting normal human intestinal flora into EAE model mice can reduce the frequency of inflammatory monocytes $CD14^{high}CD16^{low}$ and the expression of HLA-DR on dendritic cells (Tankou et al., 2018; Tankou et al., 2018), and produces myelin antigens to induce an anti-inflammatory peripheral immune response (Kasarello et al., 2015; Kasarełło et al., 2016), regulates the balance of CD4+ T cell subsets (Salehipour et al., 2017), reduces the expression of IL-1β, IL-8, IL-10 and TNF-α in the blood of patients (Tamtaji et al., 2017), and ultimately weaken the severity of the disease (Tamtaji et al., 2017; Mangalam et al., 2017; Tankou et al., 2018; Blais et al., 2021). In the current study, after OMAT treatment, the contents of Lactobacillus and Bifidobacterium, the main components of probiotics in the feces of EAE mice, were increased from 1.25% to 9.82%, and 0.71% to 4.46%, respectively. The abundance of Prevotella histicola in the EAE mice with OMAT treatment was also significantly increased from 0.013% to 1.44%, which was reported could down-regulate the pro-inflammatory Th1/Th17 response and induce regulatory CD4+FoxP3+ regulatory T cells (Treg) to inhibit the occurrence of MS (Mangalam et al., 2017; Blais et al., 2021).

SCFAs, the metabolic products of enteric bacteria, contribute directly to preserving the integrity of the intestinal epithelial barrier (Wan Saudi and Sjöblom, 2017; Feng et al., 2018), and are associated with gut microbiota dysbiosis and inflammation in MS (Zeng et al., 2019; Olsson et al., 2021; Moles et al., 2022). Although the levels of PA and BA in the study increased slightly, while IBA and IVA were dramatically decreased after taking OMAT in EAE mice, further rank regression analysis found the expression of IBA and IVA were significantly positive correlation with intestinal flora disorder, especially among Enterobacter, Escherichia-Shigella, Proteus, Clostridium_innocuum_group, Clostridium_sensu_stricto_1, Clostridioides (all R>0.5). Published data displayed IVA could reduce Na+, K+-ATPase activity (a crucial enzyme responsible for maintaining the basal potential membrane necessary for normal neurotransmission) and enzyme citrate synthase in the cerebral cortex to inhibit the citric acid cycle (Ribeiro et al., 2007; Ribeiro et al., 2009), therefore, the OMAT restores the level of VA may regulate the citric acid cycle by increased the activity of Na+, K+-ATPase and enzyme citrate synthase in EAE mice.

Indeed, alterations of enteric bacteria, will successively activate the intestinal and central immune system, and promote the release of a large number of immune-inflammatory factors, such as IL-1β, IL-6, and TNF-α, which will lead to the change in the permeability of the intestinal

mucosal barrier and blood-brain barrier. This view is supported by alterations of intestinal permeability and signs of systemic inflammation in patients with MS which appear to be correlated with the disability status of the disease (Miyauchi et al., 2020; Sorboni et al., 2022). In our study, the relative abundance of the KO05100 pathway (Bacterial invasion of epithelial cells) was significantly increased in EAE mice and reversed by OMAT treatment. Meanwhile, the mRNA expression of IL-1β, IL-6, and TNF-α both in the brains and colons of EAE mice were dramatically increased, and almost completely reversed by OMAT. Consistent with literature reports, the intercellular junction complexes which ensure the integrity of the epithelial barrier and regulate paracellular permeability, including Occludin, ZO-1 (Camara-Lemarroy et al., 2018), located around the apical surface of adjacent epithelial cells, were significantly decreased in EAE mice and reversed by OMAT treatment, and the regulatory effect of OMAT on the IEB or BBB function in EAE model mice was also confirmed in DSS model mice (Yao et al., 2021) or early brain injury rats (Liu et al., 2016).

Surprisingly, the study found OMAT has certain intestinal toxicity to normal mice, but not to EAE mice. Combined with the reported liver protection and liver toxicity, neuroprotection, and neurotoxicity of OMAT in the literature (You et al., 2020; Li et al., 2021), it suggests that the toxic mechanism of OMAT maybe its pharmacodynamic effect in the treatment of MS, hepatitis, and other diseases, which is also in line with an old saying of traditional Chinese medicine "The principle that if there is enough reason, a toxic medicine can also be used without harm".

Based on the 16S rRNA data, the study concludes that the ecological and functional microenvironment of the gut is differentially altered in the EAE mice and is reversed by OMAT. Collective data indicate that the alterations of the gut microbiome in the OMAT-treated EAE mice may be partly explained by changes in the metabolic pathway of infectious diseases, energy metabolism, immune system, and nervous system. Among the information showed by Figure 10, regulating gut microbiota disorder and reducing BBB permeability may be the potential mechanism of OAMT in the treatment of MS.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

Ethics statement

The animal study was reviewed and approved by The First Affiliated Hospital of Henan University of Chinese Medicine (YFYDW2020002).

Author contributions

J-FT conceived and designed the experiments. M-LZ performed the experiments, analyzed the data, and wrote the manuscript. C-ZW, D-XK, BW, S-QZ, and K-RF were responsible for helping with the collection of experimental samples. Y-LC and X-YW helped to detect the gut microflora and short-chain fatty acids. HZ, L-QY, and LN helped with data analysis. Y-LW and W-XL reviewed the paper. All authors have reviewed the manuscript and approved the final version of the manuscript. All authors have read, revised, and approved the final manuscript.

Funding

This work was supported by the National Natural Science Foundation of China (No. 82004021, No. 82173993, No. U1904129), the science and technology research project of Henan Provincial Department of Education (grant number 202102310185), special research project on Traditional Chinese Medicine in Henan Province (grant number 2019ZY2144), Henan province traditional Chinese medicine talented person training program (prepared Chinese medicine science and education [2018] no. 35), Science and Technology Innovation Team in Vuiversities of Henan Province (No: 23IRTSTHN026).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2022.1095053/full#supplementary-material

References

Antonazzo, I. C., Poluzzi, E., Forcesi, E., Riise, T., Bjornevik, K., Baldin, E., et al. (2019). Liver injury with drugs used for multiple sclerosis: A contemporary analysis of the FDA adverse event reporting system. *Mult Scler* 25 (12), 1633–1640. doi: 10.1177/1352458518799598

Berer, K., Gerdes, L. A., Cekanaviciute, E., Jia, X., Xiao, L., Xia, Z., et al. (2017). Gut microbiota from multiple sclerosis patients enables spontaneous autoimmune encephalomyelitis in mice. *Proc. Natl. Acad. Sci. U.S.A.* 114 (40), 10719–10724. doi: 10.1073/pnas.1711233114

Blais, L. L., Montgomery, T. L., Amiel, E., Deming, P. B., and Krementsov, D. N. (2021). Probiotic and commensal gut microbial therapies in multiple sclerosis and its animal models: A comprehensive review. *Gut Microbes* 13 (1), 1943289. doi: 10.1080/19490976.2021.1943289

Bossu, J. L., Wioland, L., Doussau, F., Isope, P., Popoff, M. R., and Poulain, B. (2020). Epsilon toxin from clostridium perfringens causes inhibition of potassium inward rectifier (Kir) channels in oligodendrocytes. *Toxins (Basel)* 12 (1), 36. doi: 10.3390/toxins12010036

Calderón, K., Spor, A., Breuil, M. C., Bru, D., Bizouard, F., Violle, C., et al. (2017). Effectiveness of ecological rescue for altered soil microbial communities and functions. *Isme J.* 11 (1), 272–283. doi: 10.1038/ismej.2016.86

Camara-Lemarroy, C. R., Metz, L., Meddings, J. B., Sharkey, K. A., and Wee Yong, V. (2018). The intestinal barrier in multiple sclerosis: Implications for pathophysiology and therapeutics. *Brain* 141 (7), 1900–1916. doi: 10.1093/brain/awy131

Cekanaviciute, E., Yoo, B. B., Runia, T. F., Debelius, J. W., Singh, S., Nelson, C. A., et al. (2017). Gut bacteria from multiple sclerosis patients modulate human T cells and exacerbate symptoms in mouse models. *Proc. Natl. Acad. Sci. U.S.A.* 114 (40), 10713–10718. doi: 10.1073/pnas.1711235114

Chu, F., Shi, M., Lang, Y., Shen, D., Jin, T., Zhu, J., et al. (2018). Gut microbiota in multiple sclerosis and experimental autoimmune encephalomyelitis: Current applications and future perspectives. *Mediators Inflammation* 2018, 8168717. doi: 10.1155/2018/8168717

Dou, M., Zhou, X., Li, L., Zhang, M., Wang, W., Wang, M., et al. (2021). Illumination of molecular pathways in multiple sclerosis lesions and the immune mechanism of matrine treatment in EAE, a mouse model of MS. *Front. Immunol.* 12, 640778. doi: 10.3389/fimmu.2021.640778

Feng, Y., Wang, Y., Wang, P., Huang, Y., and Wang, F. (2018). Short-chain fatty acids manifest stimulative and protective effects on intestinal barrier function through the inhibition of NLRP3 inflammasome and autophagy. *Cell Physiol. Biochem.* 49 (1), 190–205. doi: 10.1159/000492853

Jiao-Yan, Y., Qing-Qing, L., Xi, L., Mei, Z., Ting, S., Na, H., et al. (2021). Oxymatrine improves blood-brain barrier integrity after cerebral ischemia-reperfusion injury by downregulating CAV1 and MMP9 expression. *Phytomedicine* 84, 153505. doi: 10.1016/j.phymed.2021.153505

Kanehisa, M., Sato, Y., Furumichi, M., Morishima, K., and Tanabe, M. (2019). New approach for understanding genome variations in KEGG. *Nucleic Acids Res.* 47 (D1), D590–d595. doi: 10.1093/nar/gky962

Kasarello, K., Kwiatkowska-Patzer, B., Lipkowski, A. W., Bardowski, J. K., and Szczepankowska, A. K. (2015). Oral administration of lactococcus lactis expressing synthetic genes of myelin antigens in decreasing experimental autoimmune encephalomyelitis in rats. *Med. Sci. Monit* 21, 1587–1597. doi: 10.12659/MSM.892764

Kasarełło, K., Szczepankowska, A., Kwiatkowska-Patzer, B., Lipkowski, A. W., Gadamski, R., Sulejczak, D., et al. (2016). Effect of recombinant lactococcus lactis producing myelin peptides on neuroimmunological changes in rats with experimental allergic encephalomyelitis. *Folia Neuropathol.* 54 (3), 249–258. doi: 10.5114/fn.2016.62534

Kou, S., Zheng, Q., Wang, Y., Zhao, H., Zhang, Q., Li, M., et al. (2014). Zuo-gui and you-gui pills, two traditional Chinese herbal formulas, downregulated the expression of NogoA, NgR, and RhoA in rats with experimental autoimmune encephalomyelitis. *J. Ethnopharmacol* 158 (Pt A), 102–112. doi: 10.1016/j.jep.2014.10.007

Leprun, P. M. B., and Clarke, G. (2019). The gut microbiome and pharmacology: A prescription for therapeutic targeting of the gut-brain axis. *Curr. Opin. Pharmacol.* 49, 17–23. doi: 10.1016/j.coph.2019.04.007

Li, P., Lei, J., Hu, G., Chen, X., Liu, Z., and Yang, J. (2019). Matrine mediates inflammatory response *via* gut microbiota in TNBS-induced murine colitis. *Front. Physiol.* 10, 28. doi: 10.3389/fphys.2019.00028

Linden, J. R., Ma, Y., Zhao, B., Harris, J. M., Rumah, K. R., Schaeren-Wiemers, N., et al. (2015). Clostridium perfringens epsilon toxin causes selective death of mature oligodendrocytes and central nervous system demyelination. *mBio* 6 (3), e02513. doi: 10.1128/mBio.02513-14

Li, X., Tang, Z., Wen, L., Jiang, C., and Feng, Q. (2021). Matrine: A review of its pharmacology, pharmacokinetics, toxicity, clinical application and preparation researches. *J. Ethnopharmacol* 269, 113682. doi: 10.1016/j.jep.2020.113682

Liu, N., Kan, Q.-c., Zhang, X.-j., Xv, Y.-m., Zhang, S., Zhang, G.-X., et al. (2014). Upregulation of immunomodulatory molecules by matrine treatment in experimental autoimmune encephalomyelitis. *Exp. Mol. Pathol.* 97 (3), 470–476. doi: 10.1016/j.yexmp.2014.10.004

Liu, X., Zhang, X., Ma, K., Zhang, R., Hou, P., Sun, B., et al. (2016). Matrine alleviates early brain injury after experimental subarachnoid hemorrhage in rats: Possible involvement of PI3K/Akt-mediated NF-κB inhibition and Keap1/Nrf2-dependent HO-1 inductionn. *Cell Mol. Biol. (Noisy-le-grand)* 62 (11), 38–44.

Lynch, S. V., and Pedersen, O. (2016). The human intestinal microbiome in health and disease. *N Engl. J. Med.* 375 (24), 2369–2379. doi: 10.1056/NEJMra1600266

Mangalam, A., Shahi, S. K., Luckey, D., Karau, M., Marietta, E., Luo, N., et al. (2017). Human gut-derived commensal bacteria suppress CNS inflammatory and demyelinating disease. *Cell Rep.* 20 (6), 1269–1277. doi: 10.1016/j.celrep.2017.07.031

Mindur, J. E., Yadav, S. K., Ito, N., Senoh, M., Kato, H., Dhib-Jalbut, S., et al. (2020). Surface layer protein a expressed in clostridioides difficile DJNS06-36 possesses an encephalitogenic mimotope of myelin basic protein. *Microorganisms* 9 (1), 34. doi: 10.3390/microorganisms9010034

Miyauchi, E., Kim, S. W., Suda, W., Kawasumi, M., Onawa, S., Taguchi-Atarashi, N., et al. (2020). Gut microorganisms act together to exacerbate inflammation in spinal cords. *Nature* 585 (7823), 102–106. doi: 10.1038/s41586-020-2634-9

Moiola, L., Rommer, P. S., and Zettl, U. K. (2020). Prevention and management of adverse effects of disease modifying treatments in multiple sclerosis. *Curr. Opin. Neurol.* 33 (3), 286–294. doi: 10.1097/WCO.0000000000000824

Moles, L., Delgado, S., Gorostidi-Aicua, M., Sepúlveda, L., Alberro, A., Iparraguirre, L., et al. (2022). Microbial dysbiosis and lack of SCFA production in a Spanish cohort of patients with multiple sclerosis. *Front. Immunol.* 13, 960761. doi: 10.3389/fimmu.2022.960761

Olsson, A., Gustavsen, S., Nguyen, T. D., Nyman, M., Langkilde, A. R., Hansen, T. H., et al. (2021). Serum short-chain fatty acids and associations with inflammation in newly diagnosed patients with multiple sclerosis and healthy controls. *Front. Immunol.* 12, 661493. doi: 10.3389/fimmu.2021.661493

Preziosi, G., Raptis, D. A., Raeburn, A., Thiruppathy, K., Panicker, J., and Emmanuel, A. (2013). Gut dysfunction in patients with multiple sclerosis and the role of spinal cord involvement in the disease. *Eur. J. Gastroenterol. Hepatol.* 25 (9), 1044–1050. doi: 10.1097/MEG.0b013e328361eaf8

Rashid, H. U., Xu, Y., Muhammad, Y., Wang, L., and Jiang, J. (2019). Research advances on anticancer activities of matrine and its derivatives: An updated overview. *Eur. J. Med. Chem.* 161, 205–238. doi: 10.1016/j.ejmech.2018.10.037

Ribeiro, C. A., Balestro, F., Grando, V., and Wajner, M. (2007). Isovaleric acid reduces na+, k+-ATPase activity in synaptic membranes from cerebral cortex of young rats. *Cell Mol. Neurobiol.* 27 (4), 529–540. doi: 10.1007/s10571-007-9143-3

Ribeiro, C. A., Leipnitz, G., Amaral, A. U., de Bortoli, G., Seminotti, B., and Wajner, M. (2009). Creatine administration prevents Na+,K+-ATPase inhibition induced by intracerebroventricular administration of isovaleric acid in cerebral cortex of young rats. *Brain Res.* 1262, 81–88. doi: 10.1016/j.brainres.2009.01.005

Rumsey, D. J. (2016). *Statistics for dummies. 2nd ed.* Ed. N. J. Hoboken (Wiley Publishing).

Salehipour, Z., Haghmorad, D., Sankian, M., Rastin, M., Nosratabadi, R., Soltan Dallal, M. M., et al. (2017). Bifidobacterium animalis in combination with human origin of lactobacillus plantarum ameliorate neuroinflammation in experimental model of multiple sclerosis by altering CD4+ T cell subset balance. *BioMed. Pharmacother.* 95, 1535–1548. doi: 10.1016/j.biopha.2017.08.117

Sorboni, S. G., Moghaddam, H. S., Jafarzadeh-Esfehani, R., and Soleimanpour, S. (2022). A comprehensive review on the role of the gut microbiome in human neurological disorders. *Clin. Microbiol. Rev.* 35 (1), e0033820. doi: 10.1128/CMR.00338-20

Stanisavljevic, S., Dinic, M., Jevtic, B., Dedovic, N., Momcilovic, M., Dokic, J., et al. (2018). Gut microbiota confers resistance of albino Oxford rats to the induction of experimental autoimmune encephalomyelitis. *Front. Immunol.* 9, 942. doi: 10.3389/fimmu.2018.00942

Tamtaji, O. R., Kouchaki, E., Salami, M., Aghadavod, E., Akbari, E., Tajabadi-Ebrahimi, M., et al. (2017). The effects of probiotic supplementation on gene expression related to inflammation, insulin, and lipids in patients with multiple sclerosis: A randomized, double-blind, placebo-controlled trial. *J. Am. Coll. Nutr.* 36 (8), 660–665. doi: 10.1080/07315724.2017.1347074

Tankou, S. K., Regev, K., Healy, B. C., Cox, L. M., Tjon, E., Kivisakk, P., et al. (2018). Investigation of probiotics in multiple sclerosis. *Mult Scler* 24 (1), 58–63. doi: 10.1177/1352458517737390

Tankou, S. K., Regev, K., Healy, B. C., Tjon, E., Laghi, L., Cox, L. M., et al. (2018). A probiotic modulates the microbiome and immunity in multiple sclerosis. *Ann. Neurol.* 83 (6), 1147–1161. doi: 10.1002/ana.25244

Thompson, A. J., Baranzini, S. E., Geurts, J., Hemmer, B., and Ciccarelli, O. (2018). Multiple sclerosis. *Lancet* 391 (10130), 1622–1636. doi: 10.1016/S0140-6736(18)30481-1

Wan Saudi, W. S., and Sjöblom, M. (2017). Short-chain fatty acids augment rat duodenal mucosal barrier function. *Exp. Physiol.* 102 (7), 791–803. doi: 10.1113/EP086110

Wu, H., Chen, Q., Liu, J., Chen, X., Luo, H., Ye, Z., et al. (2021). Microbiome analysis reveals gut microbiota alteration in mice with the effect of matrine. *Microb. Pathog.* 156, 104926. doi: 10.1016/j.micpath.2021.104926

Xu, L., Zhang, C., He, D., Jiang, N., Bai, Y., et al. (2020). Rapamycin and MCC950 modified gut microbiota in experimental autoimmune encephalomyelitis mouse by brain gut axis. *Life Sci.* 253, 117747. doi: 10.1016/j.lfs.2020.117747

Yao, H., Shi, Y., Yuan, J., Sa, R., Chen, W., and Wan, X. (2021). Matrine protects against DSS-induced murine colitis by improving gut barrier integrity, inhibiting the PPAR-α signaling pathway, and modulating gut microbiota. *Int. Immunopharmacol* 100, 108091. doi: 10.1016/j.intimp.2021.108091

You, L., Yang, C., Du, Y., Wang, W., Sun, M., Liu, J., et al. (2020). A systematic review of the pharmacology, toxicology and pharmacokinetics of matrine. *Front. Pharmacol.* 11, 01067. doi: 10.3389/fphar.2020.01067

Zeng, Q., Junli, G., Liu, X., Chen, C., Sun, X., Li, H., et al. (2019). Gut dysbiosis and lack of short chain fatty acids in a Chinese cohort of patients with multiple sclerosis. *Neurochem. Int.* 129, 104468. doi: 10.1016/j.neuint.2019.104468

Zha, Z., Gao, Y. F., Ji, J., Sun, Y. Q., Li, J. L., Qi, F., et al. (2021). Bu shen yi sui capsule alleviates neuroinflammation and demyelination by promoting microglia toward M2 polarization, which correlates with changes in miR-124 and miR-155 in experimental autoimmune encephalomyelitis. *Oxid. Med. Cell Longev* 2021, 5521503. doi: 10.1155/2021/5521503

Zhang, H., Chen, L., Sun, X., Yang, Q., Wan, L., and Guo, C. (2020). Matrine: A promising natural product with various pharmacological activities. *Front. Pharmacol.* 11, 588. doi: 10.3389/fphar.2020.00588

Zhang, M. L., Zhang, X. J., Kang, J., Zhang, H. J., Chen, X. L., Liu, N., et al. (2017). Matrine promotes NT3 expression in CNS cells in experimental autoimmune encephalomyelitis. *Neurosci. Lett.* 649, 100–106. doi: 10.1016/j.neulet.2017.04.005

Zhang, Y. B., Zhan, L. Q., Li, G. Q., Wang, F., Wang, Y., Li, Y. L., et al. (2016). Dimeric matrine-type alkaloids from the roots of sophora flavescens and their anti-hepatitis b virus activities. *J. Org Chem.* 81 (15), 6273–6280. doi: 10.1021/acs.joc.6b00804

Zhao, G., Nyman, M., and Jönsson, J. A. (2006). Rapid determination of short-chain fatty acids in colonic contents and faeces of humans and rats by acidified water-extraction and direct-injection gas chromatography. *BioMed. Chromatogr* 20 (8), 674–682. doi: 10.1002/bmc.580

Zhou, L., and Fan, Y. (2015). Randomized trial of erhuangfang for relapsing multiple sclerosis. *Neurol. Res.* 37 (7), 633–637. doi: 10.1179/1743132815Y.0000000011

frontiers | Frontiers in Cellular and Infection Microbiology

TYPE Review
PUBLISHED 27 January 2023
DOI 10.3389/fcimb.2023.1092118

OPEN ACCESS

EDITED BY
Chuanxing Xiao,
Xiamen University, China

REVIEWED BY
Jianan Zhao,
Shanghai University of Traditional Chinese Medicine, China
Xinwang Duan,
Second Affiliated Hospital of Nanchang University, China
Zhijun Xie,
Zhejiang Chinese Medical University, China

*CORRESPONDENCE
Gabriel Horta-Baas
gabho@hotmail.com

SPECIALTY SECTION
This article was submitted to Intestinal Microbiome, a section of the journal Frontiers in Cellular and Infection Microbiology

RECEIVED 07 November 2022
ACCEPTED 16 January 2023
PUBLISHED 27 January 2023

CITATION
Romero-Figueroa MdS, Ramírez-Durán N, Montiel-Jarquín AJ and Horta-Baas G (2023) Gut-joint axis: Gut dysbiosis can contribute to the onset of rheumatoid arthritis *via* multiple pathways. *Front. Cell. Infect. Microbiol.* 13:1092118. doi: 10.3389/fcimb.2023.1092118

Gut-joint axis: Gut dysbiosis can contribute to the onset of rheumatoid arthritis *via* multiple pathways

María del Socorro Romero-Figueroa[1], Ninfa Ramírez-Durán[2], Alvaro José Montiel-Jarquín[3] and Gabriel Horta-Baas[4]*

[1]Centro de Investigación en Ciencias de la Salud, Universidad Anáhuac México, Mexico City, Mexico, [2]Laboratory of Medical and Environmental Microbiology, Department of Medicine, Autonomous University of the State of Mexico, Toluca, Mexico, [3]Dirección de Educación e Investigación en Salud, Hospital de Especialidades de Puebla, Instituto Mexicano del Seguro Social, Puebla, Mexico, [4]Rheumatology Service, Internal Medicine Department, Instituto Mexicano del Seguro Social, Merida, Mexico

Rheumatoid Arthritis (RA) is an autoimmune disease characterized by loss of immune tolerance and chronic inflammation. It is pathogenesis complex and includes interaction between genetic and environmental factors. Current evidence supports the hypothesis that gut dysbiosis may play the role of environmental triggers of arthritis in animals and humans. Progress in the understanding of the gut microbiome and RA. has been remarkable in the last decade. *In vitro* and *in vivo* experiments revealed that gut dysbiosis could shape the immune system and cause persistent immune inflammatory responses. Furthermore, gut dysbiosis could induce alterations in intestinal permeability, which have been found to predate arthritis onset. In contrast, metabolites derived from the intestinal microbiota have an immunomodulatory and anti-inflammatory effect. However, the precise underlying mechanisms by which gut dysbiosis induces the development of arthritis remain elusive. This review aimed to highlight the mechanisms by which gut dysbiosis could contribute to the pathogenesis of RA. The overall data showed that gut dysbiosis could contribute to RA pathogenesis by multiple pathways, including alterations in gut barrier function, molecular mimicry, gut dysbiosis influences the activation and the differentiation of innate and acquired immune cells, cross-talk between gut microbiota-derived metabolites and immune cells, and alterations in the microenvironment. The relative weight of each of these mechanisms in RA pathogenesis remains uncertain. Recent studies showed a substantial role for gut microbiota-derived metabolites pathway, especially butyrate, in the RA pathogenesis.

KEYWORDS

gut microflora, gut microbiome, rheumatoid arthritis, short-chain fatty acid, butyrate

Introduction

Although the precise etiopathogenesis of rheumatoid arthritis (RA) is not well understood, it is characterized by loss of immune tolerance and chronic inflammation (Zhang et al., 2015; Liu et al., 2016; Zhang et al., 2020; Li et al., 2022). The accumulation and activation of immune cells, including dendritic cells, macrophages, neutrophils, and T cell subsets, within the synovial tissue is a cardinal feature of RA (Buckley and McGettrick, 2018). Abnormalities in the immune response lead to dysregulated cytokine secretion and autoantibodies production. Anticitrullinated protein antibodies (ACPA) and rheumatoid factor (RF) are hallmark autoantibodies of RA (Burmester et al., 2014; Yue et al., 2019). The inflammatory environment produced by lymphocytes, macrophages, and fibroblast-like synoviocytes causes synovitis, leading to joint destruction (Opoku et al., 2022).

Although the trigger that leads to loss of immune tolerance is unknown, previous studies have shown that individuals at risk for RA showed IgA-ACPA before the onset of arthritis (Bos et al., 2014; Mankia and Emery, 2016; Yue et al., 2019). Therefore, a breach of tolerance at mucosal surfaces (lungs, gut, or oral mucosa) is considered an initial event in the pathogenesis of RA that can occur many years before disease onset. Experimental evidence has suggested that microbial factors may be possible initiators of autoimmunity (Moen et al., 2005). However, despite multiple efforts, it has not yet been possible to identify any microorganism causing RA.

Gut dysbiosis, an altered intestinal microbiota composition, has been implicated in the pathogenesis of multiple rheumatic diseases, such as RA, psoriatic arthritis, and axial spondyloarthritis (Gill et al., 2022; Wang et al., 2022b). The role of gut dysbiosis in the pathogenesis of RA has been widely studied from experimental animal models. Growing evidence has suggested the role of gut microbiota in the onset of arthritis. Studies in mice (Rosser et al., 2014; Liu et al., 2016; Jubair et al., 2018; Maeda and Takeda, 2019; Peng et al., 2019; Aa et al., 2020), rats (Huang et al., 2019; Peng et al., 2019; Yue et al., 2019; Xu et al., 2020; Xu et al., 2022a), and pigs (Mansson et al., 1971) consistently demonstrate that gut dysbiosis is associated with inflammatory arthritis development.

The germ-free condition has been found to alleviate arthritis symptoms in spontaneous mouse models of RA (K/BxN, SKG, and IL-1 receptor antagonist deficient mouse models) (Van de Wiele et al., 2016; Rogier et al., 2017). However, the introduction of segmented filamentous bacteria into germ-free mice caused the production of autoantibodies and arthritis (Ivanov et al., 2009). Furthermore, studies in rodents have shown that the intestinal microbial community undergoes marked changes in the pre-clinical immune-priming phase and precede the onset of inflammatory arthritis (Rogier et al., 2017; Jubair et al., 2018; Doonan et al., 2019; Zhang et al., 2019). In addition, differences in the gut microbiota before arthritis onset between collagen-induced arthritis (CIA)-susceptible and CIA-resistant mice are consistent with the view that bacteria can influence RA development (Liu et al., 2016).

Alteration of the gut microbiota *via* fecal microbiota transplantation (FMT) has been used to demonstrate the causal relationship between arthritis and microbiome composition. FMT from mice susceptible to CIA into germ-free mice increased the severity of arthritis. Similarly, the FMT enriched in *Prevotella copri* from RA patients exacerbates the arthritis of SKG mice (Maeda et al., 2016). In another study with mice, it was found that gut-induced dysbiosis by oral inoculation of *Porphyromonas gingivalis* exacerbated arthritis (Sato et al., 2017; Hamamoto et al., 2020). Conversely, it has been demonstrated that oral administration of *Prevotella histicola* in either preventive or therapeutic reduces arthritis severity (Marietta et al., 2016).

In a recent study, Chriswell et al. showed that *Subdoligranulum didolesgii*, a human gut commensal, triggers synovitis in the germ-free DBA/1 mice, along with deposition of complement and immunoglobulins (Chriswell et al., 2022). Significantly, mice monocolonized with *S. didolesgii* developed arthritis without an adjuvant trigger. Furthermore, serum transfer from arthritic mice into gnotobiotic mice injected intraperitoneally led to a rapid onset of arthritis.

Early administration of probiotics may be a potential strategy for moderating clinical arthritis. Treatment with *B. adolescentis* before arthritis can ameliorate inflammation through rebalancing immune responses and modulating the gut-associated responses such as gut microbiota, short-chain fatty acids (SCFAs), and gut permeability (tight-junction proteins) in the CIA mouse model (Fan et al., 2020a).

To date, limited studies have assessed the relationship between fungal gut microbiota, helminths, and RA. Lee et al. showed that intraperitoneal injections of a fungal cell wall component (zymosan or fungal β-glucan) into SKG mice in a specific pathogen-free induced autoimmune arthritis. In contrast, injections of an antifungal agent and antifungal cell wall component did not (Lee et al., 2022). In the CIA mice model (male DBA/1), gastrointestinal helminths (*Heligmosomoides polygyrus* and *Trichuris muris)* can protect against intestinal mucosa inflammatory conditions by modulating the gut microbiota and suppressing the inflammation associated with gut dysbiosis. The ability of helminths to relieve CIA has been attributed to their capacity to secrete molecules (ES-62) that exert immunoregulation and limit host pathology (Doonan et al., 2019).

These findings indicate that some gut bacteria species and fungi can induce arthritis in a genetically predisposed animal. Interestingly, significant changes in the fecal microbiota composition occur during pre-clinical and early onset arthritis stages of the CIA model. Therefore, gut dysbiosis plays a role in arthritis pathogenesis in various animal models of RA.

In recent years, studies have explored the association of gut microbiota with RA. Multiple studies have demonstrated that the gut microbiota composition on fecal samples differs between RA patients and healthy controls (HCs) (Scher et al., 2013; Zhang et al., 2015; Chen et al., 2016; Maeda et al., 2016; Pan et al., 2017; Yue et al., 2019; Kishikawa et al., 2020; He et al., 2022; Wang et al., 2022a). Despite discrepancies about the species involved, certain intestinal bacteria appear to be the link between gut dysbiosis and RA (Table 1). These findings suggested that gut bacteria can contribute to the pathogenesis of RA. In a recent meta-analysis, gut dysbiosis in RA patients was characterized by a depletion of anti-inflammatory butyrate-producing bacteria (i.e., Faecalibacterium) and enrichment of pro-inflammatory bacteria (i.e., Streptococcus) (Wang et al., 2022b).

TABLE 1 Summary of studies evaluating the role of Gut Microbiota in Rheumatoid Arthritis patients.

Autor	Country	Disease group	Case (n)	RF positive	ACPAs positive	Control group	Control (n)	Method	Overabundance	Lower abundance	Smoking	DMARDs	Diet
Alpizar-Rodríguez et al.	Switzerland	p-RA[1]	83	34%	46%	First-degree relatives	53	16sRNA	Prevotellaceae (*P.copri, P. stercorea, P. oralis, P. oulora, P. conceptionensis*) Lactobacillaceae	NA	19%	NA	NA
Rooney et al.	The UK	p-RA[2]	25	44%	100%	Healthy controls	44	16sRNA	Lachnospiraceae Helicobacteraceae Erysupelotrichaceae Bifidobacteriaceae	Bacteroidaceae Barnesiellaceae Methanobacteriaceae	60%	NA	History of vegetarian diet 12%
Scher et al.	EE.UU.	NORA e-RA	44 26	NORA: 95% e-RA: 81%	NORA: 100% e-RA 85%	Healthy controls	28	16sRNA WGS	NORA: Prevotellaceae (P. copri)	NORA Bacteroidaceae (genus Bacteroides) Lachnospiraceae Clostridiaceae	NA	NORA: Naïve 100% e-RA MTX 41% G.C.s 12% Biologic 12%	Patients with current extreme diet and probiotic use were excluded
Maeda et al.	Japan	NORA	17	82.4%	82.4%	Healthy controls	14	16sRNA	Prevotellaceae (*P. copri and P. stercorea*)	Bacteroidaceae (genus Bacteroides)	NA	Naïve 100% NSAIDs were allowed	Patients with extreme diet use were excluded
Zhang et al.	China	NORA e-RA	94 21	NA.	NA.	Unrelated healthy controls Healthy relatives	80 17	WGS MWAS	*Lactobacillus salivarius Bacteroides*, Gordonibacter pamelaear, *Eggerthella lenta*, *Clostridium asparagiforme*	Veillonella, *Hemophilus* ssp. *K. pneumoniae, Megamonas hypermegale, Sutterella wadsworthensis, Bifidobacterium bifidum*	NA	Naïve 82% Cs 18%	NA
Chen et al.	EE.UU.	e-RA	40	100%	83%	Healthy controls Healthy relatives	15 17	16sRNA	*Coriobacteriaceae (Eggerthella, Collinsella)*	Ruminococcaceae (F. prausnitzii)	NA	Cs 16.2% PDN 48.9% Biological 34%	NA
Kishikawa et al.	Japan	e-RA	82	74%	66%	Healthy controls	42	WGS	Prevotella spp. (*P. denticola, P. marshii, P. disiens, P. corporis* and *P. amnni*). *Gardnerella vaginalis. Bacteroides sartorii.*	NA	NA	Naïve 71% Cs 28% Biological 2%	Strict vegetarians were excluded
El Menofy et al.	Egypt	e-RA	45	NA	NA	Healthy	15	16sRNA	*Megasphaera, Adlercreutzia, Ruminococcus, Bacteroides, Collinsella*, and *Acidaminococcus*	*Acidaminococcus, Streptococcus, Gardenella, Anaerococcus*, and *Sphingomonas*	NA	DMARDs, GCs, and NSAIDs were allowed	NA
Ruiz-Limón et al.	Spain	e-RA	110	81.8%	80%	Healthy controls	110	16sRNA	*Collinsella Bifidobacterium*	*Oxalobacteraceae*	30%	DMARDs 100%. Biologic DMARDs 38.1%. GCs 18.2%.	Subjects with extreme diets or taking probiotics were excluded

(Continued)

10.3389/fcimb.2023.1092118

TABLE 1 Continued

Autor	Country	Disease group	Case (n)	RF positive	ACPAs positive	Control group	Control (n)	Method	Overabundance	Lower abundance	Smoking	DMARDs	Diet
Lee et al.	Korea	e-RA	99	82.8%	76.5%	Healthy controls	30	16sRNA	*Streptococcus* *Candida* spp.	*Bifidobacterium* *Blautia*	NA	csDMARDs 87.9% Biologics (40.4%)	NA
Chiang et al.	Taiwan	e-RA	138	75.8%	73.4%	Healthy controls	21	16sRNA	*A. muciniphila*		8.6%	csDMARDs Biologics (82.85) NSAIDs GCs	Subjects with extreme diets or taking probiotics were excluded
Jeong et al.	Korea	p-RA NORA	25	NA	NA	Healthy controls	25	16sRNA	Bacteroidales Prevotella (genus)	Erysipelotrichales Coriobacteriales Collinsella (genus)	NA	NSAIDs	Subjects taking probiotics or prebiotics were excluded

At the family level (at the species level)
16sRNA, 16S rRNA sequencing; Cs, conventional synthetic; DMARDs, Disease-Modifying anti-rheumatic drugs; e-RA, Established RA; MTX, methotrexate; MWAS, Metagenome-wide association study; NA, Information not available; WGS, Whole-genome shotgun sequencing; PDN, prednisone; p-RA, preclinical RA stage.
[1]Anticitrullinated protein autoantibodies (ACPAs) positivity and-or rheumatoid factor (RF) positivity or musculoskeletal symptoms with or without undifferentiated arthritis.
[2]Anti-CCP positive individuals with nonspecific musculoskeletal symptoms and without clinical evidence of synovitis.

Although the underlying mechanisms of the gut-joint axis still need to be investigated in more detail, new data suggest that gut microbiota is likely among the key players within the gut–joint axis. Gut dysbiosis precedes the onset of disease and could lead to changes in systemic immune responses, loss of tolerance, and the development of arthritis (Wang et al., 2022b). Notably, modifications in gut microbiota showed beneficial effects on symptom relief in animal models of RA, which were associated with the modulation of the immune response (Horta-Baas et al., 2021). However, the precise underlying mechanisms by which gut dysbiosis induces the development of arthritis remain unknown. This review aimed to highlight the mechanisms by which gut dysbiosis plays a role in the pathogenesis of RA.

Mechanisms that account for the gut-joint axis in RA

Researchers have continued exploring the underlying mechanisms linking gut dysbiosis to RA in recent decades. Evidence suggests that gut dysbiosis can contribute to arthritis susceptibility through multiple pathways. Alterations in gut barrier function, molecular mimicry, alterations in the ratio of T helper 17 (Th17)/regulatory T (Treg) cells, an imbalance of T follicular helper cells (Tfh)/T follicular regulatory (Tfr) cells, cross-talk between microbiota-derived metabolites and immune cells, and alterations in the gut microenvironment are the mechanism proposed to explain a gut-joint axis through the interaction of gut microbiota with the host immune system (Figure 1) (Larsen, 2017; Picchianti-Diamanti et al., 2018; Zhang et al., 2020; Iljazovic et al., 2021a).

Alterations in gut barrier function

Gut barrier function is part of the host's defense against microorganisms, preventing pathogens from invading the intestine into the systemic circulation and extra-intestinal tissues and triggering immune responses (Correa-Oliveira et al., 2016; Xu et al., 2022b). The gut mucosal barrier, a monolayer of intestinal epithelial cells connect *via* tight junctions, separates the host from enormous amounts of antigens of both dietary and microbial origin. Mucus plays a vital part in this barrier by permitting access to host tissue for many diffusive molecules while limiting both the entry and colonization of microbes (Foster et al., 2017).

Zonulin is an enterotoxin secreted by enterocytes in response to dietary and microbial stimuli that disengages proteins zonula occludens-1 (ZO-1) and occludin from the tight junction (TJ) complex, leading to intestinal barrier damage, an increased permeability, translocation of bacterial products in the blood, and initiation of inflammatory responses (Xu et al., 2022b; Tajik et al., 2020; Audo et al., 2022). Zonulin induces T-cell-mediated mucosal inflammation and may control immune cells' transmigration from the gut into the joints (Tajik et al., 2020).

The gut barrier is controlled by fine-tuned communications between gut microbiota and the host immune system (Litvak et al., 2018). Luminal antigen sampling by enterocytes *via* the transcellular pathway and dendritic cells regulates molecular trafficking between

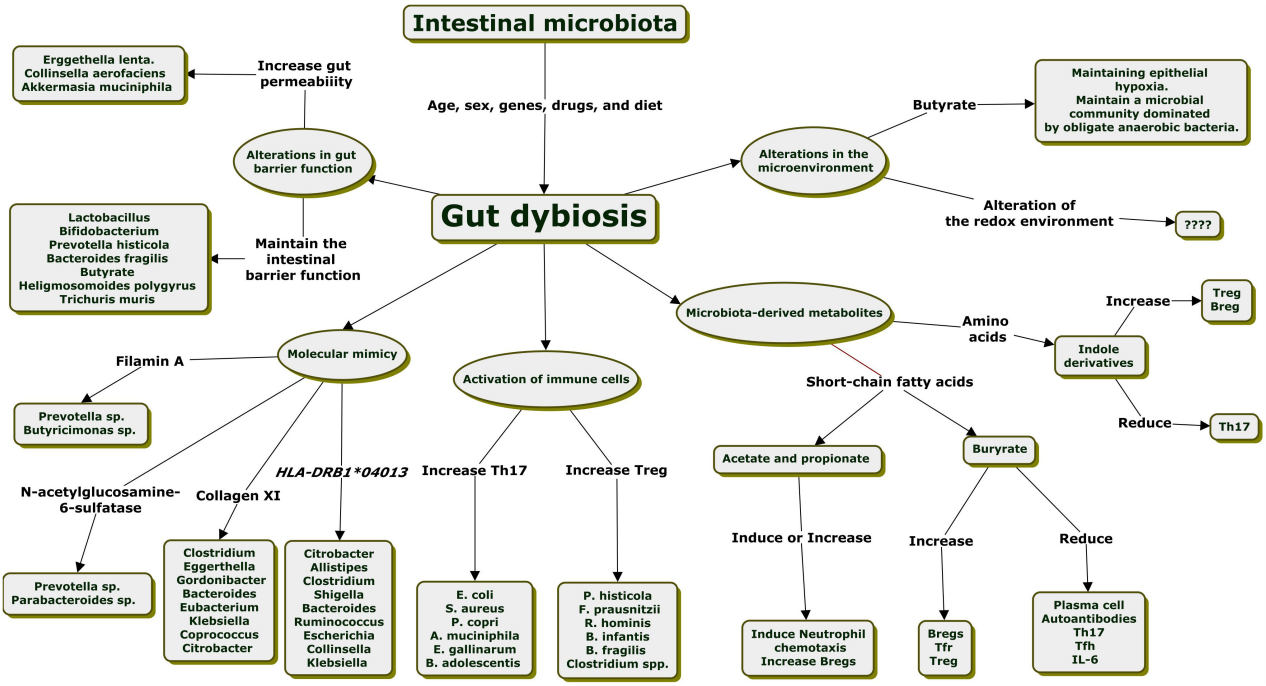


FIGURE 1
Mechanisms explained the relationship between intestinal dysbiosis and the development of rheumatoid arthritis.

the intestinal lumen and the submucosa, leading to either tolerance or immune response to non-self. The loss of mucosal barrier function affects bacterial and antigen trafficking and allows microbes and their products to cross into the lamina propria and sub-epithelial spaces. The interaction of Toll-like receptors (TLR) and pathogen-associated molecular patterns (PAMP) on microbes have the potential to activate the immune system, leading to the production of pro-inflammatory cytokines such as interleukin (IL)-6 (IL-6), tumor necrosis factor-alpha (TNF-α), or IL-1β to eliminate the pathogen (Chiang et al., 2019; Opoku et al., 2022; Parantainen et al., 2022).

The disruption of the epithelial barrier function occurs in the pre-clinical phase of RA in murine models and humans (Tajik et al., 2020; Audo et al., 2022). Gut dysbiosis might trigger the breakdown of gut barrier integrity and the leakage of microbiota or their metabolites into gut tissue and even venous or lymphatic circulation, enabling exposure of the immune cells to bacterial antigens leading to local and systemic inflammation, increased pro-inflammatory cytokines such as TNF-α and IL-17A, and differentiation of autoreactive Th17 cells (Figure 2) (Berthelot et al., 2019; Chiang et al., 2019; Man et al., 2020; Tajik et al., 2020; Garabatos and Santamaria, 2022; Zhao et al., 2022). The migration of self-reactive cells to the joints can cause cartilage and bone damage (Zhao et al., 2022).

Translocation of gut bacteria (dead or alive) to joints from the intestine may lead to joint inflammation in RA patients. In agreement with these findings, some studies demonstrated that DNA from a variety of bacterial species (*Prevotella*, *Fusobacterium*, *Porphyromonas*, and *Bacteroides*) or bacterial cell wall constituents had been observed in serum and synovial fluids from RA patients (van der Heijden et al., 2000; Reichert et al., 2013; Zhang et al., 2015; Larsen, 2017; Zhao et al., 2018; Cheng et al., 2022). A recent study demonstrated that microbial invasion of the joint synovial fluid happens in the fourth stage of RA (RAS4) and that *Prevotella copri* was also found abundant in most synovial fluid samples of RA patients in RAS4 (Cheng et al., 2022).

It is still unclear how this bacterial nucleic acid (and perhaps still living bacteria from microbiota) reaches cartilage. Possible mechanisms, including bacteria or their components, are transported to the joints *via* the mesenteric lymphoid organs or the bloodstream. Another possibility includes that bacterial DNA is secondary to the migration of immune cells trafficking from the intestine harboring DNA (macrophages or leukocytes) (van der Heijden et al., 2000; Martinez-Martinez et al., 2009; Temoin et al., 2012; Berthelot et al., 2019; Cheng et al., 2022). Tajik et al. show that zonulin-dependent transmigration of immune cells from the gut into the joints occurs during the onset of arthritis. Furthermore, larazotide (a zonulin antagonist) treatment attenuated the enhanced intestinal permeability and blocked the migration of immune cells from the intestine to the joints (Tajik et al., 2020).

Enterobacteriaceae and *Klebsiella's* lipopolysaccharide (LPS) could promote inflammation by increasing intestinal permeability (Chiang et al., 2019). The fiber-containing diet in mice colonized with *P. copri* increased inflammatory cytokine production, adaptive immunity activation, and gut barrier dysfunction (Jiang et al., 2022). In rodents, intestinal inflammation occurred earlier than the onset of arthritis, and restoration of the intestinal barrier by probiotics (*Bifidobacterium adolescentis*), butyrate, or using larazotide, was found to attenuate arthritis (Tajik et al., 2020; Fan et al., 2020a; Audo et al., 2022).

New-onset RA (NORA) patients had altered gut barrier integrity with lower expression of TJ proteins occludin and claudin-1 in intestinal epithelial cells on ileal mucosal and decreased ZO-1 in

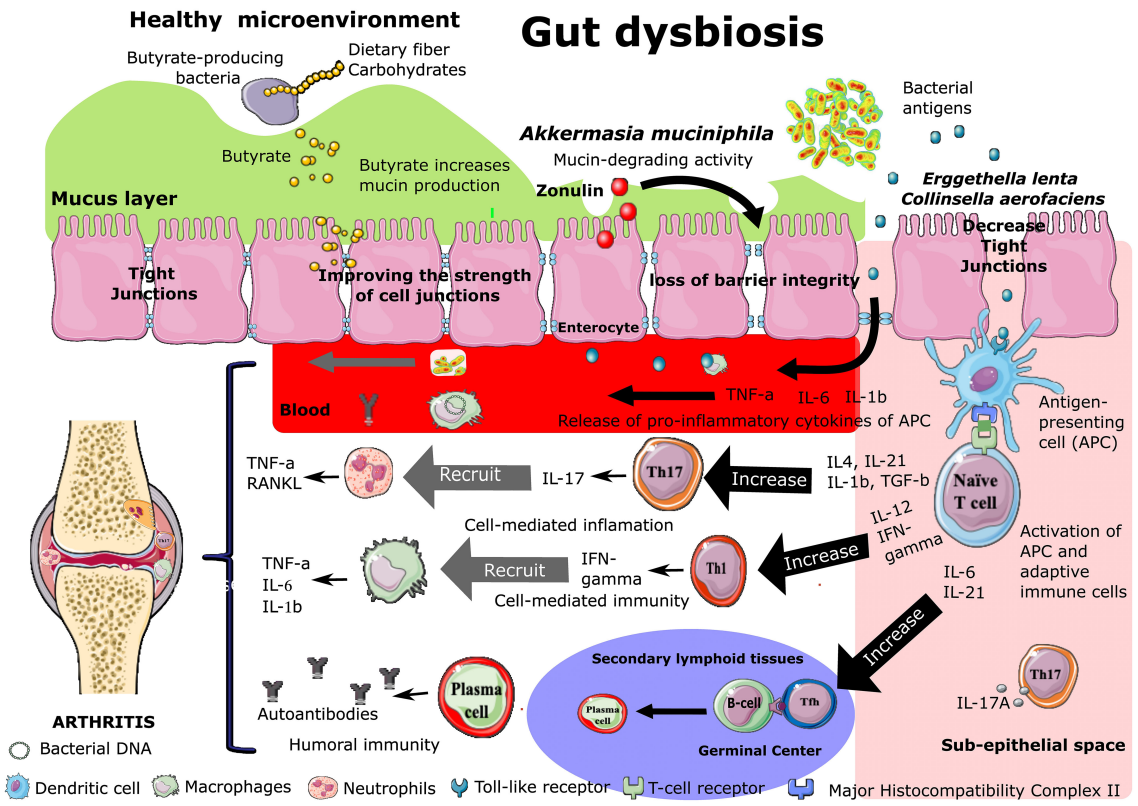


FIGURE 2
Effect of intestinal dysbiosis on intestinal permeability and B and T cell polarization during the development of rheumatoid arthritis. Increased Zonulin secretion is followed by increased intestinal permeability ("leaky gut") associated with the disassembly of ZO-1 protein from the tight junction complex. Bacteria or their components are transported to the joints *via* secondary lymphoid organs or the bloodstream. After encountering the microbiota-derived antigen presented by antigen-presenting cells (APCs), naive CD4+ T cells differentiate into various subsets, including at least Th1, Th17, and Tfh cells.

the colon, as well as increased serological gut permeability markers (i.e., zonulin/ZRPs, LBP and sCD14) (Tajik et al., 2020; Audo et al., 2022).

The gut microbiota produces metabolites that can maintain intestinal barrier function. Butyrate, a microbiota-derived metabolite, contributed to the intestinal barrier function by multiple mechanisms. Butyrate increased the expression of the TJ protein claudin-1 and induced the redistribution of the TJ proteins occludin and ZO-1 in the cellular membrane (Tajik et al., 2020; Xu et al., 2022b). These protective effects seem largely concentration-dependent, with higher doses causing epithelial barrier disruption (Blaak et al., 2020).

Furthermore, butyrate is an essential regulator of intestinal barrier function through stimulation of mucin synthesis and quality (Blaak et al., 2020; Marazzato et al., 2022). Luminal-derived butyrate is a primary form of energy for the epithelial cells; 70% of the total amount of oxygen consumed by human colonocytes *in vitro* was used for butyrate oxidation (Correa-Oliveira et al., 2016; Van de Wiele et al., 2016; Kang et al., 2017; Lin and Zhang, 2017; Cani, 2018; Blaak et al., 2020; Fan et al., 2020b). These findings showed that butyrate is essential for a healthy colonic epithelium. Similarly, Indole-3-formaldehyde (3-IALD), a tryptophan metabolite, plays a role in maintaining intestinal epithelial barrier integrity and suppressing inflammatory responses dependent on AHR/IL-22 in mice (Xu et al., 2022b).

Some studies have investigated the mechanisms by which intestinal bacteria can alter the permeability of the intestinal barrier. Studies *in vitro* and in murine models of arthritis have demonstrated the arthrogenic role of *Collinsella*. *In vitro* experiments showed that the CACO-2 cell line cultured in the presence of *Collinsella aerofaciens* enhances gut permeability by decreasing the expression of tight junction protein ZO-1 in epithelial cells (Chen et al., 2016). In the humanized murine model of arthritis, DQ8 mice orally gavaged with *C. aerofaciens* showed an increase in gut permeability, and inoculation of *C. aerofaciens* into CIA-susceptible mice induces severe arthritis (Zhang et al., 2015; Chen et al., 2016). The overabundance of *Collinsella* in the gut microbiome has been reported in RA patients (Chen et al., 2016; El Menofy et al., 2022; Ruiz-Limon et al., 2022). In a recent study, *C. aerofaciens* was elevated exclusively in early RA (Cheng et al., 2022). Therefore, the overabundance of *C. aerofaciens* might contribute to the early breach in gut barrier integrity. In another study, the expansion of *Collinsella* was independently associated with inflammatory activity in RA patients (Ruiz-Limon et al., 2022). These findings suggest that the genus *Collinsella* seems to have an essential role in the pathogenesis of RA and its severity.

Similarly, the mucin-degrading activity of *Akkermansia muciniphila* can affect gut barrier function (Lin and Zhang, 2017). In the CIA mouse model, an overabundance of *A. muciniphila* was observed at the onset of arthritis (Peng et al., 2019). In one study,

patients with active RA had a higher relative abundance of *Akkermansia* than those with inactive RA (Chiang et al., 2019). This effect may be related to its ability to degrade mucus and thus increase the exposure of resident immune cells to gut microbial antigens (Chiang et al., 2019). Furthermore, *Akkermansia* has also been associated with pro-inflammatory pathways, including the upregulation of B- and T-cell receptor signaling and the induction of M1-like macrophage response (Fan et al., 2021).

On the other hand, a probiotics-rich diet has been reported to ameliorate some RA symptoms by restoring barrier mechanisms in the gut mucosal (Opoku et al., 2022). Therapeutic administration of human gut-derived *Prevotella histicola* reduced the incidence and severity of CIA. *P. histicola* increased expression of ZO-1, preserving gut epithelium integrity in the context of inflammation (Marietta et al., 2016). *Lactobacillus* and *Bifidobacterium* could limit the development of autoimmune diseases in genetically susceptible individuals by increasing the expression of TJ proteins (Hills et al., 2019). *Bacteroides fragilis* sphingolipids promote gut barrier integrity (Garabatos and Santamaria, 2022).

Molecular mimicry

Gut bacteria can activate the immune system and trigger T-cell responses against self-antigens by molecular mimicry (Jethwa and Abraham, 2017; Zhang et al., 2020). The molecular mimicry or crossreactivity hypothesis proposes that an exogenous substance (i.e., a microbial agent with antigenic similarity to self-antigens) may trigger an immune response against self-antigens (Rashid and Ebringer, 2012). *Prevotella* contributes to arthritis development in mice by activating autoreactive T cells specific for the arthritis-relevant autoantigen Ribosomal Protein L23a (RPL23A) (Garabatos and Santamaria, 2022). Similarly, peptides derived from *Bacteroides fragilis*, *Candida albicans*, and *Streptococcus sanguis* are similar to collagen-type-II and induced cross-reactive responses in the CIA model (Costalonga et al., 2002; Yordanov et al., 2005; Zheng et al., 2020; Zhou et al., 2020).

Pianta et al., using discovery-based proteomics to detect HLA-DR-presented peptides in synovia or peripheral blood mononuclear cells, identified N-acetylglucosamine-6-sulfatase (GNS) and filamin A (FLNA) as targets of T and B cell responses in 52% and 56% of RA patients, respectively. GNS and FLNA were present in synovial fluid and inflamed synovial tissue (Pianta et al., 2017). The HLA-DR-presented GNS peptide has an evident homology with epitopes from *Prevotella* sp. (arylsulfatase protein) and *Parabacteroides* sp. (protein *N*-acetylgalactosamine-6-sulfatase). Similarly, the HLA-DR-presented FLNA peptide has homology with epitopes from proteins of *Prevotella* sp. (WP_028897633) and *Butyricimonas* sp. (WP_065219401.1). Therefore, sequence homology between T cell epitopes of two self-proteins and multiple gut microbial peptide epitopes may link gut microbiota and autoimmunity in RA.

Zhang et al. (Zhang et al., 2015) describe several gut microbial proteins as molecular mimicry for human self-antigens (collagen XI and *HLA-DR4/1*). Molecular mimicry of RA-associated antigens such as Collagen XI by gut microbial genes from *Clostridium, Eggerthella, Gordonibacter, Bacteroides, Eubacterium, Klebsiella, Coprococcus*, and *Citrobacter* was also suggested, with a number of the genes belonging to metagenomic linkage groups enriched in RA gut samples. RA-enriched genes from *Citrobacter, Allistipes, Clostridium, Shigella, Bacteroides, Ruminococcus, Escherichia, Collinsella*, and *Klebsiella* mimicked motifs in *HLA-DRB1*04013*.

Microbial antigens can be presented to CD4+ T cells by dendritic cells and macrophages, leading to the differentiation of inflammatory T cell subtypes. Therefore, molecular mimicry may partly explain the relationship between alterations in intestinal barrier function and the development of autoimmunity in RA patients.

Gut dysbiosis induces the development of chronic inflammation and autoimmunity

The leading site of inflammation RA is the synovium, which includes a cellular surface layer of macrophages and fibroblast-like synoviocytes and an underlying tissue layer that contains fibroblasts, blood vessels, and lymphatics arrayed within a loose collagenous matrix. Immune cells ingress into the synovium is a critical process in the pathogenesis of RA (Qu et al., 2019). Pro-inflammatory cytokines and chemokines stimulate macrophages, neutrophils, T cells, and B cell infiltration (Block et al., 2016). Th1 and Th17 cells produce excessive pro-inflammatory cytokines, stimulating B cells to produce autoantibodies and macrophages to produce pro-inflammatory cytokines (Wang et al., 2019c; Fan et al., 2020a). These cytokines lead to synovial hyperplasia, pannus formation, and destruction of cartilage and joints. Pro-inflammatory cytokines induce fibroblasts to produce matrix metalloproteinases and RANKL (receptor activator of nuclear factor kB ligand), which mediate the destruction of bone and cartilage tissue, leading to the development of RA (Zhao et al., 2022).

Gut dysbiosis can lead to inflammation in the intestinal mucosa and tissue damage, promoting the loss of immune tolerance and the development of autoimmunity (Inda et al., 2019). Gut microbiota, primarily through microbiota-derived metabolites, has a role in regulating T cell functions and could disrupt gut immune homeostasis through abnormal antigen presentation and modulating the adaptive immunity, especially in the polarization of näive T cells to Th17 cells and generation of autoreactive B cells (Lin and Zhang, 2017; Wang et al., 2019b; Di Gangi et al., 2020; Zhang et al., 2020; Garabatos and Santamaria, 2022; Marazzato et al., 2022). Gut dysbiosis leads to inflammation by alterations in the ratio of Th17/Treg cells and an imbalance of Tfh/Tfr cells.

Gut microbiota can modulate the Th17/Treg balance

Bacterial strains from the human intestine can regulate the differentiation and activation of Th17 and Treg cells (Narushima et al., 2014). Intestinal mucosa contains many Th17 and Treg cells (Zhao et al., 2022). Th17 cells usually are in the gut in a microbiota-dependent manner, maintaining tissue homeostasis and fighting against extracellular bacteria and fungi. Contrarily, intestinal Treg cells maintain immune tolerance to dietary antigens and gut microbiota, retain tolerance to self-antigens, and suppress the activation and proliferation of self-reactive effector T cells (Horta-Baas et al., 2017; Haase et al., 2018; Schinnerling et al., 2019; Sun et al.,

2019). Microbiota-induced Tregs attenuate intestinal damage caused by exaggerated immune responses against pathogens (Kamada et al., 2013). Under physiological conditions, the functions of Th17 and Treg cells are in balance (Chiang et al., 2019).

Th17 mainly secrete IL-17 and IL-22 and intervenes in developing chronic immune-mediated inflammatory diseases, including RA (Feng et al., 2022). IL-17A is a potent inducer of matrix metalloproteinases, recruits neutrophils to the joint, and stimulates osteoclastogenesis resulting in cartilage and bone destruction (Amdekar et al., 2013; Pineda et al., 2014; Lee et al., 2015; du Teil Espina et al., 2019; Huang et al., 2019; Marazzato et al., 2022). Alteration in the ratio between Th17 and Treg cells plays a crucial role in the early phase of RA development (Figure 2) (Lee et al., 2015; Marazzato et al., 2022).

Gut-derived Th17 cells are thought to be essential in the link between gut microbiota and RA (Buckley and McGettrick, 2018). In animal models of arthritis, a pathogenic role of gut-derived Th17 cells has been demonstrated (Table 2). Microbiota from CIA-susceptible mice showed an altered ratio of Th17/Tregs cells, characterized by increased Th17 cells and reduced Treg cells (Pineda et al., 2014; Liu et al., 2016; Wu et al., 2018). Before the onset of CIA, Th17 cells aggregate in germinal centers. The release of autoantibodies and cytokines into circulation carries them to tissues and organs, leading to the activation of macrophages culminating in the release of pro-inflammatory cytokines (IL-6, IL-1, TNF-α, and IL-17). Germ-free mice conventionalized with the gut microbiota from CIA-susceptible mice, which have higher levels of serum IL-17, develop greater severity of arthritis (Liu et al., 2016). Maeda et al. demonstrated that FMT from RA patients in germ-free SKG mice could activate autoreactive T cells and an increased number of Th17 cells in the intestine compared with SKG mice inoculated with fecal microbiota of HCs (Maeda et al., 2016).

On the contrary, some bacterial gut microbiota species exert an anti-inflammatory effect by stimulating Treg cells. Therapeutic administration (preventive or therapeutic approach) of human gut-derived *P. histicola* reduced the incidence and severity of CIA in HLA DQ8-transgenic mice by triggering the generation of IL-10-producing Treg cells, decreasing Th17 responses in the intestine and CD11c+CD103+ dendritic cells in the gut and the spleen (Marietta et al., 2016). Rats orally gavaged with *B. adolescentis* before immunization had significantly higher Tregs frequency and lower TNF-α than that in the late *B. adolescentis* treated group (Fan et al., 2020a).

In the mouse gut, colonic Treg induced by Clostridium bacteria are vital players in gut homeostasis and prevent colitis (Alameddine et al., 2019). In the human colon microbiota, Clostridium IV *Faecalibacterium prausnitzii* induces the formation of Treg cells *via* the activation of dendritic cells and causes the secretion of IL-10 by T cells (Garabatos and Santamaria, 2022; Wang et al., 2022b). Another gut bacteria, Bacteroides fragilis, *via* its carbohydrate antigen polysaccharide A (PSA), may promote the differentiation of Treg *in vitro* or mice through dendritic cell modulation. Furthermore, PSA stimulates Treg cells and suppresses Th17 cell responses through an IL-2-dependent mechanism (Horta-Baas et al., 2017).

In humans, gut bacteria have been shown to influence the polarization of T-cell subpopulations. At the phylum level, *Verrucomicrobiota* showed a positive correlation with the absolute number of Tregs, while *Firmicutes* showed a negative correlation with the total number of Th17 cells in RA patients (Wang et al., 2022a). Increased abundance of *Prevotella* and *Collinsella* in patients with RA are correlated with the production of Th17 cell cytokines. Bacterial species associated with increased Th17 or Treg are presented in Figure 1 (Liu et al., 2016; Sun et al., 2019; Fan et al., 2020b; Garabatos and Santamaria, 2022; Yang et al., 2022). Human-derived *Clostridia* are potent inducers of Treg cells (Narushima et al., 2014). Bacterial strains belonging to Clostridia cluster IV and XIVa stimulate the secretion of transforming growth factor beta (TGF-β) by intestinal epithelial cells, promoting the expansion of

TABLE 2 Summary of possible underlying mechanisms by which intestinal dysbiosis contributes to the development of arthritis in rodents with collagen-induced arthritis.

	Model	Animal	Key findings
Marietta et al.	CIA	DBA/1 mice	Mice gavaged with *P. histicola* showed reduced IL-2, IL-17, TNF-a, and increased IL-4 and IL-10. Mice treated with *P. histicola* showed a reduction in anti-CII antibodies. Mice treated with *P. histicola* had increased numbers of CD103+ intestinal dendritic cells. *P. histicola* treated mice had a significantly lower gut permeability.
Hiu et al.	CIA	DBA/1J mice	The butyrate treatment alleviated arthritis severity. IL-1β, IL-6, and IL-17A were significantly downregulated in the butyrate group. In contrast, butyrate upregulated the mRNA expression level of IL-10 in synovial tissues. Butyrate promoted the polarization of Treg but not Th17 cells.
Xu et al.	CIA	Sprague–Dawley rats	Did not find a correlation between changes in gut bacteria and changes in amino acids metabolites (tryptophan, histidine, and phenylalanine) Gut dysbiosis was characterized by bacteria related to butyrate metabolism. Tripterygium glycosides could lead to a variation in metabolites in the tryptophan and phenylalanine pathways.
Jiang et al.	CIA	DBA/1J mice	P. copri was capable of activating the TLR4 pathway and producing LPS-induced inflammation. The fiber-containing diet-fed (FCD) mice displayed elevated levels of anti-collagen antibodies and more Th17 cells in the mesenteric lymph nodes.
Tajik et al.	CIA	DBA/1J mice	Intestinal inflammation and an increase in intestinal permeability precede the onset of arthritis. Th1 and Th17 cells accumulate in the intestine before arthritis onset. Butyrate levels drop before the onset of arthritis, Reducing intestinal barrier permeability attenuates arthritis.

Treg cells in the colonic lamina propria (Kamada et al., 2013; Lin and Zhang, 2017; Sun et al., 2019).

The Th17/Treg cells ratio is skewed in favor of Th17 cells in RA patients compared to controls. One study demonstrated an alteration in Th17/Treg balance, with higher Th17 levels and lower Treg levels in the peripheral blood, from early RA patients compared to HCs (Marazzato et al., 2022). Furthermore, RA patients present an impaired function of circulating Treg cells and an increase in Th17 cells in plasma and synovial fluid (Horta-Baas et al., 2017). Treg cells in RA patients show a decreased suppressive activity, which can be related to the potential of Treg cells to convert into Th1-like Treg cells, secreting interferon-gamma (INF-γ) as well as Th17-like Treg cells, secreting IL-17 (Haase et al., 2018; Fan et al., 2020b). In a recent study, Wang et al. found that the number of Tregs and Th17/Tregs ratio were negatively correlated with disease activity in RA patients (Wang et al., 2022a). In another study, Chiang et al. demonstrated a positive correlation between the abundance of the phylum Euryarchaeota with serum levels of IL-6 or IL-17A (Chiang et al., 2019). These results indicate a correlation between gut microbiota and RA disease activity.

Gut microbiota can modulate the Tfh/Tfr balance

The production of antibodies occurs through B cells, which require Tfh cells for activation. B cells produce antibodies against extracellular pathogens and toxins. Antibodies are produced within germinal centers, regulated by interactions between B, Tfh, and Tfr cells (Ribeiro et al., 2022). Tfh cells are a CD4 T cell lineage that interacts with B cells to form germinal centers, promote differentiation into plasma cells, promote class-switching, somatic hypermutation, and the generation of high-affinity antigen-specific memory B cells and antibody-producing cells (Diamanti et al., 2016; Wang et al., 2019b; Zeng et al., 2022). Therefore, Tfh cells control initiation and the outcome of the germinal center B cell response. IL-6 and IL-21 can induce naive CD4+ T cells to differentiate into Tfh cells. Furthermore, IL-21 produced by Tfh cells is a factor that potently promotes B cell activation (Xie et al., 2019).

Microbial antigens can induce differentiating of B cells, with the help of Tfh cells, to plasma cells. Segmented filamentous bacteria are responsible for the induction of Tfh cells in Peyer's patches (PP). Using the K/BxN mice model, Teng et al. demonstrated that PP Tfh cells were essential for segmented filamentous bacteria-induced arthritis despite producing auto-antibodies occurring in systemic lymphoid tissues, not PP. Consequently, gut microbiota can regulate arthritis development by driving the induction and gut Tfh cells migration to the systemic lymphoid tissues and inducing autoantibody production (Teng et al., 2016).

Excessive Tfh cell activity can lead to autoimmunity. The proper regulation of Tfh cell differentiation is essential for normal immune function and for preventing autoimmune disease. Tfr cells can suppress Tfh cell-mediated humoral immunity by downregulating the production of effector cytokines such as IL-4, IFN-γ, and IL-21, which are essential for B cell activation and class switch recombination (Wang et al., 2019b; Takahashi et al., 2020). Consequently, Tfr cells maintain tolerance during the B cell response. The increase of Tfh and decrease in the number of Tfr cells are associated with the growth of self-reactive B cells, which lead to the production of high levels of self-reactive autoantibodies (Xie et al., 2019; Wang et al., 2019c; Cao et al., 2020; Takahashi et al., 2020).

Self-reactive antibodies are present in approximately 70 to 80% of RA patients (Table 1). Some studies show that the imbalance of Tfh and Tfr cells may be involved in the association between intestinal dysbiosis and RA pathogenesis. Block et al. demonstrated that antibiotic treatment of IL-17-deficient mice inhibited arthritis, refuting the concept of a role for Th17 cells in gut-regulated K/BxN mice-induced arthritis. Instead, the authors proposed that the ability of the gut microbiota to regulate arthritis was dependent on Tfh cells (Block et al., 2016). In another study, Zeng et al. showed that Tfh and Tfr cells were increased in spleen germinal centers in the CIA mice model and their levels and functions returned to normal after the anti-TNF-a and anti-IL-1β treatment (Zeng et al., 2022). Although the Tfh/Tfr ratio did not change significantly, the relative enhancement of B cell function remained as the final result, which may be related to the relatively higher Tfh cell level.

Two studies in experimental models report a relationship between microbiota-derivated metabolite butyrate and alterations with Tfh/Tfr cells. Dietary butyrate supplementation conferred anti-inflammatory benefits in a CIA mice model (DBA/1). A butyrate-rich diet started on the first day of collagen immunization significantly lowers the overall incidence of arthritis and reduces the severity of joint inflammation. These effects were explained by rebalancing Tfh cells and Tregs and reducing antibody production (He et al., 2022). In another study, butyrate prevented arthritis development in the CIA and SKG mice model. However, butyrate does not prevent collagen antibody-induced arthritis (CAIA) or the development of CIA when butyrate begins after booster immunization. These findings suggested that butyrate suppresses the initial phase of Tfh cell-mediated autoimmune responses rather than the effector phase of arthritis development (Takahashi et al., 2020).

In RA patients, a reduced number of Tfr cells has been associated with the elevation of autoantibodies and disease severity (Takahashi et al., 2020). Wang et al. have shown that both circulating Tfh and Tfr cells were increased in RA patients compared with HCs. The percent Tfh cells positively correlated with the serum levels of serum RF, ACPA, and disease activity score in 28 joints (DAS28) index. Conversely, the Tfr/Tfh ratio was negatively correlated with the level of serum RF, ACPA, and DAS28 (Wang et al., 2019c). Similarly, Cao et al. found that peripheral blood Tfh cells were increased in RA patients, while the frequency of Tfr cells and the ratio of Tfr/Tfh were significantly decreased compared to HCs. Furthermore, the Tfr/Tfh ratio was positively correlated with RF and negatively correlated with the DAS28 index (Cao et al., 2020).

In another study, Ribeiro et al. reported that the frequency of circulating Tfh and Tfr cells was decreased in patients with RA and that the Tfr/Tfh ratio was similar to HCs (Ribeiro et al., 2022). These results show inconsistent results on the role of the Tfr/Tfh ratio in the pathogenesis of RA. Further studies are required to determine the role of alterations in Tfh and Tfr cells in RA's pathogenesis and whether the gut microbiota modulates these cells during the development of arthritis.

Cross-talk between microbiota-derived metabolites and immune cells

Recent works revealed that the relationship between gut dysbiosis and RA could be mediated by gut microbiota-derived metabolites (Yu et al., 2021; Yao et al., 2022). Microbiota-derived metabolites are critical for immune regulation (Haase et al., 2018; Iljazovic et al., 2021b; Yang et al., 2022). Gut dysbiosis may lead to alterations in fecal metabolites, and a deficiency of beneficial bacteria and their metabolites may stimulate the inflammatory response (Yu et al., 2021). Among gut bacterial metabolites, SCFAs, amino acids, and their metabolites have been implicated in the pathogenesis of RA (Chen et al., 2021).

Short-chain fatty acids

SCFAs are small organic acids produced by intestinal bacteria through the fermentation of the cecum and colon's undigested food components (mainly dietary fiber and carbohydrates) (Marazzato et al., 2022). SCFAs can regulate multiple metabolic pathways both in the gut and outside the intestine and are associated with a variety of physiological processes, such as energy balance, maintenance of the intestinal barrier, sugar/lipid metabolism, and immunomodulatory properties, thus contributing to disease prevention (Xu et al., 2022b).

The main SCFAs produced by intestinal bacteria in the human gut are acetate, propionate, and butyrate. Other SCFAs, include pentanoate, hexanoate, and heptylate (Jiang et al., 2022). Gram-negative bacteria, such as *Bacteroides*, primarily generate propionate and acetate, whereas gram-positive bacteria, such as *Firmicutes*, produce large amounts of butyrate (Lin and Zhang, 2017; Lee et al., 2019; Zhang and Frenette, 2019; Marazzato et al., 2022). Propionate and acetate are absorbed at the gut level and pass through bloodstream circulation, reaching and affecting distant tissues. Conversely, butyrate carries on its functions within the gut (Marazzato et al., 2022). The concentration of fecal SCFAs depends on dietary intake, the host's gut microbiota community and host-microbiota metabolite flux, and the liver's and small intestine's absorptivity (Fan et al., 2020a).

The immunomodulatory properties of SCFAs are related to their effect on the innate and acquired immune system cells by inhibiting histone deacetylase (HDACs) (Correa-Oliveira et al., 2016; Lee et al., 2019). SCFAs could regulate neutrophils and macrophages and thus modulate the magnitude of inflammatory responses (Zhang and Frenette, 2019; Man et al., 2020; Wang et al., 2020). Acetate and propionate activate cell surface receptor GPR43 to induce neutrophil chemotaxis (Hills et al., 2019). Both *in vivo* and *in vitro* studies have demonstrated that SCFAs stimulate the polarization of M2 macrophages, which mainly exert an anti-inflammatory function (Jiao et al., 2020). At the level of intestinal macrophages, SCFAs cause down-regulation of the pro-inflammatory cytokine profile (Jiao et al., 2020). Furthermore, SCFAs play a role in colonic Treg cell homeostasis, reduced IgG, IgA, and IgE secretion, and plasma cell differentiation in human B cells in a dose-dependent manner (Xu et al., 2022b)

Neutrophils play essential roles in the pathogenesis of RA by promoting inflammation and facilitating autoantibody production (Cecchi et al., 2018; Zhang and Frenette, 2019; Aa et al., 2020). In RA, increased recruitment of neutrophils in synovial fluid occurs at the onset of this disorder (Zhang et al., 2015). Macrophages are one of the most abundant cell types in the synovium and are centrally involved in the pathogenesis of RA (Mondanelli et al., 2019; Yang et al., 2020). Activated synovial macrophages produce cytokines (IL-1β, IL-6, and TNF-α) that promote T-cell polarization and inflammation by activating a wide range of immune and non-immune cells (e.g., fibroblast and osteoclast) (Yang et al., 2020).

Results on differences between fecal SCFAs concentrations in RA patients compared to HCs demonstrate a reduced amount of SCFAs in samples of RA patients. In one study, levels of acetate, propionate, butyrate, and valerate were decreased in RA patients (Yao et al., 2022). In another study, early-RA patients presented significantly reduced propionate levels (Marazzato et al., 2022). Similarly, Takahashi et al. and Rosser et al. showed that the stool concentrations of butyrate were significantly lower in new-onset RA patients and inactive RA patients, respectively (Rosser et al., 2020; Takahashi et al., 2020). He et al. reported significant reductions in serum and stool butyrate levels in RA patients (He et al., 2022). Conversely, in Rosser et al. study, there was no difference in propionate or butyrate but a significant increase in acetate levels in serum samples of RA patients compared to HCs.

SCFAs play a role in colonic Treg cell homeostasis. Administration of SCFAs to mice with CIA can reduce the severity of arthritis by their ability to increase Foxp3+IL-10–producing Tregs (Smith et al., 2013). In another study, SCFAs positively correlated with Tregs and negatively correlated with pro-inflammatory cytokines (IL-17A, IL-6, TNF-a) in CIA rats (Fan et al., 2020a). In RA patients, the levels of acetate, propionate, and butyrate positively correlated with the frequency of B cells (Yao et al., 2022). SCFAs can diminish B cell differentiation and the production of autoantibodies (Piper et al., 2019; Yao et al., 2022). In addition, the production of SCFAs is one of the proposed mechanisms by which gut microbiota affects Treg cell differentiation (Yang et al., 2022). Colonization with *Clostridia* induces differentiation of peripheral Treg cells that have a critical role in suppressing inflammatory responses (Lin and Zhang, 2017).

Butyrate

Butyrate is the most extensively investigated SCFAs (Hui et al., 2019). Butyrogenic bacteria are strictly anaerobic and oxygen-sensitive saccharolytic bacteria from the *Firmicutes* phylum. *Clostridia* clusters IV and XIVa, *Bacteroides fragilis*, *Ruminococcaceae*, and *Eubacterium* are the mainly intestinal bacteria producers of butyrate (Mizuno et al., 2017; Rogier et al., 2019; Takahashi et al., 2020; Wang et al., 2020).

Butyrate is critically involved in maintaining mucosal integrity and immune regulation (Guo et al., 2019; Lee et al., 2019; Garabatos and Santamaria, 2022; Wang et al., 2022b). The butyrate drives the metabolism of surface colonocytes toward mitochondrial beta-

oxidation of fatty acids, which is essential for maintaining epithelial hypoxia (Litvak et al., 2018). Gut epithelial cells directly take up butyrate, and a lack of butyrate is associated with immune dysregulation in the intestine (Foster et al., 2017).

Butyrate has anti-inflammatory properties by regulating inflammatory gene expression and induction of Treg cells (Foster et al., 2017). Butyrate regulates pro-inflammatory cytokine expression (e.g., IL-1, IL-6, TNF-α), inhibits the expression of LPS-induced cytokines, inhibits LPS-mediated macrophage migration, modulates the function of dendritic cells (increased phagocytic activity and reduced T-cell stimulatory capacity), promoted conversion of naive T-cells into immunosuppressive Treg (Hui et al., 2019; Blaak et al., 2020; Wang et al., 2020). Butyrate suppresses pro-inflammatory effectors in lamina propria, macrophages, neutrophils, and differentiation of dendritic cells (DCs) from bone marrow stem cells *via* HDACs inhibition or suppressing the NF-kB activation (Correa-Oliveira et al., 2016; Koh et al., 2016; Wang et al., 2020).

There is mechanistic evidence for the effect of butyrate on mucosal immunity and inflammation, mainly from cell lines and animal models. *In vitro*, DCs treated with butyrate increase the expression of indoleamine 2,3-dioxygenase 1 and aldehyde dehydrogenase 1A2. These enzymes attenuate the immune activation through tryptophan depletion and the generation of retinoic acid, a molecule with immunosuppressive properties (Correa-Oliveira et al., 2016). Butyrate increased IL-10 and IL-23 production by macrophages and DCs (Correa-Oliveira et al., 2016; Mizuno et al., 2017; Wang et al., 2019a).

The effects of butyrate in relieving arthritis appear to occur indirectly by modulating the function of immune cells, especially Treg cells. In cell cultures, the treatment of butyrate on naïve T cells cultured under the Treg-cell-polarizing conditions promoted the IL-10 expression of Treg cells and further inhibited the pro-inflammatory cytokines secreted by Th17 cells (Hui et al., 2019).

In CIA, butyrate treatment attenuated arthritis onset, decreased serum zonulin concentrations, and reduced inflammation-mediated small intestinal shortening (Tajik et al., 2020). In the antigen-induced model of arthritis (AIA), in stool samples, there was a reduction of butyrate and acetate levels during the acute and remission phase of arthritis compared to pre-arthritic mice (Rosser et al., 2020).

The effects of supplementation with butyrate in the pathogenesis of RA have been evaluated in experimental mouse models. Dietary butyrate supplementation conferred anti-inflammatory benefits in a mouse model of arthritis by rebalancing Tfh cells and Tregs and reducing antibody production. He et al. compared a butyrate-rich diet (started on the first day of collagen immunization) to normal chow in the CIA model (He et al., 2022). The butyrate supplementation increased butyrate levels in stool and blood, accompanied by a significantly lower overall incidence of arthritis, reduced severity of joint inflammation, and milder arthritis. Dietary butyrate supplementation increased serum IL-10 levels and decreased serum IL-6 and autoantibodies. Butyrate increased the number of Tfr cells, especially in the draining lymph nodes, and reduced germinal center B cells. The anti-inflammatory benefits of butyrate in the DBA/1 mice model were explained by rebalancing Tfh cells and Tregs and reducing antibody production.

Yao et al. demonstrated that supplementation of the three SCFAs before the onset of CIA in mice improved arthritic symptoms, increased the Bregs frequency, and decreased transitional B and follicular B cell frequency (Yao et al., 2022). These therapeutic effects were dependent on FFA2 receptors in CD19+ B cells. The fecal levels of acetate, propionate, and butyrate were positively correlated with the frequency of Bregs peripheral blood but not Tregs. Interestingly, treatment before the onset of CIA significantly improved joint inflammation and bone damage in mice, while administration after the start of CIA was less effective (Yao et al., 2022). Similarly, Rosser et al. reported that the supplementation with butyrate reduces the severity of arthritis in a Breg-dependent manner. The supplementation with butyrate before disease induction, but not acetate and propionate, reduced arthritis in Wild-Type mice compared to control mice (Rosser et al., 2020). However, butyrate supplementation failed to suppress disease in B-cell-deficient mice. These findings suggest that Bregs are necessary for the butyrate-mediated suppression of arthritis. Butyrate activates aryl-hydrocarbon receptor (AhR)-dependent gene transcription in B cells, supporting Breg function and inhibiting germinal center B cell and plasma cell differentiation. Nevertheless, butyrate no suppresses arthritis severity in Ahrfl/-Mb1cre/+, which has a B cell-specific deletion of AhR. Butyrate supplementation was associated with reduced TNF-α, IL-6, IL-17 production, and Th17 cell frequency. Interestingly, butyrate-mediated suppression was decreased in mice after Treg was depleted with an anti-CD25 depleting antibody treatment. Therefore, Treg also plays a role in mediating the suppression of arthritis by butyrate. These findings are consistent with the pleiotropic immunomodulatory effect of butyrate (Figure 3).

A recent study showed that the effect of microbial fermentation of fiber on host health could be context-dependent and species-dependent. Colonization of *P. copri* and a high-fiber diet led to the overproduction of organic acids, including fumarate, succinate, and SCFAs. Succinate promoted pro-inflammatory responses in macrophages. Furthermore, supplementation with succinate exacerbated arthritis in the CIA model. In patients with RA, succinate is abundantly present in synovial fluids, and these fluids elicit IL-1β release from macrophages (Jiang et al., 2022).

Gut dysbiosis in RA patients is characterized by a deficiency of butyrate-producing bacteria and an overwhelming number of butyrate bacteria consumers (He et al., 2022). In RA patients, higher butyrate levels were associated with increased Treg levels (Xu et al., 2022b). Patients with NORA displayed an increase in *Bacteroidetes* and a decrease in *Firmicutes*, *Proteobacteria*, and *Actinobacteria* compared to levels in HCs (Sun et al., 2019). It is possible that the reduction of *Firmicutes* can lead to inflammation in RA patients (Wang et al., 2022a).

In RA patients, total abundances of intestinal bacteria butyrate producers were lower in patients ACPA-positive compared to ACPA-negative patients. Conversely, butyrate consumers bacteria were higher in ACPA-positive than ACPA-negative patients. Furthermore, the increased abundance of butyrate-producing bacteria was associated with a lower incidence of deformed joint count and ACPA-positive, suggesting the potential roles of butyrate in alleviating inflammation. These anti-inflammatory effects may be

attributed to increased Treg polarization, decreased Tfh and Th17 (but not Th1 or Th2) cell numbers, and a decrease in the production of pro-inflammatory cytokines. A higher proportion of circulating Treg was associated with high levels of stool butyrate (He et al., 2022).

Amino acids

Metabolism of amino acids by intestinal bacteria may regulate inflammation and exert modulatory effects on the immune system (Mondanelli et al., 2019; Panfili et al., 2020; Yu et al., 2021). The cross-talk between amino acid metabolites and the immune cells has emerged as a possible mechanism by which gut dysbiosis could lean toward the development of inflammation or autoimmunity during the development of arthritis.

Microbiota-dependent tryptophan catabolites are abundantly produced within the intestine and are known to affect the maintenance of epithelial barrier function and immune homeostasis. The gut microbiota can also metabolize dietary tryptophan into indole derivatives. In host tissues, indole derivatives are known as ligands for the AhR, a ligand-activated transcription factor. AhR signaling contributes to immune homeostasis by modulating T cell differentiation. Indole derivatives are implicated in immune cell maturation and promote Treg differentiation while suppressing Th17 differentiation (Wang et al., 2019a; Yu et al., 2021; Hanlon et al., 2022; Yang et al., 2022). AhR expression and activation in DCs or T cells translate into Treg cell-mediated immunoregulatory effects, which dampen immune responses. However, in the presence of 6-formylindolo[3,2-b] carbazole, activation of AhR can promote the development of Th17 cells. Therefore, AhR plays a dual depending on the ligand nature, cell expression, and presence of other signals in the cell microenvironment (Panfili et al., 2020).

B cell-specific deletion of AhR in mice exacerbated arthritis, diminished IL-10 production by Bregs cells, and reduced the frequency of Tregs cells and expansion of inflammatory Th1 and Th17 cells compared with B cell AhR-sufficient mice (Piper et al., 2019). Rosser et al. demonstrated that butyrate reduced experimental arthritis severity *via* an increase in 5-hydroxy indole-3-acetic acid (5-HIAA), an indole derived from the decomposition of serotonin. The activation of AhR promoted the differentiation of B cells into Breg cells (Rosser et al., 2020).

Tryptophan metabolism would exert protective effects in experimental models of arthritis but not in all RA patients. RA patients may have reduced concentrations of tryptophan, 3-hydroxykynurenine (3-HK), and 3-hydroxyanthranilic acid (3-HAA), along with increased concentrations of kynurenine and xanthurenic acid, indicating that the kynurenine pathway is active in

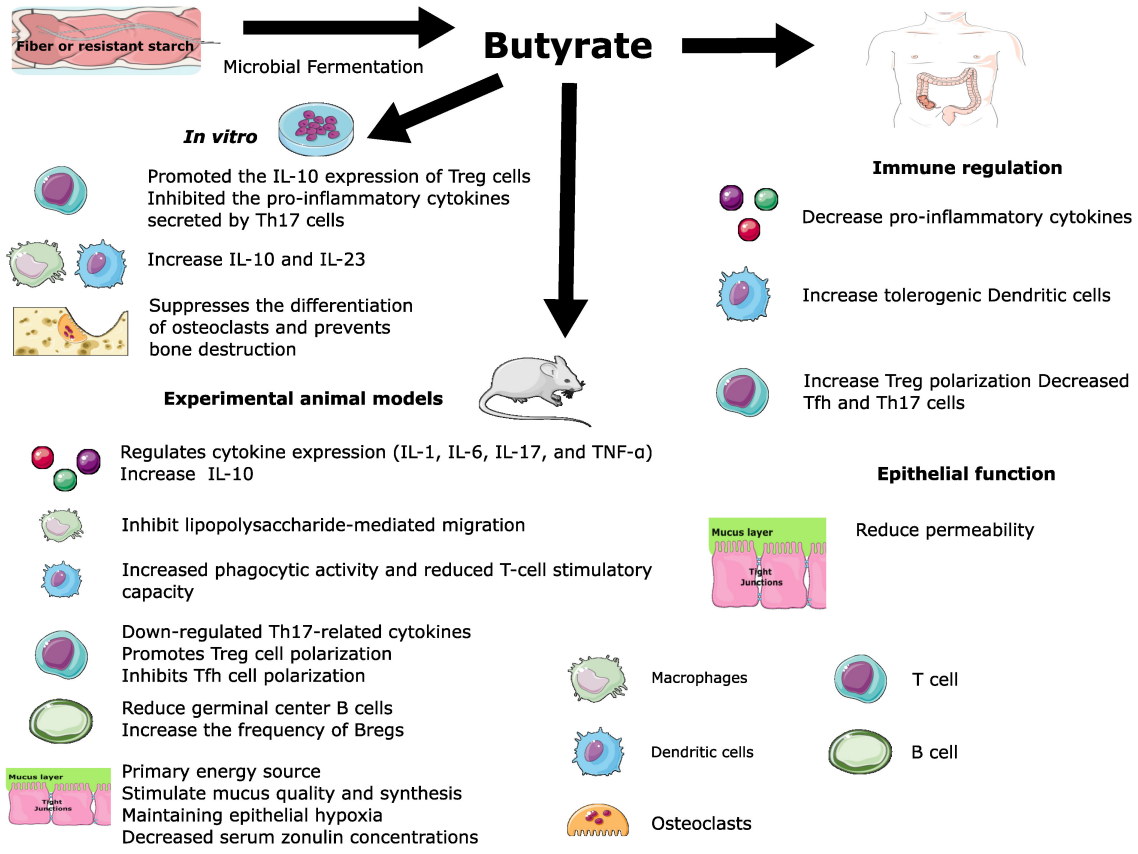


FIGURE 3
Pleiotropic immunomodulatory effect of butyrate.

RA patients (Panfili et al., 2020). Few studies have evaluated the relationship between amino acid metabolites produced by gut microbiota and the pathogenesis of RA.

Recent work describes that the most highly enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway in RA patients was amino acid metabolism (e.g., alanine, aspartate, and glutamate) (Wang et al., 2022a). Two studies in Chinese RA patients reported that the amino acid pathways were significantly altered between the RA patients compared to HCs. Wang et al. (Wang et al., 2018) demonstrated decreased levels of tryptophan and glycine in RA patients compared to HCs; treatment with methotrexate returned amino acid levels to baseline. In another study by Yu et al., according to KEGG pathway enrichment analysis, the amino acid biosynthesis pathways were depleted in the RA group. These amino acids included L-arginine and ornithine, aromatic amino acids, and branched amino acids. Furthermore, RA patients exhibited lower levels of tryptophan metabolites in feces (Yu et al., 2021).

Alterations in the gut microenvironment

The metabolic activity of the microbiota could also affect pathogen colonization. The butyrate influences the gut microbiota by driving the metabolism of surface colonocytes toward mitochondrial beta-oxidation of fatty acids, which is essential for maintaining epithelial hypoxia. The consequent epithelial hypoxia helps maintain a microbial community dominated by obligate anaerobic bacteria, which benefit from converting fiber into SCFAs (Litvak et al., 2018).

The metagenomic analysis from stool samples of RA patients demonstrates an altered redox environment (Scher et al., 2013; Zhang et al., 2015; Kishikawa et al., 2020). Iron transport-related genes were enriched in early RA patients (Jeong et al., 2019). Kishikawa et al. showed that the abundance of the R6FCZ7 gene, related to the redox reaction, was significantly decreased in the metagenome of RA patients compared to HCs (Kishikawa et al., 2020). The R6FCZ7 sequences were linked to *Bacteroides uniformis*, *Bacteroides rodentium*, Bacteroides fragilis, and *Bacteroides* spp. These findings have suggested that the redox function of the microbiome, especially the genus *Bacteroides*, may have an essential role in the pathology of RA (Kishikawa et al., 2020).

Discussion

This review highlights the multiple mechanisms by which alterations in the gut microbiota contribute to the pathogenesis of RA. The relationship between gut dysbiosis and joint diseases, called the 'gut–joint axis,' has been suggested to be involved in the pathogenesis of arthritis, such as RA, Psoriatic Arthritis, and Spondyloarthritis. The association of gut dysbiosis with chronic inflammation and the fact that gut dysbiosis is essential to trigger arthritis in experimental mice models suggest a role of gut dysbiosis in the onset of RA. It has been hypothesized that the interactions between gut microbiota and host lead to mucosal inflammation and the breaking of immune tolerance (Chiang et al., 2019).

There is evidence that RA may be associated with changes in the composition of fecal bacterial communities. However, some studies have demonstrated the association between fungal microbiota, gastrointestinal helminths, and RA. Findings derived from animal models suggest that gut dysbiosis is related to the onset of RA, a stage in which activation of the autoimmune system occurs, leading to chronic inflammation (Burmester et al., 2014).

Growing evidence reveals the mechanisms underlying the link between gut microbiota, their metabolites, and cells (immune and non-immune) involved in RA pathogenesis. Gut dysbiosis affects the functions of the intestine and other organs, including joints. Consequently, persistent gut dysbiosis is associated with intestinal inflammation and increased Th17/Treg cell ratio. It can contribute to a break in immunological tolerance and tissue damage by various mechanisms, including translocation of bacteria across the gut barrier, T helper cell skewing, and crossreactivity with autoantigens. A possible hypothesis could be that gut dysbiosis trigger the migration of self-reactive B or T cells from intestinal sites to secondary lymphoid organs and arthritic joints. However, the mechanisms by which gut dysbiosis can contribute to RA onset are still incompletely understood and remains to be further elucidated.

Experimental animal models have been helpful in the understanding of the mechanism associated between gut dysbiosis and arthritis. Most studies have focused on the effects of a specific family or strain of bacteria or gut microbiota derivatives on the differentiation of Treg and Th17 cells. In contrast, other types of cells have been less well-studied (i.e., neutrophils, osteoclasts, or fibroblast-like synoviocytes).

Evidence suggests that gut dysbiosis is involved in the pathogenesis of RA, but to date, finding proof of causality is still a significant challenge in this field. This review showed the recent findings highlighting the complex regulatory networks between gut microbiota and the immune system. Gut microbiota diversity is easily altered by multiple factors such as drugs, diet, health status, hygiene, and surrounding environmental microorganisms. Furhermore, the inflammatory and metabolic pathways are complex networks context-dependent by various factors, including genetics, diet, cell status, and environmental factors (Yang et al., 2022). The mechanism of gut microbiota involvement in the occurrence and development of inflammatory diseases is very complex, and research on how intestinal metabolites and the host interact to affect diseases is a hot topic (Xu et al., 2022a). Further studies are needed to assess the impact of intestinal dysbiosis and gut microbiota-derived metabolites rather than specific bacterial species to understand the mechanisms involved in RA pathogenesis.

A more comprehensive understanding of the underlying mechanisms in the relationship between gut dysbiosis and RA will help to develop new treatment strategies. The study of gut microbiota-derivated metabolites is of great interest due to their therapeutic potential (He et al., 2022). The beneficial effects of butyrate obtained in animal studies warrant further investigation of its therapeutic potential in the form of butyrate-rich diets or by butyrate supplementation. Similarly, considerable evidence shows that

alterations of the intestinal barrier are related to the onset of AR, and that gut dysbiosis could influence the inflammatory activity of RA patients through the regulation of gut permeability. Therefore, future studies may employ strategies to avoid a leaky gut (e.g., diet, SCFAs supplementation, or zonulin antagonists).

The authors hope this review's results can provide a valuable resource for future research to advance our understanding of the possible underlying mechanism in the relationship between gut dysbiosis and RA.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Funding

This research received no specific grant from public, commercial, or not-for-profit funding agencies.

Acknowledgments

The Figures were partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Aa, L. X., Fei, F., Qi, Q., Sun, R. B., Gu, S. H., Di, Z. Z., et al. (2020). Rebalancing of the gut flora and microbial metabolism is responsible for the anti-arthritis effect of kaempferol. *Acta Pharmacol. Sin.* 41 (1), 73–81. doi: 10.1038/s41401-019-0279-8

Alameddine, J., Godefroy, E., Papargyris, L., Sarrabayrouse, G., Tabiasco, J., Bridonneau, C., et al. (2019). Faecalibacterium prausnitzii skews human DC to prime IL10-producing T cells through TLR2/6/JNK signaling and IL-10, IL-27, CD39, and IDO-1 induction. *Front. Immunol.* 10. doi: 10.3389/fimmu.2019.00143

Amdekar, S., Singh, V., Kumar, A., Sharma, P., and Singh, R. (2013). Lactobacillus casei and lactobacillus acidophilus regulate inflammatory pathway and improve antioxidant status in collagen-induced arthritic rats. *J. Interferon Cytokine Res.* 33 (1), 1–8. doi: 10.1089/jir.2012.0034

Audo, R., Sanchez, P., Riviere, B., Mielle, J., Tan, J., Lukas, C., et al. (2022). Rheumatoid arthritis is associated with increased gut permeability and bacterial translocation which are reversed by inflammation control. *Rheumatol. (Oxford).* doi: 10.1093/rheumatology/keac454

Berthelot, J. M., Sellam, J., Maugars, Y., and Berenbaum, F. (2019). Cartilage-gut-microbiome axis: a new paradigm for novel therapeutic opportunities in osteoarthritis. *RMD Open* 5 (2), e001037. doi: 10.1136/rmdopen-2019-001037

Blaak, E. E., Canfora, E. E., Theis, S., Frost, G., Groen, A. K., Mithieux, G., et al. (2020). Short chain fatty acids in human gut and metabolic health. *Benef. Microbes* 11 (5), 411–455. doi: 10.3920/BM2020.0057

Block, K. E., Zheng, Z., Dent, A. L., Kee, B. L., and Huang, H. (2016). Gut microbiota regulates K/BxN autoimmune arthritis through follicular helper T but not Th17 cells. *J. Immunol.* 196 (4), 1550–1557. doi: 10.4049/jimmunol.1501904

Bos, W. H., van de Stadt, L. A., Sohrabian, A., Ronnelid, J., and van Schaardenburg, D. (2014). Development of anti-citrullinated protein antibody and rheumatoid factor isotypes prior to the onset of rheumatoid arthritis. *Arthritis Res. Ther.* 16 (2), 405. doi: 10.1186/ar4511

Buckley, C. D., and McGettrick, H. M. (2018). Leukocyte trafficking between stromal compartments: lessons from rheumatoid arthritis. *Nat. Rev. Rheumatol.* 14 (8), 476–487. doi: 10.1038/s41584-018-0042-4

Burmester, G. R., Feist, E., and Dorner, T. (2014). Emerging cell and cytokine targets in rheumatoid arthritis. *Nat. Rev. Rheumatol.* 10 (2), 77–88. doi: 10.1038/nrrheum.2013.168

Cani, P. D. (2018). Human gut microbiome: hopes, threats and promises. *Gut* 67 (9), 1716–1725. doi: 10.1136/gutjnl-2018-316723

Cao, G., Wang, P., Cui, Z., Yue, X., Chi, S., Ma, A., et al. (2020). An imbalance between blood CD4(+)CXCR5(+)Foxp3(+) tfr cells and CD4(+)CXCR5(+)Tfh cells may contribute to the immunopathogenesis of rheumatoid arthritis. *Mol. Immunol.* 125, 1–8. doi: 10.1016/j.molimm.2020.06.003

Cecchi, I., Arias de la Rosa, I., Menegatti, E., Roccatello, D., Collantes-Estevez, E., Lopez-Pedrera, C., et al. (2018). Neutrophils: Novel key players in rheumatoid arthritis. current and future therapeutic targets. *Autoimmun. Rev.* 17 (11), 1138–1149. doi: 10.1016/j.autrev.2018.06.006

Cheng, M., Zhao, Y., Cui, Y., Zhong, C., Zha, Y., Li, S., et al. (2022). Stage-specific roles of microbial dysbiosis and metabolic disorders in rheumatoid arthritis. *Ann. Rheum. Dis.* 81 (12), 1669–1677. doi: 10.1136/ard-2022-222871

Chen, Y., Ma, C., Liu, L., He, J., Zhu, C., Zheng, F., et al. (2021). Analysis of gut microbiota and metabolites in patients with rheumatoid arthritis and identification of potential biomarkers. *Aging (Albany NY)* 13 (20), 23689–23701. doi: 10.18632/aging.203641

Chen, J., Wright, K., Davis, J. M., Jeraldo, P., Marietta, E. V., Murray, J., et al. (2016). An expansion of rare lineage intestinal microbes characterizes rheumatoid arthritis. *Genome Med.* 8 (1), 43. doi: 10.1186/s13073-016-0299-7

Chiang, H. I., Li, J. R., Liu, C. C., Liu, P. Y., Chen, H. H., Chen, Y. M., et al. (2019). An association of gut microbiota with different phenotypes in Chinese patients with rheumatoid arthritis. *J. Clin. Med.* 8 (11), 1770. doi: 10.3390/jcm8111770

Chriswell, M. E., Lefferts, A. R., Clay, M. R., Hsu, A. R., Seifert, J., Feser, M. L., et al. (2022). Clonal IgA and IgG autoantibodies from individuals at risk for rheumatoid arthritis identify an arthritogenic strain of subdoligranulum. *Sci. Transl. Med.* 14 (668), eabn5166. doi: 10.1126/scitranslmed.abn5166

Correa-Oliveira, R., Fachi, J. L., Vieira, A., Sato, F. T., and Vinolo, M. A. (2016). Regulation of immune cell function by short-chain fatty acids. *Clin. Transl. Immunol.* 5 (4), e73. doi: 10.1038/cti.2016.17

Costalonga, M., Hodges, J. S., and Herzberg, M. C. (2002). Streptococcus sanguis modulates type II collagen-induced arthritis in DBA/1J mice. *J. Immunol.* 169 (4), 2189–2195. doi: 10.4049/jimmunol.169.4.2189

Diamanti, A. P., Manuela Rosado, M., Lagana, B., and D'Amelio, R. (2016). Microbiota and chronic inflammatory arthritis: an interwoven link. *J. Transl. Med.* 14 (1), 233. doi: 10.1186/s12967-016-0989-3

Di Gangi, A., Di Cicco, M. E., Comberiati, P., and Peroni, D. G. (2020). Go with your gut: The shaping of T-cell response by gut microbiota in allergic asthma. *Front. Immunol.* 11. doi: 10.3389/fimmu.2020.01485

Doonan, J., Tarafdar, A., Pineda, M. A., Lumb, F. E., Crowe, J., Khan, A. M., et al. (2019). The parasitic worm product ES-62 normalises the gut microbiota bone marrow axis in inflammatory arthritis. *Nat. Commun.* 10 (1), 1554. doi: 10.1038/s41467-019-09361-0

du Teil Espina, M., Gabarrini, G., Harmsen, H. J. M., Westra, J., van Winkelhoff, A. J., and van Dijl, J. M. (2019). Talk to your gut: the oral-gut microbiome axis and its immunomodulatory role in the etiology of rheumatoid arthritis. *FEMS Microbiol. Rev.* 43 (1), 1–18. doi: 10.1093/femsre/fuy035

El Menofy, N. G., Ramadan, M., Abdelbary, E. R., Ibrahim, H. G., Azzam, A. I., Ghit, M. M., et al. (2022). Bacterial compositional shifts of gut microbiomes in patients with rheumatoid arthritis in association with disease activity. *Microorganisms* 10 (9), 1820. doi: 10.3390/microorganisms10091820

Fan, L., Xu, C., Ge, Q., Lin, Y., Wong, C. C., Qi, Y., et al. (2021). A. muciniphila suppresses colorectal tumorigenesis by inducing TLR2/NLRP3-mediated M1-like TAMs. *Cancer Immunol. Res.* 9 (10), 1111–1124. doi: 10.1158/2326-6066.CIR-20-1019

Fan, Z., Yang, B., Ross, R. P., Stanton, C., Shi, G., Zhao, J., et al. (2020a). Protective effects of bifidobacterium adolescentis on collagen-induced arthritis in rats depend on timing of administration. *Food Funct.* 11 (5), 4499–4511. doi: 10.1039/d0fo00077a

Fan, Z., Yang, B., Ross, R. P., Stanton, C., Zhao, J., Zhang, H., et al. (2020b). The prophylactic effects of different lactobacilli on collagen-induced arthritis in rats. *Food Funct.* 11 (4), 3681–3694. doi: 10.1039/c9fo02556a

Feng, Y., Chen, Z., Tu, S. Q., Wei, J. M., Hou, Y. L., Kuang, Z. L., et al. (2022). Role of interleukin-17A in the pathomechanisms of periodontitis and related systemic chronic inflammatory diseases. *Front. Immunol.* 13. doi: 10.3389/fimmu.2022.862415

Foster, K. R., Schluter, J., Coyte, K. Z., and Rakoff-Nahoum, S. (2017). The evolution of the host microbiome as an ecosystem on a leash. *Nature* 548 (7665), 43–51. doi: 10.1038/nature23292

Garabatos, N., and Santamaria, P. (2022). Gut microbial antigenic mimicry in autoimmunity. *Front. Immunol.* 13. doi: 10.3389/fimmu.2022.873607

Gill, T., Stauffer, P., Asquith, M., Laderas, T., Martin, T. M., Davin, S., et al. (2022). Axial spondyloarthritis patients have altered mucosal IgA response to oral and fecal microbiota. *Front. Immunol.* 13. doi: 10.3389/fimmu.2022.965634

Guo, L. X., Wang, H. Y., Liu, X. D., Zheng, J. Y., Tang, Q., Wang, X. N., et al. (2019). Saponins from clematis mandshurica rupr. regulates gut microbiota and its metabolites during alleviation of collagen-induced arthritis in rats. *Pharmacol. Res.* 149, 104459. doi: 10.1016/j.phrs.2019.104459

Haase, S., Haghikia, A., Wilck, N., Muller, D. N., and Linker, R. A. (2018). Impacts of microbiome metabolites on immune regulation and autoimmunity. *Immunology* 154 (2), 230–238. doi: 10.1111/imm.12933

Hamamoto, Y., Ouhara, K., Munenaga, S., Shoji, M., Ozawa, T., Hisatsune, J., et al. (2020). Effect of porphyromonas gingivalis infection on gut dysbiosis and resultant arthritis exacerbation in mouse model. *Arthritis Res. Ther.* 22 (1), 249. doi: 10.1186/s13075-020-02348-z

Hanlon, M. M., Canavan, M., Barker, B. E., and Fearon, U. (2022). Metabolites as drivers and targets in rheumatoid arthritis. *Clin. Exp. Immunol.* 208 (2), 167–180. doi: 10.1093/cei/uxab021

He, J., Chu, Y., Li, J., Meng, Q., Liu, Y., Jin, J., et al. (2022). Intestinal butyrate-metabolizing species contribute to autoantibody production and bone erosion in rheumatoid arthritis. *Sci. Adv.* 8 (6), eabm1511. doi: 10.1126/sciadv.abm1511

Hills, R. D.Jr., Pontefract, B. A., Mishcon, H. R., Black, C. A., Sutton, S. C., and Theberge, C. R. (2019). Gut microbiome: Profound implications for diet and disease. *Nutrients* 11 (7), 1613. doi: 10.3390/nu11071613

Horta-Baas, G., Romero-Figueroa, M. D. S., Montiel-Jarquin, A. J., Pizano-Zarate, M. L., Garcia-Mena, J., and Ramirez-Duran, N. (2017). Intestinal dysbiosis and rheumatoid arthritis: A link between gut microbiota and the pathogenesis of rheumatoid arthritis. *J. Immunol. Res.* 2017, 4835189. doi: 10.1155/2017/4835189

Horta-Baas, G., Sandoval-Cabrera, A., and Romero-Figueroa, M. D. S. (2021). Modification of gut microbiota in inflammatory arthritis: Highlights and future challenges. *Curr. Rheumatol. Rep.* 23 (8), 67. doi: 10.1007/s11926-021-01031-9

Huang, Y., Li, M., Zhou, L., Xu, D., Qian, F., Zhang, J., et al. (2019). Effects of qingluo tongbi decoction on gut flora of rats with adjuvant-induced arthritis and the underlying mechanism. *Evid Based Complement Alternat Med.* 2019, 6308021. doi: 10.1155/2019/6308021

Hui, W., Yu, D., Cao, Z., and Zhao, X. (2019). Butyrate inhibit collagen-induced arthritis *via* Treg/IL-10/Th17 axis. *Int. Immunopharmacol.* 68, 226–233. doi: 10.1016/j.intimp.2019.01.018

Iljazovic, A., Amend, L., Galvez, E. J. C., de Oliveira, R., and Strowig, T. (2021a). Modulation of inflammatory responses by gastrointestinal prevotella spp. - from associations to functional studies. *Int. J. Med. Microbiol.* 311 (2), 151472. doi: 10.1016/j.ijmm.2021.151472

Iljazovic, A., Roy, U., Galvez, E. J. C., Lesker, T. R., Zhao, B., Gronow, A., et al. (2021b). Perturbation of the gut microbiome by prevotella spp. enhances host susceptibility to mucosal inflammation. *Mucosal Immunol.* 14 (1), 113–124. doi: 10.1038/s41385-020-0296-4

Inda, M. E., Broset, E., Lu, T. K., and de la Fuente-Nunez, C. (2019). Emerging frontiers in microbiome engineering. *Trends Immunol.* 40 (10), 952–973. doi: 10.1016/j.it.2019.08.007

Ivanov, I. I., Atarashi, K., Manel, N., Brodie, E. L., Shima, T., Karaoz, U., et al. (2009). Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* 139 (3), 485–498. doi: 10.1016/j.cell.2009.09.033

Jeong, Y., Kim, J. W., You, H. J., Park, S. J., Lee, J., Ju, J. H., et al. (2019). Gut microbial composition and function are altered in patients with early rheumatoid arthritis. *J. Clin. Med.* 8 (5), 693. doi: 10.3390/jcm8050693

Jethwa, H., and Abraham, S. (2017). The evidence for microbiome manipulation in inflammatory arthritis. *Rheumatol. (Oxford)* 56 (9), 1452–1460. doi: 10.1093/rheumatology/kew374

Jiang, L., Shang, M., Yu, S., Liu, Y., Zhang, H., Zhou, Y., et al. (2022). A high-fiber diet synergizes with prevotella copri and exacerbates rheumatoid arthritis. *Cell Mol. Immunol.* 19 (12), 1414–1424. doi: 10.1038/s41423-022-00934-6

Jiao, Y., Wu, L., Huntington, N. D., and Zhang, X. (2020). Crosstalk between gut microbiota and innate immunity and its implication in autoimmune diseases. *Front. Immunol.* 11. doi: 10.3389/fimmu.2020.00282

Jubair, W. K., Hendrickson, J. D., Severs, E. L., Schulz, H. M., Adhikari, S., Ir, D., et al. (2018). Modulation of inflammatory arthritis in mice by gut microbiota through mucosal inflammation and autoantibody generation. *Arthritis Rheumatol.* 70 (8), 1220–1233. doi: 10.1002/art.40490

Kamada, N., Seo, S. U., Chen, G. Y., and Nunez, G. (2013). Role of the gut microbiota in immunity and inflammatory disease. *Nat. Rev. Immunol.* 13 (5), 321–335. doi: 10.1038/nri3430

Kang, Y., Cai, Y., Zhang, X., Kong, X., and Su, J. (2017). Altered gut microbiota in RA: implications for treatment. *Z Rheumatol.* 76 (5), 451–457. doi: 10.1007/s00393-016-0237-5

Kishikawa, T., Maeda, Y., Nii, T., Motooka, D., Matsumoto, Y., Matsushita, M., et al. (2020). Metagenome-wide association study of gut microbiome revealed novel aetiology of rheumatoid arthritis in the Japanese population. *Ann. Rheum. Dis.* 79 (1), 103–111. doi: 10.1136/annrheumdis-2019-215743

Koh, A., De Vadder, F., Kovatcheva-Datchary, P., and Backhed, F. (2016). From dietary fiber to host physiology: Short-chain fatty acids as key bacterial metabolites. *Cell* 165 (6), 1332–1345. doi: 10.1016/j.cell.2016.05.041

Larsen, J. M. (2017). The immune response to prevotella bacteria in chronic inflammatory disease. *Immunology* 151 (4), 363–374. doi: 10.1111/imm.12760

Lee, E. H., Kim, H., Koh, J. H., Cha, K. H., Lee, K. K., Kim, W. U., et al. (2022). Dysbiotic but nonpathogenic shift in the fecal mycobiota of patients with rheumatoid arthritis. *Gut Microbes* 14 (1), 2149020. doi: 10.1080/19490976.2022.2149020

Lee, S., Koh, J., Chang, Y., Kim, H. Y., and Chung, D. H. (2019). Invariant NKT cells functionally link microbiota-induced butyrate production and joint inflammation. *J. Immunol.* 203 (12), 3199–3208. doi: 10.4049/jimmunol.1801314

Lee, J. S., Tato, C. M., Joyce-Shaikh, B., Gulen, M. F., Cayatte, C., Chen, Y., et al. (2015). Interleukin-23-Independent IL-17 production regulates intestinal epithelial permeability. *Immunity* 43 (4), 727–738. doi: 10.1016/j.immuni.2015.09.003

Lin, L., and Zhang, J. (2017). Role of intestinal microbiota and metabolites on gut homeostasis and human diseases. *BMC Immunol.* 18 (1), 2. doi: 10.1186/s12865-016-0187-3

Litvak, Y., Byndloss, M. X., and Baumler, A. J. (2018). Colonocyte metabolism shapes the gut microbiota. *Science* 362 (6418), eaat9076. doi: 10.1126/science.aat9076

Liu, X., Zeng, B., Zhang, J., Li, W., Mou, F., Wang, H., et al. (2016). Role of the gut microbiome in modulating arthritis progression in mice. *Sci. Rep.* 6, 30594. doi: 10.1038/srep30594

Li, H., Yu, L., Zhang, X., Shang, J., and Duan, X. (2022). Exploring the molecular mechanisms and shared gene signatures between rheumatoid arthritis and diffuse large b cell lymphoma. *Front. Immunol.* 13. doi: 10.3389/fimmu.2022.1036239

Maeda, Y., Kurakawa, T., Umemoto, E., Motooka, D., Ito, Y., Gotoh, K., et al. (2016). Dysbiosis contributes to arthritis development *via* activation of autoreactive T cells in the intestine. *Arthritis Rheumatol.* 68 (11), 2646–2661. doi: 10.1002/art.39783

Maeda, Y., and Takeda, K. (2019). Host-microbiota interactions in rheumatoid arthritis. *Exp. Mol. Med.* 51 (12), 1–6. doi: 10.1038/s12276-019-0283-6

Mankia, K., and Emery, P. (2016). Pre-clinical rheumatoid arthritis: Progress toward prevention. *Arthritis Rheumatol.* 68 (4), 779–788. doi: 10.1002/art.39603

Mansson, I., Norberg, R., Olhagen, B., and Bjorklund, N. E. (1971). Arthritis in pigs induced by dietary factors. microbiologic, clinical and histologic studies. *Clin. Exp. Immunol.* 9 (5), 677–693.

Man, A. W. C., Zhou, Y., Xia, N., and Li, H. (2020). Involvement of gut microbiota, microbial metabolites and interaction with polyphenol in host immunometabolism. *Nutrients* 12 (10), 3054. doi: 10.3390/nu12103054

Marazzato, M., Iannuccelli, C., Guzzo, M. P., Nencioni, L., Lucchino, B., Radocchia, G., et al. (2022). Gut microbiota structure and metabolites, before and after treatment in early rheumatoid arthritis patients: A pilot study. *Front. Med. (Lausanne)* 9. doi: 10.3389/fmed.2022.921675

Marietta, E. V., Murray, J. A., Luckey, D. H., Jeraldo, P. R., Lamba, A., Patel, R., et al. (2016). Suppression of inflammatory arthritis by human gut-derived prevotella histicola in humanized mice. *Arthritis Rheumatol.* 68 (12), 2878–2888. doi: 10.1002/art.39785

Martinez-Martinez, R. E., Abud-Mendoza, C., Patino-Marin, N., Rizo-Rodriguez, J. C., Little, J. W., and Loyola-Rodriguez, J. P. (2009). Detection of periodontal bacterial DNA in serum and synovial fluid in refractory rheumatoid arthritis patients. *J. Clin. Periodontol.* 36 (12), 1004–1010. doi: 10.1111/j.1600-051X.2009.01496.x

Mizuno, M., Noto, D., Kaga, N., Chiba, A., and Miyake, S. (2017). The dual role of short fatty acid chains in the pathogenesis of autoimmune disease models. *PloS One* 12 (2), e0173032. doi: 10.1371/journal.pone.0173032

Moen, K., Brun, J. G., Eribe, E. K., Olsen, I., and Jonsson, R. (2005). Oral bacterial DNAs in synovial fluids of arthritis patients. *Microb. Ecol. Health Dis.* 17 (1), 2–8.

Mondanelli, G., Iacono, A., Carvalho, A., Orabona, C., Volpi, C., Pallotta, M. T., et al. (2019). Amino acid metabolism as drug target in autoimmune diseases. *Autoimmun. Rev.* 18 (4), 334–348. doi: 10.1016/j.autrev.2019.02.004

Narushima, S., Sugiura, Y., Oshima, K., Atarashi, K., Hattori, M., Suematsu, M., et al. (2014). Characterization of the 17 strains of regulatory T cell-inducing human-derived clostridia. *Gut Microbes* 5 (3), 333–339. doi: 10.4161/gmic.28572

Opoku, Y. K., Asare, K. K., Ghartey-Quansah, G., Afrifa, J., Bentsi-Enchill, F., Ofori, E. G., et al. (2022). Intestinal microbiome-rheumatoid arthritis crosstalk: The therapeutic role of probiotics. *Front. Microbiol.* 13. doi: 10.3389/fmicb.2022.996031

Panfili, E., Gerli, R., Grohmann, U., and Pallotta, M. T. (2020). Amino acid metabolism in rheumatoid arthritis: Friend or foe? *Biomolecules* 10 (9), 1280. doi: 10.3390/biom10091280

Pan, H., Li, R., Li, T., Wang, J., and Liu, L. (2017). Wheter probiotic supplementation benefits rheumatoid arthritis patients: A systematic review and meta-analysis. *engineering* 3, 115–121. doi: 10.1016/J.ENG.2017.01.006

Parantainen, J., Barreto, G., Koivuniemi, R., Kautiainen, H., Nordstrom, D., Moilanen, E., et al. (2022). The biological activity of serum bacterial lipopolysaccharides associates with disease activity and likelihood of achieving remission in patients with rheumatoid arthritis. *Arthritis Res. Ther.* 24 (1), 256. doi: 10.1186/s13075-022-02946-z

Peng, J., Lu, X., Xie, K., Xu, Y., He, R., Guo, L., et al. (2019). Dynamic alterations in the gut microbiota of collagen-induced arthritis rats following the prolonged administration of total glucosides of paeony. *Front. Cell Infect. Microbiol.* 9. doi: 10.3389/fcimb.2019.00204

Pianta, A., Arvikar, S. L., Strle, K., Drouin, E. E., Wang, Q., Costello, C. E., et al. (2017). Two rheumatoid arthritis-specific autoantigens correlate microbial immunity with autoimmune responses in joints. *J. Clin. Invest.* 127 (8), 2946–2956. doi: 10.1172/JCI93450

Picchianti-Diamanti, A., Panebianco, C., Salemi, S., Sorgi, M. L., Di Rosa, R., Tropea, A., et al. (2018). Analysis of gut microbiota in rheumatoid arthritis patients: Disease-related dysbiosis and modifications induced by etanercept. *Int. J. Mol. Sci.* 19 (10), 2938. doi: 10.3390/ijms19102938

Pineda, M. A., Rodgers, D. T., Al-Riyami, L., Harnett, W., and Harnett, M. M. (2014). ES-62 protects against collagen-induced arthritis by resetting interleukin-22 toward resolution of inflammation in the joints. *Arthritis Rheumatol.* 66 (6), 1492–1503. doi: 10.1002/art.38392

Piper, C. J. M., Rosser, E. C., Oleinika, K., Nistala, K., Krausgruber, T., Rendeiro, A. F., et al. (2019). Aryl hydrocarbon receptor contributes to the transcriptional program of IL-10-Producing regulatory b cells. *Cell Rep.* 29 (7), 1878–1892 e1877. doi: 10.1016/j.celrep.2019.10.018

Qu, F., Guilak, F., and Mauck, R. L. (2019). Cell migration: implications for repair and regeneration in joint disease. *Nat. Rev. Rheumatol.* 15 (3), 167–179. doi: 10.1038/s41584-018-0151-0

Rashid, T., and Ebringer, A. (2012). Autoimmunity in rheumatic diseases is induced by microbial infections *via* crossreactivity or molecular mimicry. *Autoimmune Dis.* 2012, 539282. doi: 10.1155/2012/539282

Reichert, S., Haffner, M., Keysser, G., Schafer, C., Stein, J. M., Schaller, H. G., et al. (2013). Detection of oral bacterial DNA in synovial fluid. *J. Clin. Periodontol.* 40 (6), 591–598. doi: 10.1111/jcpe.12102

Ribeiro, F., Romao, V. C., Rosa, S., Jesus, K., Agua-Doce, A., Barreira, S. C., et al. (2022). Different antibody-associated autoimmune diseases have distinct patterns of T follicular cell dysregulation. *Sci. Rep.* 12 (1), 17638. doi: 10.1038/s41598-022-21576-8

Rogier, R., Ederveen, T. H. A., Wopereis, H., Hartog, A., Boekhorst, J., van Hijum, S., et al. (2019). Supplementation of diet with non-digestible oligosaccharides alters the intestinal microbiota, but not arthritis development, in IL-1 receptor antagonist deficient mice. *PLoS One* 14 (7), e0219366. doi: 10.1371/journal.pone.0219366

Rogier, R., Evans-Marin, H., Manasson, J., van der Kraan, P. M., Walgreen, B., Helsen, M. M., et al. (2017). Alteration of the intestinal microbiome characterizes pre-clinical inflammatory arthritis in mice and its modulation attenuates established arthritis. *Sci. Rep.* 7 (1), 15613. doi: 10.1038/s41598-017-15802-x

Rosser, E. C., Oleinika, K., Tonon, S., Doyle, R., Bosma, A., Carter, N. A., et al. (2014). Regulatory b cells are induced by gut microbiota-driven interleukin-1beta and interleukin-6 production. *Nat. Med.* 20 (11), 1334–1339. doi: 10.1038/nm.3680

Rosser, E. C., Piper, C. J. M., Matei, D. E., Blair, P. A., Rendeiro, A. F., Orford, M., et al. (2020). Microbiota-derived metabolites suppress arthritis by amplifying aryl-hydrocarbon receptor activation in regulatory b cells. *Cell Metab.* 31 (4), 837–851 e810. doi: 10.1016/j.cmet.2020.03.003

Ruiz-Limon, P., Mena-Vazquez, N., Moreno-Indias, I., Manrique-Arija, S., Lisbona-Montanez, J. M., Cano-Garcia, L., et al. (2022). Collinsella is associated with cumulative inflammatory burden in an established rheumatoid arthritis cohort. *BioMed. Pharmacother.* 153, 113518. doi: 10.1016/j.biopha.2022.113518

Sato, K., Takahashi, N., Kato, T., Matsuda, Y., Yokoji, M., Yamada, M., et al. (2017). Aggravation of collagen-induced arthritis by orally administered porphyromonas gingivalis through modulation of the gut microbiota and gut immune system. *Sci. Rep.* 7 (1), 6955. doi: 10.1038/s41598-017-07196-7

Scher, J. U., Sczesnak, A., Longman, R. S., Segata, N., Ubeda, C., Bielski, C., et al. (2013). Expansion of intestinal prevotella copri correlates with enhanced susceptibility to arthritis. *Elife* 2, e01202. doi: 10.7554/eLife.01202

Schinnerling, K., Rosas, C., Soto, L., Thomas, R., and Aguillon, J. C. (2019). Humanized mouse models of rheumatoid arthritis for studies on immunopathogenesis and preclinical testing of cell-based therapies. *Front. Immunol.* 10. doi: 10.3389/fimmu.2019.00203

Smith, P. M., Howitt, M. R., Panikov, N., Michaud, M., Gallini, C. A., Bohlooly, Y. M., et al. (2013). The microbial metabolites, short-chain fatty acids, regulate colonic treg cell homeostasis. *Science* 341 (6145), 569–573. doi: 10.1126/science.1241165

Sun, Y., Chen, Q., Lin, P., Xu, R., He, D., Ji, W., et al. (2019). Characteristics of gut microbiota in patients with rheumatoid arthritis in shanghai, China. *Front. Cell Infect. Microbiol.* 9. doi: 10.3389/fcimb.2019.00369

Tajik, N., Frech, M., Schulz, O., Schalter, F., Lucas, S., Azizov, V., et al. (2020). Targeting zonulin and intestinal epithelial barrier function to prevent onset of arthritis. *Nat. Commun.* 11 (1), 1995. doi: 10.1038/s41467-020-15831-7

Takahashi, D., Hoshina, N., Kabumoto, Y., Maeda, Y., Suzuki, A., Tanabe, H., et al. (2020). Microbiota-derived butyrate limits the autoimmune response by promoting the differentiation of follicular regulatory T cells. *EBioMedicine* 58, 102913. doi: 10.1016/j.ebiom.2020.102913

Temoin, S., Chakaki, A., Askari, A., El-Halaby, A., Fitzgerald, S., Marcus, R. E., et al. (2012). Identification of oral bacterial DNA in synovial fluid of patients with arthritis with native and failed prosthetic joints. *J. Clin. Rheumatol.* 18 (3), 117–121. doi: 10.1097/RHU.0b013e3182500c95

Teng, F., Klinger, C. N., Felix, K. M., Bradley, C. P., Wu, E., Tran, N. L., et al. (2016). Gut microbiota drive autoimmune arthritis by promoting differentiation and migration of peyer's patch T follicular helper cells. *Immunity* 44 (4), 875–888. doi: 10.1016/j.immuni.2016.03.013

van der Heijden, I. M., Wilbrink, B., Tchetverikov, I., Schrijver, I. A., Schouls, L. M., Hazenberg, M. P., et al. (2000). Presence of bacterial DNA and bacterial peptidoglycans in joints of patients with rheumatoid arthritis and other arthritides. *Arthritis Rheum.* 43 (3), 593–598. doi: 10.1002/1529-0131(200003)43:3<593::AID-ANR16>3.0.CO;2-1

Van de Wiele, T., Van Praet, J. T., Marzorati, M., Drennan, M. B., and Elewaut, D. (2016). How the microbiota shapes rheumatic diseases. *Nat. Rev. Rheumatol.* 12 (7), 398–411. doi: 10.1038/nrrheum.2016.85

Wang, J., Chen, W. D., and Wang, Y. D. (2020). The relationship between gut microbiota and inflammatory diseases: The role of macrophages. *Front. Microbiol.* 11. doi: 10.3389/fmicb.2020.01065

Wang, M., Huang, J., Fan, H., He, D., Zhao, S., Shu, Y., et al. (2018). Treatment of rheumatoid arthritis using combination of methotrexate and tripterygium glycosides tablets-a quantitative plasma pharmacochemical and pseudotargeted metabolomic approach. *Front. Pharmacol.* 9. doi: 10.3389/fphar.2018.01051

Wang, G., Huang, S., Wang, Y., Cai, S., Yu, H., Liu, H., et al. (2019a). Bridging intestinal immunity and gut microbiota by metabolites. *Cell Mol. Life Sci.* 76 (20), 3917–3937. doi: 10.1007/s00018-019-03190-6

Wang, Y., Wei, J., Zhang, W., Doherty, M., Zhang, Y., Xie, H., et al. (2022b). Gut dysbiosis in rheumatic diseases: A systematic review and meta-analysis of 92 observational studies. *EBioMedicine* 80, 104055. doi: 10.1016/j.ebiom.2022.104055

Wang, X., Yang, C., Xu, F., Qi, L., Wang, J., and Yang, P. (2019c). Imbalance of circulating Tfr/Tfh ratio in patients with rheumatoid arthritis. *Clin. Exp. Med.* 19 (1), 55–64. doi: 10.1007/s10238-018-0530-5

Wang, Q., Zhang, S. X., Chang, M. J., Qiao, J., Wang, C. H., Li, X. F., et al. (2022a). Characteristics of the gut microbiome and its relationship with peripheral CD4(+) T cell subpopulations and cytokines in rheumatoid arthritis. *Front. Microbiol.* 13. doi: 10.3389/fmicb.2022.799602

Wang, L., Zhu, L., and Qin, S. (2019b). Gut microbiota modulation on intestinal mucosal adaptive immunity. *J. Immunol. Res.* 2019, 4735040. doi: 10.1155/2019/4735040

Wu, X., Tian, J., and Wang, S. (2018). Insight into non-pathogenic Th17 cells in autoimmune diseases. *Front. Immunol.* 9. doi: 10.3389/fimmu.2018.01112

Xie, M. M., Liu, H., Corn, C., Koh, B. H., Kaplan, M. H., Turner, M. J., et al. (2019). Roles of T follicular helper cells and T follicular regulatory cells in autoantibody production in IL-2-Deficient mice. *Immunohorizons* 3 (7), 306–316. doi: 10.4049/immunohorizons.1900034

Xu, H., Cao, J., Li, X., Lu, X., Xia, Y., Fan, D., et al. (2020). Regional differences in the gut microbiota and gut-associated immunologic factors in the ileum and cecum of rats with collagen-induced arthritis. *Front. Pharmacol.* 11. doi: 10.3389/fphar.2020.587534

Xu, H., Pan, L. B., Yu, H., Han, P., Fu, J., Zhang, Z. W., et al. (2022a). Gut microbiota-derived metabolites in inflammatory diseases based on targeted metabolomics. *Front. Pharmacol.* 13. doi: 10.3389/fphar.2022.919181

Xu, X., Wang, M., Wang, Z., Chen, Q., Chen, X., Xu, Y., et al. (2022b).). the bridge of the gut-joint axis: Gut microbial metabolites in rheumatoid arthritis. *Front. Immunol.* 13. doi: 10.3389/fimmu.2022.1007610

Yang, X., Chang, Y., and Wei, W. (2020). Emerging role of targeting macrophages in rheumatoid arthritis: Focus on polarization, metabolism and apoptosis. *Cell Prolif* 53 (7), e12854. doi: 10.1111/cpr.12854

Yang, W., Yu, T., and Cong, Y. (2022). CD4(+) T cell metabolism, gut microbiota, and autoimmune diseases: implication in precision medicine of autoimmune diseases. *Precis Clin. Med.* 5 (3), pbac018. doi: 10.1093/pcmedi/pbac018

Yao, Y., Cai, X., Zheng, Y., Zhang, M., Fei, W., Sun, D., et al. (2022). Short-chain fatty acids regulate b cells differentiation *via* the FFA2 receptor to alleviate rheumatoid arthritis. *Br. J. Pharmacol.* 179 (17), 4315–4329. doi: 10.1111/bph.15852

Yordanov, M., Tchorbanov, A., and Ivanovska, N. (2005). Candida albicans cell-wall fraction exacerbates collagen-induced arthritis in mice. *Scand. J. Immunol.* 61 (4), 301–308. doi: 10.1111/j.1365-3083.2005.01575.x

Yu, D., Du, J., Pu, X., Zheng, L., Chen, S., Wang, N., et al. (2021). The gut microbiome and metabolites are altered and interrelated in patients with rheumatoid arthritis. *Front. Cell Infect. Microbiol.* 11. doi: 10.3389/fcimb.2021.763507

Yue, M., Tao, Y., Fang, Y., Lian, X., Zhang, Q., Xia, Y., et al. (2019). The gut microbiota modulator berberine ameliorates collagen-induced arthritis in rats by facilitating the generation of butyrate and adjusting the intestinal hypoxia and nitrate supply. *FASEB J.* 33 (11), 12311–12323. doi: 10.1096/fj.201900425RR

Zeng, X., Lu, S., Li, M., Zheng, M., Liu, T., Kang, R., et al. (2022). Inflammatory cytokine-neutralizing antibody treatment prevented increases in follicular helper T cells

and follicular regulatory T cells in a mouse model of arthritis. *J. Inflammation Res.* 15, 3997–4011. doi: 10.2147/JIR.S355720

Zhang, X., Chen, B. D., Zhao, L. D., and Li, H. (2020). The gut microbiota: Emerging evidence in autoimmune diseases. *Trends Mol. Med.* 26 (9), 862–873. doi: 10.1016/j.molmed.2020.04.001

Zhang, D., and Frenette, P. S. (2019). Cross talk between neutrophils and the microbiota. *Blood* 133 (20), 2168–2177. doi: 10.1182/blood-2018-11-844555

Zhang, L., Song, P., Zhang, X., Metea, C., Schleisman, M., Karstens, L., et al. (2019). Alpha-glucosidase inhibitors alter gut microbiota and ameliorate collagen-induced arthritis. *Front. Pharmacol.* 10. doi: 10.3389/fphar.2019.01684

Zhang, X., Zhang, D., Jia, H., Feng, Q., Wang, D., Liang, D., et al. (2015). The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. *Nat. Med.* 21 (8), 895–905. doi: 10.1038/nm.3914

Zhao, Y., Chen, B., Li, S., Yang, L., Zhu, D., Wang, Y., et al. (2018). Detection and characterization of bacterial nucleic acids in culture-negative synovial tissue and fluid samples from rheumatoid arthritis or osteoarthritis patients. *Sci. Rep.* 8 (1), 14305. doi: 10.1038/s41598-018-32675-w

Zhao, T., Wei, Y., Zhu, Y., Xie, Z., Hai, Q., Li, Z., et al. (2022). Gut microbiota and rheumatoid arthritis: From pathogenesis to novel therapeutic opportunities. *Front. Immunol.* 13. doi: 10.3389/fimmu.2022.1007165

Zheng, D., Liwinski, T., and Elinav, E. (2020). Interaction between microbiota and immunity in health and disease. *Cell Res.* 30 (6), 492–506. doi: 10.1038/s41422-020-0332-7

Zhou, C., Zhao, H., Xiao, X. Y., Chen, B. D., Guo, R. J., Wang, Q., et al. (2020). Metagenomic profiling of the pro-inflammatory gut microbiota in ankylosing spondylitis. *J. Autoimmun.* 107, 102360. doi: 10.1016/j.jaut.2019.102360

frontiers | Frontiers in Cellular and Infection Microbiology

TYPE Original Research
PUBLISHED 06 February 2023
DOI 10.3389/fcimb.2023.1124256

OPEN ACCESS

EDITED BY
Xiangtian Yu,
Shanghai Jiao Tong University, China

REVIEWED BY
Hengameh Chloè Mirsepasi-Lauridsen,
Statens Serum Institut, Denmark
Chunlin Ke,
Fujian Medical University, China

*CORRESPONDENCE
Yuantao Li
liyuantao@treatgut.com
Hailing Tang
tanghl@yeah.net
Bangzhou Zhang
geebzbz@xmu.edu.cn

†These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION
This article was submitted to Intestinal Microbiome, a section of the journal Frontiers in Cellular and Infection Microbiology

RECEIVED 14 December 2022
ACCEPTED 16 January 2023
PUBLISHED 06 February 2023

CITATION
Liu J, Lin H, Cao M, Lin T, Lin A, Xu W, Wang H, He J, Li Y, Tang H and Zhang B (2023) Shifts and importance of viable bacteria in treatment of DSS-induced ulcerative colitis mice with FMT. *Front. Cell. Infect. Microbiol.* 13:1124256. doi: 10.3389/fcimb.2023.1124256

Shifts and importance of viable bacteria in treatment of DSS-induced ulcerative colitis mice with FMT

Jinglong Liu[1†], Hao Lin[2†], Man Cao[3,4†], Tan Lin[2], Aiqiang Lin[3], Wei Xu[2,3], Han Wang[3], Jianquan He[3], Yuantao Li[3*], Hailing Tang[5*] and Bangzhou Zhang[3,4,6*]

[1]Department of Gastroenterology, Shanxi Provincial People's Hospital, Taiyuan, China, [2]Center for Microecological Medical Technology, Xiamen Institute of Union Respiratory Health, Xiamen, China, [3]Center for Research and Development, Xiamen Treatgut Biotechnology Co., Ltd., Xiamen, China, [4]Department of Gastroenterology, The Second Affiliated Hospital of Fujian University of Traditional Chinese Medicine, Fuzhou, China, [5]Division of Gastroenterology, Xi'an Central Hospital, Xi'an, China, [6]School of Pharmacy, Fujian University of Traditional Chinese Medicine, Fuzhou, China

Background and Aims: Ulcerative colitis (UC) has become a global public health concern, and is in urgent need of novel therapies. Fecal microbiota transplantation (FMT) targeting gut microbiota has recently been applied to the treatment of UC. Despite its recent successes, it is still largely unknown how FMT functionally modulates the gut microbiota and improves the disease.

Methods: We prospectively collected fecal samples from the 40 mice (30 mice for dextran sulfate sodium (DSS)-induced, 10 for controls), followed by Propidium monoazide treatment for 16S rRNA gene sequencing. These 30 mice were divided equally into 3 groups, which were transplanted with original donor microbiota (DO), inactivated donor microbiota (DI) and saline, respectively. Subsequently, we used 16S rRNA gene sequencing to analyze the viable gut bacteria of ulcerative colitis (UC) mice and histological analysis to evaluate the effects of fecal microbiota transplantation (FMT) with viable microbiota.

Results: We demonstrated that the community structure of viable bacteria was significantly different from fecal bacteria based on total DNA. Furthermore, the intestinal viable microbiota and colonic mucosal structure of mice were significantly changed by DSS induction. The histological analysis showed that only the mice treated with original donor microbiota group (HF) achieved a significant improvement. Compared with inactivated donor microbiota group (IF) and saline (NF), Lactobacillus and Halomonas were significantly enriched in the HF group.

Conclusion: We inferred that only live bacteria from human donor reversed the histopathology and symptoms of UC in mice and altered the gut microbiota. The activity of gut microbiota in donor samples should be considered in FMT and that detailed analysis of viable microbiota is essential to understand the mechanisms by which FMT produces therapeutic effects in the future.

KEYWORDS

viable gut microbiota, PMA, FMT, DSS-induced colitis, 16S rRNA gene sequencing

Introduction

Ulcerative colitis (UC) is one subtype of inflammatory bowel disease (IBD) that has a high incidence and prevalence in the worldwide (Lima et al., 2021). It is also thought to be intricately caused by variable factors, including genetic (Hedin et al., 2015), immunological and environmental aspects (Braun and Wei, 2007), of which the gut microbiota dysbiosis may play important roles (Sheehan et al., 2015; Kump et al., 2018; Zhang et al., 2022). Despite available therapies, including corticosteroids, anti-tumor necrosis factor alpha (TNF-α) agents, aminosalicylates, immunomodulators, and surgery (Weisshof et al., 2018), development of new therapies and investigation of alternative strategies are in urgent need for amount of patients who are unresponsive to these existing treatments or present secondary failure during treatment.

Fecal microbiota transplantation (FMT) is a novel treatment method which is to transfer the functional microbiota from normal feces to an unbalanced gastrointestinal tract, reconstruct a new intestinal flora, and resume the host function (Costello et al., 2017; Liu et al., 2021) This technique has proven effective impact in many microbiota-related metabolic (Lee et al., 2019; Que et al., 2021; Gong et al., 2022), infectious (Smillie et al., 2018), and inflammatory diseases (Weingarden and Vaughn, 2017; Zhang et al., 2019). In recent research, the patients have recurrent *Clostridium difficile* infection were extremely effective treated by FMT (about 90% cure rate) (Le Bastard et al., 2018). However, the effectiveness of FMT varied among different studies (Li et al., 2016; Smillie et al., 2018), in which specified donors may play crucial roles. These differences not only exist between individuals but sometimes even within a same person (McOrist et al., 2011). For example, one study showed no improvement in clinical and endoscopic remission at 12 weeks following two infusions of FMT from healthy donors *via* a nasogastric tube, while another study showed higher endoscopic remission at 7 weeks in patients treated with weekly FMT enemas (Moayyedi et al., 2015; Rossen et al., 2015). Therefore, the detailed analysis of the composition and numbers of microbiota transplanted is essential to understand the differences in therapeutic effectiveness and mechanisms of FMT from donor samples.

The gut microbiota is a complex ecosystem with a range of bacterial genera that perform many important functions in the host, including maintaining gut homeostasis, intestinal epithelial barrier, immune system development and providing essential metabolic substrates for colon cells (Pushalkar et al., 2018), play important roles in UC progress (Ni et al., 2017; Liu et al., 2021). Gut microbiota manipulation by FMT has demonstrated promising effectiveness in UC remission in experimental colitis mice model trials. For instance, Zhang et al. evaluated the FMT effect on the composition of the colonic microbiota to determine whether changes in the gut microbiota were associated with the protective effect of FMT in DSS-induced mice (Zhang et al., 2022). Lima et al. identified the speices *Odoribacter splanchnicus*, which plays a key role in FMT, by performing the immune response use omic analysis in donor and recipient fecal samples (pre- and post-intervention), Further through mouse experiments, they proved that *Odoribacter splanchnicus* is the key bacterium (Lima et al., 2021). Similarly, Li et al. treated a mouse model of DSS-induced colitis with FMT in combination with a 16S rRNA analysis, revealing that FMT ultimately alleviates colitis by regulating the flora (Li et al., 2021a). Generally, in all of these analyses, the changes in metagenome-based strategy include 16S rRNA sequencing of total bacteria DNA were observed, while changes in the live bacteria DNA were neglected. Moreover, with the deepening of the study, living microbiota are considered to be therapeutic agents for FMT, because the colonization of these microbiota in the intestines of recipients may lead to lasting changes in patients (Khoruts et al., 2010; Seekatz et al., 2014). Therefore, it is of great significance to focus on the viable bacteria for understanding the mechanism of FMT and exploring the crucial gut microbiota.

In this paper, we presented a pioneer work to evaluate the living bacteria of gut microbiota from UC patients by FMT trials. First, 40 mice were collected for analysis. Then, 30 of them were induced with colitis by DSS and 10 were not treated with DSS served as controls. These 30 mice were also divided equally into 3 groups. Group one was transplanted with initial donor microbiota (HF), while group two was transplanted with inactivated flora (IF), and group three were transplanted with saline (NF). Subsequently, the remission rate of FMT treatment was analysed by histopathology and symptoms in mice. Meanwhile, PMA-treated donor samples were analysed by 16S rRNA gene sequencing for structural changes of total and viable bacteria DNA, as well as changes in live bacteria between DSS and controls, pre- and post- FMT, respectively. This method can accurately evaluate the changes in viable bacteria in the gut microbiota, establish a methodological basis donor screening, evaluation before and after transplantation of viable bacteria in fecal samples in the future.

Materials and methods

Preparation of donor stool sample

Samples were collected with informed consent from all participants. All participants completed a questionnaire-based interview and underwent a physical examination for screening of donors (He et al., 2021). Every subject provided fresh stool samples in a stool container on site. Fecal microbiota were extracted with an automatic fecal microbiota extractor TG-01 Extn (Treatgut, Guangzhou, China) in Xiamen Treatgut Biotechnology Co. Ltd. Fecal sludge (FS) were collected by centrifugation at 5,000 g for 5 min. The collected microbiota were then added to saline at a ratio of 1:1.1, and half of the resulting solution were autoclaved in a 250 mL Erlenmeyer flask at 121 °C for 30 min to prepare inactivated donor microbiota (DI), with the remainder serving as the original donor microbiota (DO). Total microbiota andviability were determined by flow cytometry with LIVE/DEAD™ BacLight™ Bacterial Viability Kit (Thermo Fisher Science). Analyses were carried out using a BD Accuri™ C6 Plus Flow Cytometer (BD Biosciences, USA) system. Meanwhile, the PMA-qPCR standard curve of donors were established.

Animals and experimental design

A total of 40 male C57BL/6J mice (7 weeks old, 18-20 g weight) were purchased from the Gempharmatech Co., Ltd (China). Mice

were allowed one week to acclimate prior to the study. For this period, food and water were given ad libitum and the room was ventilated, having an ambient temperature of 22 °C ± 1°C with 50% ± 10% humidity and a 12-h diurnal light cycle (lights on 07:00–19:00). 30 of 40 mice were administered a 3% dextran sulfate sodium (DSS, MP Biomedicals, USA) solution and 10 for controls not treated with DSS. These 30 DSS-induced mice were divided equally into 3 groups, which were HF, IF and NF, respectively. Mice were treated with DSS for 5 days and then gavaged for 3 days. 200 μL per dose once daily for 3 days in the HF and IF groups, and equal saline doses in the NF and control groups. Fresh fecal (250 mg) from mice in four groups were collected at the 7, 12 and 15 days, and resuspended in a 5 ml saline, vigorously shaken 3 min for subsequent analyses. All animal experiments reported in this study were approved by the Animal Care and Ethics Committee of Fujian University of Traditional Chinese Medicine Laboratory Animal Center.

Phenotype detection of mice

During the intervention period, the body weight and stool consistency of mice were observed regularly. DAI scoring criteria refers to Rangan et al. (Rangan et al., 2019). After intervention, animals were humanely sacrificed by cervical dislocation, and the colons were removed. Colon lengths and weights were measured using a ruler and an electronic analytical balance respectively. To observe detailed histopathological changes, the colons of different mice were first stored in a 10%buffered formalin solution. These were then embedded in paraffin, cut into 5 μm sections, stained with hematoxylin-eosin, and then placed under a light microscope for examination.

PMA-treated samples

Stock solution was prepared by dissolving 1 mg of PMA (US Everbright Inc,Suzhou,China) in 1 mL of 20% dimethyl sulfoxide. For PMA treatment, the FS samples treated as above method was diluted 100 times in normal saline solution. A 487.5 μL solution was weighed and transferred to an aseptic EP tube, followed by the addition of 12.5 μL PMA solution. The solution was mixed, and the tubes were incubated in dark for 10 min at room temperature. Samples were exposed to an LED light (500W) with periodic mixing at a distance of 15 cm for 10 min. In non-PMA treated control aliquots, 12.5 μL saline was added instead of PMA. Control samples underwent identical incubation and light-exposure as the matching PMA treated samples (Emerson et al., 2017). All samples was treated by PMA for further analysis.

DNA extraction

DNA was extracted from fecal samples using the QIAamp Fast DNA Stool Mini Kit (Qiagen, CA, USA) flowing the manufacturer's instructions. The concentration and purity of the isolated DNA was assessed using spectrophotometry (Multiskan™ GO, Thermo Fisher Scientific, USA). The DNA extracts were also evaluated for quality by agarose (1.5%) gel electrophoresis in 1× Tris-Acetate-EDTA buffer. DNA samples were stored at -20˚C before being used as templates for next-generation sequencing library preparation.

Library preparation and sequencing

Sequencing libraries were generated using TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA) following manufacturer's recommendations and index codes were added. The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, China) and Agilent Bioanalyzer 2100 system. At last, the library was sequenced on an Illumina MiniSeq 150 bp paired-end reads were generated.

Quantitative PCR

Bacterial 16S rRNA genes in the fecal samples were quantified using real-time qPCR on a StepOnePlus Real-Time PCR system (Thermo Fisher Scientific, Waltham, MA, China). The V4 variable regions of bacterial 16S rRNA gene were PCR-amplified using the primers (515F 5'-GTGYCAGCMGCCGCGGTAA-3',806R 5'-GGACTACNVGGGTWTCTAAT-3'). Each reaction mixture had a total volume of 20 μL. Itcontains 2 μL of sample DNA, 10 μL of ChamQ Universal SYBR qPCR Master Mix (Vazyme Biotech, NJ, China), 0.4 μL of each 10μM primer, and 7.2 μL of sterilized ultra-pure water. The cycle conditions of the real-time PCR were as follows: initial holding at 95 °C for 30 s, 40 cycles of denaturation at 95 °C for 10 s followed by annealing/elongation at 60 °C for 30 s. The specificity was determined after amplification by a melting curve analysis. All qPCR tests were performed in triplicate, and the mean values were used for analysis.

Bioinformatics and statistical analysis

First, Fast Length Adjustment of Short Reads (FLASH) (V1.2.11) was used to assemble paired-end reads for the V4 region, the -x 0.15 option was selected to control the maximum mismatched base pairs ratio in the overlap area, and the -M 150 option was selected to control the maximum length of the overlap area. Then, cutadapt (V1.13) was used to trim and filter the sequence data processed from FLASH, including removing adapter sequences and discarding sequences with fewer than the specified number of bases. Subsequently, sequences were quality filtered by Usearch with the -fastq_maxee 1.0 option. After quality control, unique sequences were obtained by eliminating redundancy, and they were sorted in descending order according to sequence abundance. Meanwhile, singletons in the sequence data were removed. To assign denovo OTUs, we removed chimeric sequences and clustered sequences with 97% similarity and using Usearch (Edgar, 2013) for individual study. The representative sequences of OTUs were aligned to the SILVA 132 database for taxonomic classification by RDP Classifier (Wang, 2007) and aggregate to various taxonomic levels.

Based on the OTU tables derived from each sample, alpha-diversity indices between every sample were calculated, including

bacterial richness (observed OTUs), shannon index, and evenness (J). Significance tests of alpha-diversity indices were conducted by the Wilcoxon test method. Then, Principal coordinates analysis (PCoA) based on Bray-Curtis distance at the OTU level was utilized for beta-diversity to visualize the differences in microbial community structure across samples. Significance tests of beta-diversity indices were determined using permutational multivariate analysis of variance (PERMANOVA) with 10^4 permutations in vegan. Linear discriminant analysis (LDA) effect size (LEfSe) was employed to identify the taxa most likely to explain the differences between groups. LEfSe uses a nonparametric Kruskal–Wallis rank sum test to assess different features with significantly different abundance between assigned taxa and performs LDA to estimate the effect size of each sequence variant, as reported by (Segata et al., 2011). Finally, the results were visualizing using the custom R script based on ggplot2 (Wickham et al., 2016). These analyses were performed using R v3.4.1, GraphPad Prism and SPSS software. A p value < 0.05 was considered statistically significant. In addition, all obtained data are expressed as the mean ± standard deviation (SD).

Results

Therapeutic effect of live microbiota in mice with DSS colitis by FMT

To investigate the alleviating effect of live FMT bacteria on colitis, we induced experimental colitis in mice (n=30) by administering 3% DSS in water for 5 consecutive days and then started FMT intervention in mice on the sixth day for 3 consecutive days (Figure 1A). The control (CON) group of mice (n=10) were in good mental condition, without diarrhoea and soft stools. The DSS mice had loose stools from day 3 of the moulding, followed by severe soft stools, bloody stools and depression on day 4. The DSS-induced mice were divided equally into three groups for FMT of DO, DI feces and saline treatment. From the Figure 1B, we could see that there was no significant difference among the HF, IF and NF groups, all of which showed a decreasing trend in body weight. Colonic length were significantly decreased compared to CON group, while there was no significant difference among the HF, IF and NF groups (Figure 1C). The DAI scores of DSS-induced mice increased on day 3, with the mice in the HF group had significantly lower DAI scores than NF group (Figure 1D). In terms of histological scores, the HF group was significantly lower than both the IF and NF groups, while there was no significant difference between the IF and NF groups (Figure 1E). Meanwhile, the histological analysis further revealed that the histopathological status of the observed colonic specimens was as shown in the Figure 1F, with normal colonic tissue morphology in the CON and HF groups, with a clear hierarchy of tissue structures, with the mucosal, submucosal and muscular layers clearly visible and the crypt and cupped cells well arranged. A variable number of inflammatory cells were seen, and the crypt was dilated near the ulcer foci. In summary, these results demonstrate that live bacteria in FMT are able to participate in and improve clinical colonic inflammatory conditions and colonic damage, whereas dead bacteria do not.

Differences between bacterial communities from total fecal DNA and PMA-treated DNA

Flow cytometric analysis showed that the original donor microbiota retained roughly 66.7% of viable bacteria, while almost all of the viable bacteria were removed after the heat-killed treatment, which is less than 0.7% (Figure 2A). Due to the low viability of the flow cytometric assay after inactivation, the dead bacteria DNA was interfered after PMA treatment and could not be amplified. Therefore, we evaluated the structure of DO and the PMA-treated original donor microbiota (DP) by 16S rRNA sequencing analysis. As shown in Figure 2C, the Observed, Shannon and evenness (J) indices were slightly reduced after PMA-treated, although the differences were not statistically significant. PCoA ordination based on Bray-Curtis distances between OTU abundance profiles shows that fecal samples after PMA were distinctly separated from the DO group (Figure 2B). At the genus level, a slightly increased abundance of *Prevotella_7*, *CAG-352*, and *Prevotella_2* and a slightly decreased abundance of *Faecalibacterium* and *Veillonella* were observed after PMA-treated in comparison to the DO group (Figure 2D). Notably, at the family level, a distinct decrease in the abundance of Ruminococcaceae, Lachnospiraceae and Veillonellaceae and an increase in the abundance of Prevotellaceae and Bacteroidaceae were observed after eradication compared to before eradication or confirmation (Figure S1). Also, we collected 11 donor stool samples to explore the correlation between bacterial load and CT values using PMA-qPCR technique (Supplementary Material). As shown in Figure S2, the activity and total bacterial load were verified by fitting standard curves based on the CT values of qPCR and flow cytometry bacterial counts. The CT values gradually decreased as the total bacterial load increased, and the correlation coefficient between their total bacterial load and CT values was close to 1 (R^2 = 0.90), indicating that the correlation between bacterial load and CT values was significant.

The histological and viable gut community differences between DSS and the controls

Histological analysis showed that compared with normal mice, DSS-induced mice formed ulcerative foci in the mucosal layer of the colon, with necrosis spreading to the entire mucosa resulting in loss of lamina propria and proliferation of connective tissue, with varying numbers of inflammatory cells infiltrating between them and dilated crypt foci near the ulcerative foci (Figure 3A). In addition, 16S rRNA gene high-throughput sequencing analysis showed that the alpha diversity indices Observe, Shannon and J were significantly decreased in UC mice compared to CON group (p<0.05, Figure 3C). We observed clear the clustering of microbial communities between colitis mice and normal group by the PCoA plot (p<0.05, Figure 3B). Further, the microbiota composition in the DSS-induced colitis mice displayed a significantly different profile at genus level from that in the controls. Sixteen taxa including *Massilia*, *Rikenella*, *Butyricicoccus*, and *Enterococcus* were decreased in cases compared to CON group, while 19 genera including *Akkermansia*, *Blautia* and *Odoribacter* were significantly increased

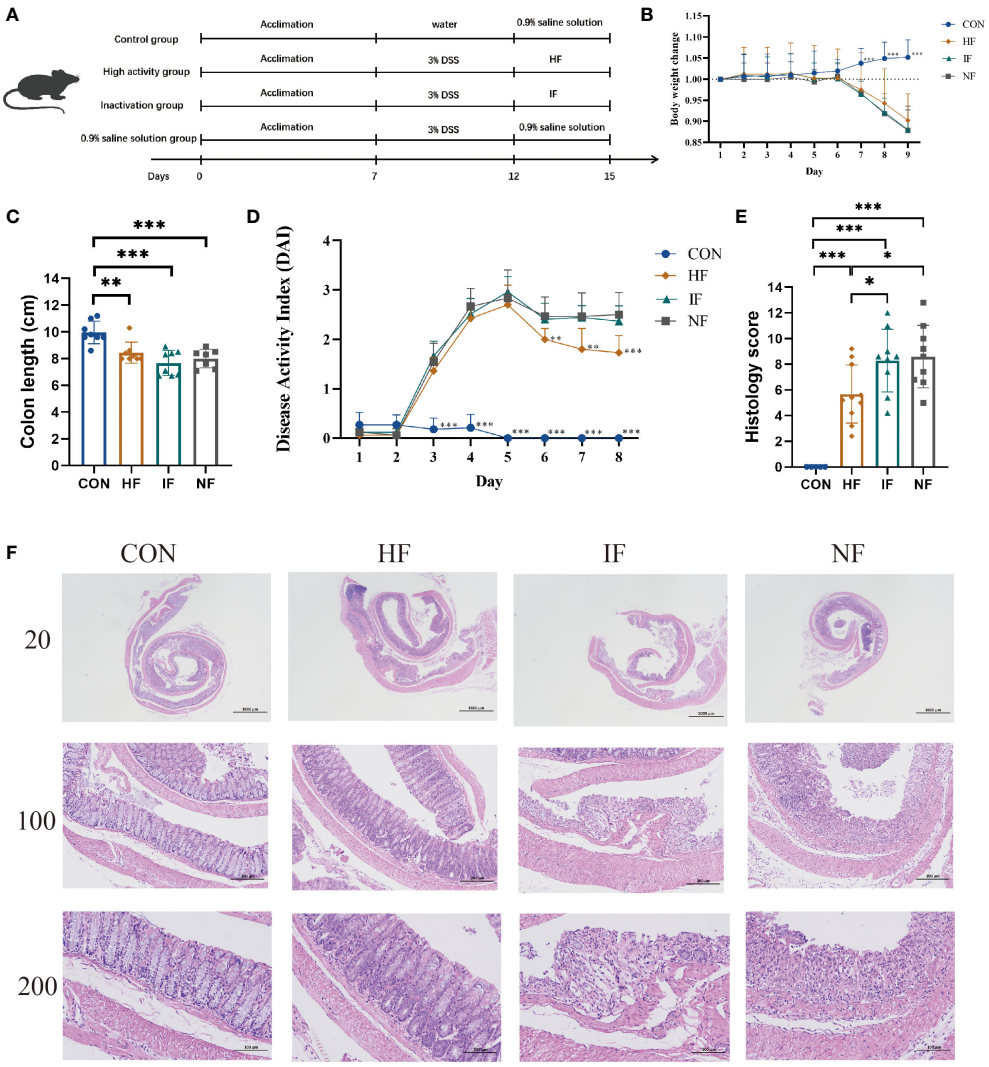


FIGURE 1
(A) The animal experimental protocol. **(B)** Daily body weight changes throughout the entire duration of the study. **(C)** the lengths of colon from each group. **(D)** Kinetics of DAI scores throughout the entire duration of the study. **(E)** Histological scores of colons. **(F)** H&E stained colon sections. Data are presented as mean ± SD. ***p < 0.001, **p < 0.01 and *p < 0.05 vs the NF group.

in DSS group (p<0.05, Figure 3D). These results indicate that the intestinal microbiota and colonic mucosal structure of mice were significantly changed by DSS induction.

Effect of FMT on the composition of the gut microbiome

Acute colitis was induced in mice with 3.0% DSS and transplanted with DO, DI group and saline respectively. Subsequently, changes in the gut microbiota of the HF, NF and IF groups were analyzed by 16S rRNA gene high-throughput sequencing. Due to the failure of library construction for one sample, only 9 mice in NF group were included in the microbiota analysis. The Observed index of the HF group was significantly lower than that of the IF and NF group, but the Shannon and evenness indices were not significantly different (Figure 4A). We also found 70 genera specific to the IF group such as *Coprobacter*, *Eggerthella* and *Erysipelatoclostridium*, which may have contributed to the elevated IF group diversity (Table S1). Pre- and post-transplantation analysis of the three groups showed no obvious change in the observed index in the HF group compared with DSSHF group, while the index was markedly change in either IF vs DSSIF or NF vs DSSNF group (Figures S1A–3A). PCoA plots analysis showed distinct differences in the gut microbiota of the three groups after treatment (p<0.05, Figure 4B). As shown in Figures S3B–5B, the gut microbiota community structure of DSS-induced mice was changed after FMT (HF, IF and NF). In addition, SPEC-OCCU plots were analyzed for microbiota in the HF, IF and NF groups (Figure 4C). Five specific genera, *Bacteroides*, *Lactobacillus*, *Halomonas*, *Bifidobacterium* and *Fusobacterium*, were identified by analyzing specificity and occupancy (≥0.7) in the HF group compared to the NF and IF groups. The relative abundance of *Fusobacterium* was significantly higher in the IF group than in the NF group (p<0.05), and the HF group was not significantly different from the other two groups. However, the relative abundance of *Halomonas* and *Lactobacillus* were significantly higher in the HF group than in the IF

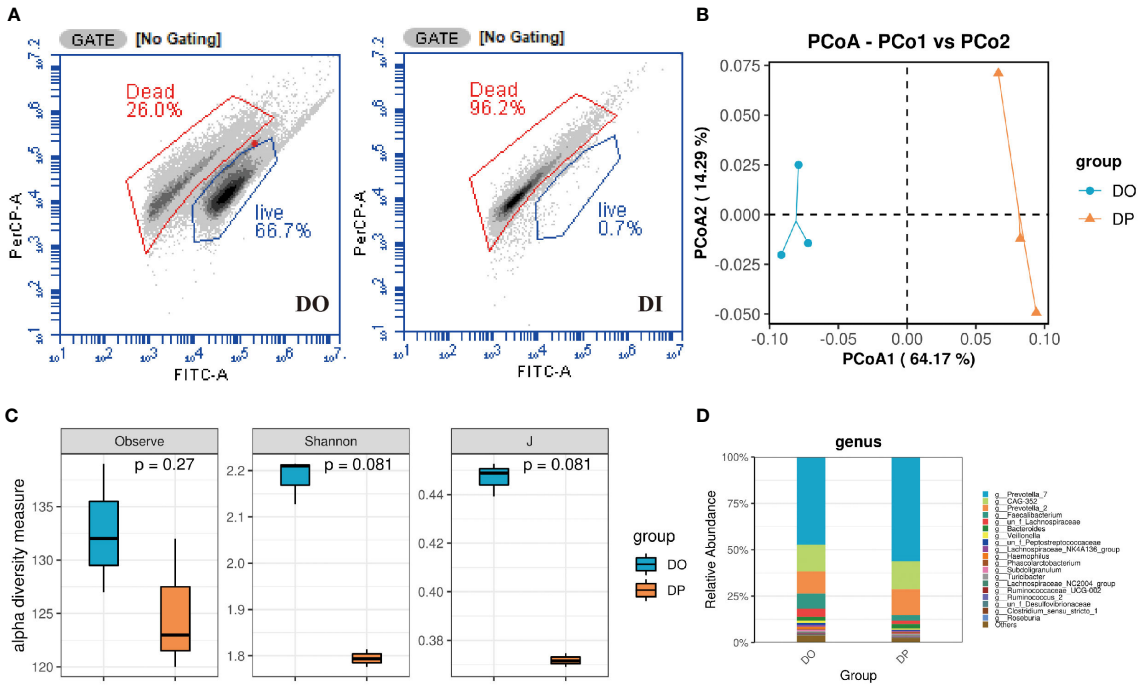


FIGURE 2
Composition of donor gut microbiota before and after PMA treatment turned out. **(A)** Flow cytometry counts of DO and DI groups. DO, DI and DP samples were analyzed based on 16S rRNA gene sequencing. **(B)** PCoA analysis; **(C)** Taxonomic profiles at the top 20 genus levels in terms of overall mean relative abundance; **(D)** microbial alpha diversity as estimated by species richness, the Shannon diversity index, and the Inverse Simpson diversity index(J) based on OTU abundance data.

group (Figure 4D). The distinct differences in taxa were observed after FMT compared to before FMT. A similar trend of *Bacteroides*, *Lactobacillus*, *Halomonas*, *Bifidobacterium* and *Fusobacterium* abundance was also observed in the before and after FMT group (Figures S3C-5C).

Discussion

In this study, we conducted a FMT trial to evaluate the importance and shifts of viable gut microbiota in DSS-induced UC mice treated by FMT from a donor sample. A total of 30 mice were divided equally into 3 groups according to the different treatments, and another 10 mice without DSS inducement were set as control. By 16S rRNA gene sequencing analysis of fecal samples treated by PMA, we found that the structure of viable bacteria DNA is different from total bacteria DNA. The intestinal viable bacteria and colonic mucosal structure of mice were significantly changed by DSS induction. The histological analysis showed that FMT with live microbiota (HF) were able to improve colonic inflammatory conditions and colonic damage, whereas effect of dead microbiota was similar with the placebo with saline. Meanwhile, we identified key genera that changed after transplantation with HF, including *Bacteroides*, *Lactobacillus*, *Halomonas*, *Bifidobacterium* and *Fusobacterium*, which provides a reference for the treatment of UC.

We found that the bacterial structure of total DNA in donor fecal sample differed from the microbial community structure after PMA. As known, most of the microorganisms in the intestinal tract are difficult to be cultured by conventional methods (Costello et al., 2015). For general molecular methods, total DNA of a sample was used as a template for PCR amplification, which is difficult to distinguish viable and dead microorganisms, resulting in false negative results. With the development of powerful and convenient high-throughput sequencing technology, 16s rRNA gene or metagenomic sequencing is a common tool for measuring the relative abundance of specific microorganisms in microbial ecology (Reuter et al., 2015; Zemb et al., 2020). Therefore,the activity and profile of gut microbiota in donor samples can be thought as an important evaluation indicator in donor screening in the future.

The changes of viable microbiota were explored in the intestine of DSS-induced and normal mice. After DSS induction, HE staining showed that the intestinal tissues were damaged, accompanied by structural changes in the intestinal flora. Alpha diversity analysis and PCoA plots indicated that the composition of the intestinal live microbiota in colitis mice changed along with the altered intestinal tissue structure, and DSS induction disrupts the stable microenvironment of the intestine. Some live bacteria may play a central role. LEfSe confirmed our hypothesis by finding a total of 19 bacterial genera with large differences in DSS group, including *Akkermansia*, *Blautia* and *Odoribacter* et al. *Akkermansia* is known as a mucin-degrading bacterium with regulatory and inflammatory properties. DSS induced disruption of the mucosal layer in the hindgut and increased infiltration of acute inflammatory immune cells (Rinaldi et al., 2019). Meanwhile, the hypothesis of *Akkermansia* as an opportunistic bacterium that may flourish after ecosystem disruption (Machiels et al., 2020), which explained the increase of live *Akkermansia.* in the intestine of mice after DSS induction in normal mice. *Odoribacter* were also increased in DSS group. Li et al.

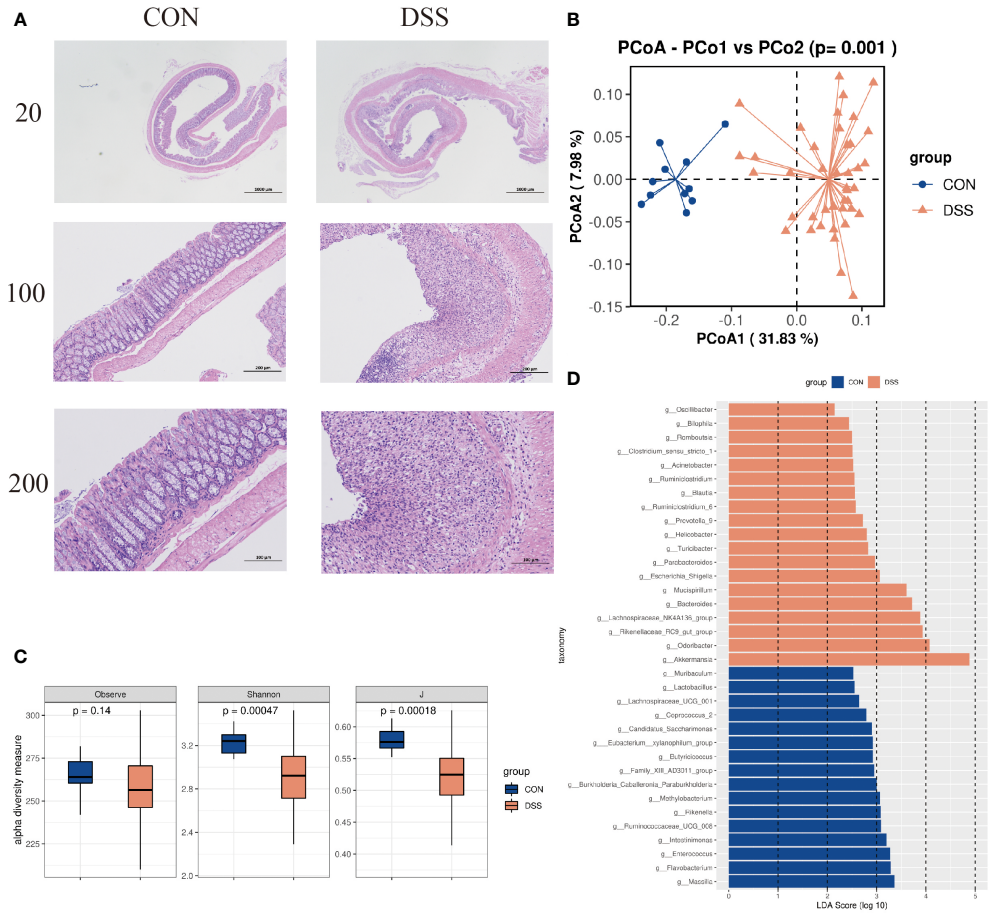


FIGURE 3
(A) HE dyeing experiment pictures. **(B)** Bacterial beta diversity. Principal Coordinates Analysis based on Bray-Curtis distances between the gut microbiota profiles of mice from the two groups. **(C)** Alpha diversity based on species richness, the Shannon diversity index, and the Inverse Simpson diversity index (J) in DSS and CON. **(D)** Significantly enriched bacterial taxa in the different groups as determined by LEfSe analysis (LDA sore >2).

found that *Odoribacter* showed a state of inhibition by other bacteria in healthy subjects, but were "unrestrained" and significantly more abundant in UC patients (Li et al., 2021b). The researchers also found a relationship between this opportunistic pathogen and pathophysiological mechanisms such as reduced SCFAs and increased inflammatory response. With a larger number of influential live bacteria identified through LEfSe method that may have potential significance for the diagnosis and treatment of UC and deserve to be further explored.

The result of DAI scores and histological examination indicated that live bacteria can participate in maintaining intestinal homeostasis, whereas dead bacteria often fail to play a role. The Observe index was significantly lower in the HF group than that in the IF and NF groups. This may be due to some dead bacteria from donor faeces, which interfered with the analysis of the live microbiota and caused differences in Observed index. 70 specific-genera was belong to the IF group such as *Coprobacter*, *Eggerthella* and *Erysipelatoclostridium*, which may have contributed to the elevated IF group diversity. The genus *Erysipelatoclostridium* is a pro-inflammatory microorganism with high potential to induce TH1 cells and high potential for intestinal inflammation (Nagayama et al., 2020). Bo Yang et al. found that *Eggerthella* may be associated with clinical symptoms of diarrhoea in a study on diarrhoeal irritable bowel syndrome and functional diarrhoea (Yang et al., 2021). Chen et al. found elevated relative abundance of *Escherichia-Shigella* in a study of the intestinal microbiota during acute necrotizing pancreatitis in rats (Chen et al., 2017). Furthermore, the PCoA plots showed that there were significant differences in the living microbiota of mice after different treatments. The differences of the intestinal structure tells that both live and dead bacteria were able to alter the intestinal structure of mice compared to the saline group. Combined with the HE staining results, the live bacteria was able to restore the intestinal health of mice, while the dead bacteria could not, probably because the dead bacteria could act as postbiotics to allow the growth of harmful bacteria.

Specificity and occupancy (≥0.7) were identified by SPEC-OCCU plots analysis of five specific genera in the HF group compared to the NF and IF groups, including *Bacteroides*, *Lactobacillus*, *Halomonas*, *Bifidobacterium* and *Fusobacterium*. In line with the He et al. study (He et al., 2013), *Bacteroides* was also substantially elevated in this trial. *Bacteroides* has good function on the improvement of endotoxaemia, reducing gut microbial lipopolysaccharide production and effectively inhibit pro-inflammatory immune responses, and low anthropoid bacteria can lead to inflammatory bowel disease (Althouse et al., 2019). Similarly, an increase in *Lactobacillus* was observed in UC mice after FMT treatment. Most

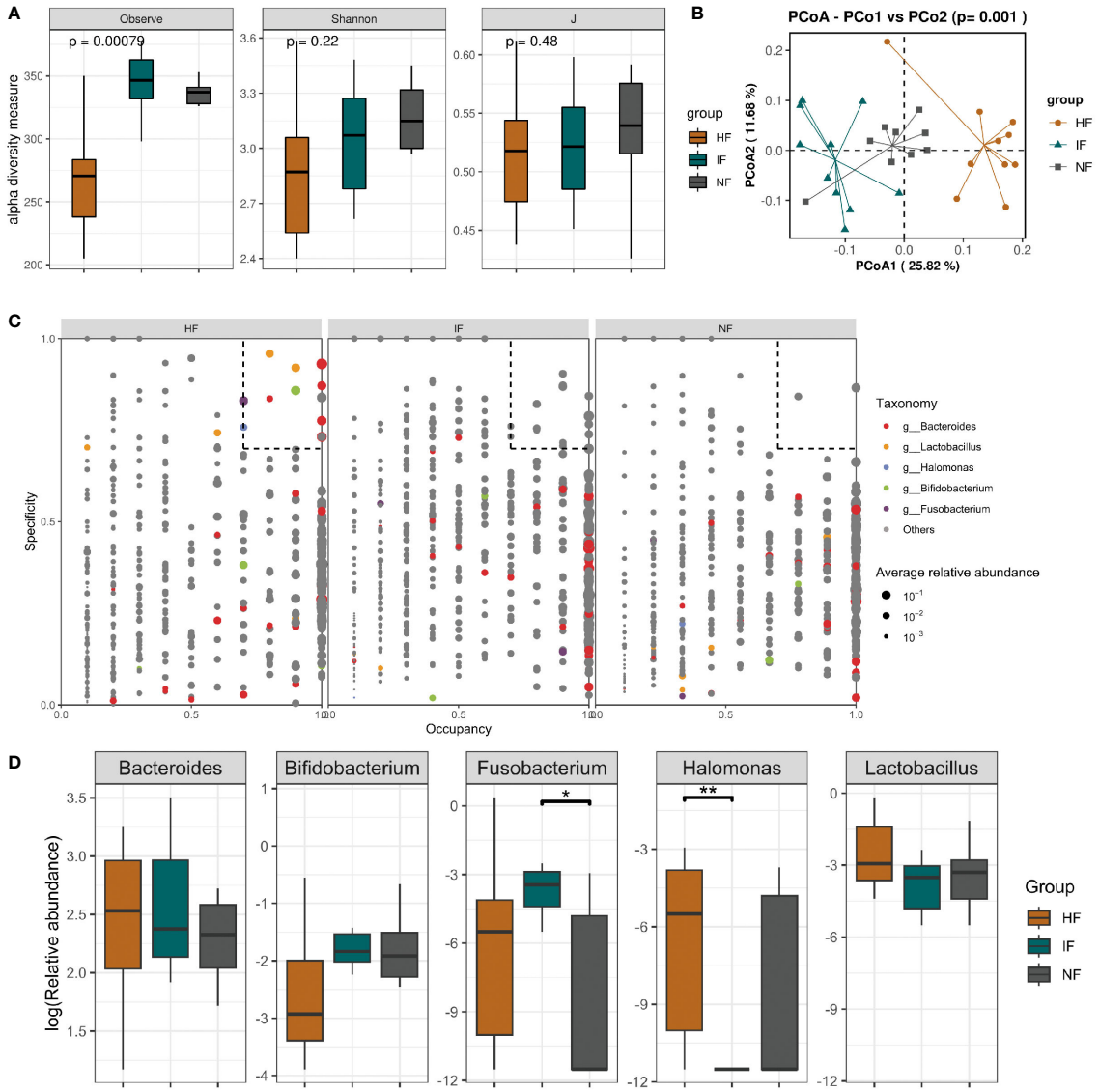


FIGURE 4
(A) Alpha diversity based on species richness, the Shannon diversity index, and J in HF, IF and NF. **(B)** Diagram of the Bray–Curtis distance principal coordinate analysis. **(C)** The SPEC-OCCU plots show the most abundant OTUs corresponding genera in HF; the x-axis represents occupancy; and the y-axis represents specificity. **(D)** The relative abundance of 5 specific genera among three groups. **p < 0.01 and *p < 0.05.

of the current results prove that *Lactobacillus* is also the main genus used for the treatment of UC (Yun et al., 2020). For example, Liu et al. reduced intestinal lining inflammation by rectal enemas of *Lactobacillus*. It is inferred that *Lactobacillus* inhibits the onset of colitis in mice and may reduce the onset of stress-induced colitis (Liu et al., 2018). Liu et al. reported that *Halomonas* is the predominant genus associated with the jejunal and ileal mucosa of goats and speculated, and *Halomonas* may play a role in promoting immune development in the gut (Liu et al., 2019). Consistent with the present experiment, there was a substantial increase in live bacteria of the genus *Halomonas*. For *Bifidobacterium*, it has been used extensively in the treatment of inflammatory bowel diseases, such as UC (Xie et al., 2022). *Bifidobacterium* in human intestine can synthesise many vitamins such as vitamin B1/B2/B6, nikonic acid, pantothenic acid, folic acid and biotin. Once synthesised, these vitamins are then absorbed by the mucosal cells and contribute to the body's metabolism and health maintenance (Huang et al., 2019). For *Fusobacterium*, it is a recognized pro-inflammatory bacterium that does not act in a simple one-way relationship with other bacteria, but may form mutually beneficial relationships that promote dysbiosis (microbial imbalance) in the community (Agarwal et al., 2020). From this we can infer that the live intestinal microbiota increased in beneficial bacteria, thus reducing the pro-inflammatory effect of *Fusobacterium*. In terms of relative abundance, the difference in *Bacteroides* and *Lactobacillus* between the groups was not significant, probably because the acute UC model was used for this modeling and the mice recovered naturally. That means live bacteria accelerate the healing of intestinal losses. So, higher levels of *Bacteroides*, *Lactobacillus*, *Halomonas* and *Bifidobacterium* in the live intestinal microbiota may be associated with the recovery of UC intestinal tissues, with the surviving live flora playing a major role. In addition to the vital importance of viable microbiota for the

treatment of UC mice with FMT, SCFA produced by these microbiota may also play a key role in inhibiting intestinal inflammation, anti-tumor effects and regulating immune response (Mirsepasi-Lauridsen, 2022). For example, *Bifidobacterium* was known to produce the acetate that can protect against enteric infection in mice (Rabbani et al., 1999; Fukuda et al., 2011; Sepúlveda et al., 2018); *Bacteroides* was also the main bacteria involved in producing SCFA and play an important role (Kaakoush et al., 2014; Comstock, 2009). *Lactobacillus* produced butyrate by altering the intestinal microbiota, which maintains homeostasis in the gut, reduces inflammatory responses and serves as a source of energy for the renewal of intestinal epithelial cells (Jhun et al., 2021; Tian et al., 2019; Jena et al., 2020). As shown in Figure S6, it was found that SCFA-producing genera in the donor, such as *Bifidobacterium* and *Bacteroides* that colonized mice, may play a key role in the treatment of UC mice.

However, the current dose of FMT is based on the weight of the bacterial sludge (Zhang et al., 2020). The number of live organisms in the slurry is a key factor in judging the merit of the FMT product as well as its effectiveness in improving efficacy while reducing the number of doses taken by the patient and making it less difficult for the patient to take the medicine. Therefore, high bacterial level is the key to the efficacy of FMT. The usual analysis of total bacteria indicates is inaccuracy and can result in false positive results, so the live microbiota analysis its biological significance is greater compared to the total microbiota analysis. What's more, many UC-associated inflammatory factors have been reported and it is important to explore the mechanisms of inflammation by detecting and observing changes in these inflammatory factors (Rubin et al., 2019). As most of the signaling pathways are significantly affected by the disease, further exploration to detect the expression of key factors in the signaling pathways can follow. The interrelationship between inflammatory factors and signaling pathways merits further investigation, which may provide further insights into targeting the microbial groups as a therapeutic strategy for UC and other diseases associated with the gut microbiota.

In summary, by H&E stained and 16S rRNA gene sequencing analysis of 30 DSS-induced mice and 10 controls with PMA treatment, we observed the significantly difference and identified UC-related viable genera in groups. After treatment of UC mice with DO, DI and raw saline group transplants, it was found that live bacteria played a key role in the treatment of UC mice. Most importantly, it would be useful to assess the composition of donor transplant material by viability assays to ensure that the microbiota composition includes a broad range of live bacteria, some of which may be important in mediating the therapeutic efficacy of FMT for microbiome-related disease. Therefore, we recommend that the activity of donor microbiota should be considered in FMT, and that detailed analysis of the types and numbers of live bacteria transplanted is essential to understanding the mechanisms of which FMT produces or fails to produce therapeutic effects.

Data availability statement

The datasets presented in this study can be found in NCBI under accession number PRJNA911460.

Ethics statement

The animal study was reviewed and approved by Animal Care and Ethics Committee of Fujian University of Traditional Chinese Medicine Laboratory Animal Center.

Author contributions

YL, HL, MC, and JL conducted the mice experiments, managed the participants, interpreted the data, and drafted and reviewed the manuscript. MC, HT, and BZ analyzed the microbiota samples, analyzed the microbiome data, interpreted the data, and reviewed and contributed to the manuscript. HL, TL, and WX analyzed the tissue samples, analyzed the data, interpreted the data, and reviewed and contributed to the manuscript. AL, HW, MC, HL, and JH performed the analyses and sample processing, interpreted the data, and reviewed and contributed to the manuscript. YL, HT, and BZ conceived the study, analyzed the microbiome data, interpreted the data, and reviewed and contributed to the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This study was funded by National Natural Science Foundation of China (82004433), Science Foundation of Fujian Province in China (2021J011327), Youth Foundation of Fujian Provincial Health Commission (2020QNB060) and Fujian University Industry-University-Research Joint Innovation Project (2022Y4007).

Conflict of interest

MC, AL, WX, HW, JH, YL, and BZ were employed by company Xiamen Treatgut Biotechnology Co., Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2023.1124256/full#supplementary-material

References

Agarwal, K., Robinson, L. S., Aggarwal, S., Foster, L. R., Hernandez-Leyva, A., Lin, H., et al. (2020). Glycan cross-feeding supports mutualism between fusobacterium and the vaginal microbiota. *PloS Biol.* 18 (8), e3000788. doi: 10.1371/journal.pbio.3000788

Althouse, M. H., Stewart, C., Jiang, W., Moorthy, B., and Lingappan, K. (2019). Impact of early life antibiotic exposure and neonatal hyperoxia on the murine microbiome and lung injury. *Sci. Rep.* 9 (1), 14992. doi: 10.1038/s41598-019-51506-0

Braun, J., and Wei, B. (2007). Body traffic: Ecology, genetics, and immunity in inflammatory bowel disease. *Annu. Rev. Pathol.* 2, 401–429. doi: 10.1146/annurev.pathol.1.110304.100128

Chen, J., Huang, C., Wang, J., Hui, Z., Lu, Y., Lou, L., et al. (2017). Dysbiosis of intestinal microbiota and decrease in paneth cell antimicrobial peptide level during acute necrotizing pancreatitis in rats. *PLoS One* 8 (4), e0176583. doi: 10.1371/journal.pone.0176583

Comstock, L. E. (2009). Importance of glycans to the host-bacteroides mutualism in the mammalian intestine. *Cell Host Microbe* 5, 522–526. doi: 10.1016/j.chom.2009.05.010

Costello, S., Conlon, M., Vuaran, M., Roberts-Thomson, I., and Andrews, J. (2015). Faecal microbiota transplant for recurrent clostridium difficile infection using long-term frozen stool is effective: Clinical efficacy and bacterial viability data. *Alimentary Pharmacol. Ther.* 42 (8), 1011–1018. doi: 10.1111/apt.13366

Costello, S. P., Soo, W., Bryant, R. V., Jairath, V., Hart, A. L., and Andrews, J. M. (2017). Systematic review with meta-analysis: Faecal microbiota transplantatio n for the induction of remission for active ulcerative colitis. *Alimentary Pharmacol. Ther.* 46 (3), 213–224. doi: 10.1111/apt.14173

Edgar, R. C. (2013). UPARSE: Highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods.* 10(10), 996–8. doi: 10.1038/nmeth.2604

Emerson, J. B., Adams, R. I., Román, C. M. B., Brooks, B., Coil, D. A., Dahlhausen, K., et al. (2017). Schrödinger's microbes: Tools for distinguishing the living from the dead in microbial ecosystems. *Microbiome* 5 (1), 86. doi: 10.1186/s40168-017-0285-3

Fukuda, S., Toh, H., Hase, K., Oshima, K., Nakanishi, Y., Yoshimura, K., et al. (2011). Bifidobacteria can protect from enteropathogenic infection through production of acetate. *Nature* 469, 543–547. doi: 10.1038/nature09646

Gong, J., Shen, Y., Zhang, H., Cao, M., Guo, M., He, J., et al. (2022). Gut microbiota characteristics of people with obesity by meta-analysis of existing datasets. *Nutrients* 14 (14), 2993. doi: 10.3390/nu14142993

Hedin, C., Gast, C. J. V. D., Rogers, G. B., Cuthbertson, L., and Whelan, K. (2015). Siblings of patients with crohn's disease exhibit a biologically relevant dysbiosis in mucosal microbial metacommunities. *Gut* 65 (6), 944–953. doi: 10.1136/gutjnl-2014-308896

He, J., He, X., Ma, Y., Yang, L., Fang, H., Shang, S., et al. (2021). A comprehensive approach to stool donor screening for faecal microbiota transplantation in China. *Microbial Cell Factories* 20 (1), 216. doi: 10.1186/s12934-021-01705-0

He, Q., Wang, L., Wang, F., Wang, C., Tang, C., Li, Q., et al. (2013). Microbial fingerprinting detects intestinal microbiota dysbiosis in zebrafish models with chemically-induced enterocolitis. *BMC Microbiol.* 13, 289. doi: 10.1186/1471-2180-13-289

Huang, X., Gao, J., Zhao, Y., He, M., Ke, S., Wu, J., et al. (2019). Dramatic remodeling of the gut microbiome around parturition and its relationship with host serum metabolic changes in sows. *Front. Microbiol.* 10. doi: 10.3389/fmicb.2019.02123

Jena, P. K., Sheng, L., Nguyen, M., Di Lucente, J., Hu, Y., Li, Y., et al. (2020). Dysregulated bile acid receptor-mediated signaling and IL-17A induction are implicated in diet-associated hepatic health and cognitive function. *biomark. Res.* 8 (1), 59. doi: 10.1186/s40364-020-00239-8

Jhun, J., Cho, K. H., Lee, D. H., Kwon, J. Y., Woo, J. S., Kim, J., et al. (2021). Oral administration of lactobacillus rhamnosus ameliorates the progression of osteoarthritis by inhibiting joint pain and inflammation. *Cells* 10 (5), 1057. doi: 10.3390/cells10051057

Kaakoush, N. O., Sodhi, N., Chenu, J. W., Cox, J. M., Riordan, S. M., and Mitchell, H. M. (2014). The interplay between campylobacter and helicobacter species and other gastrointestinal microbiota of commercial broiler chickens. *Gut Pathog.* 6, 18. doi: 10.1186/1757-4749-6-18

Khoruts, A., Dicksved, J., Jansson, J. K., and Sadowsky, M. J. (2010). Changes in the composition of the human fecal microbiome after bacteriotherapy for recurrent clostridium difficile-associated diarrhea. *J. Clin. Gastroenterol.* 44 (5), 354–360. doi: 10.1097/MCG.0b013e3181c87e02

Kump, P., Wurm, P., Gröchenig, H. P., Wenzl, H., Petritsch, W., Halwachs, B., et al. (2018). The taxonomic composition of the donor intestinal microbiota is a major factor influencing the efficacy of faecal microbiota transplantation in therapy refractory ulcerative colitis. *Alimentary Pharmacol. Ther.* 47 (1), 67–77. doi: 10.1111/apt.14387

Le Bastard, Q., Ward, T., Sidiropoulos, D., Hillmann, B. M., Chun, C. L., Sadowsky, M. J., et al. (2018). Fecal microbiota transplantation reverses antibiotic and chemotherapy-induced gut dysbiosis in mice. *Sci. Rep.* 8 (1), 6219. doi: 10.1038/s41598-018-24342-x

Lee, P., Yacyshyn, B. R., and Yacyshyn, M. B. (2019). Gut microbiota and obesity: An opportunity to alter obesity through faecal microbiota transplant (FMT). *Diabetes Obes. Metab.* 21 (3), 479–490. doi: 10.1111/dom.13561

Li, M., Guo, W., Dong, Y., Wang, W., Tian, C., Zhang, Z., et al. (2021a). Beneficial effects of celastrol on immune balance by modulating gut microbiota in dextran sodium sulfate-induced ulcerative colitis. *Cold Spring Harbor Lab*. doi: 10.1101/2021.09.28.462065

Lima, S., Gogokhia, L., Viladomiu, M., Chou, L., Putzel, G., Jin, W., et al. (2022). Transferable immunoglobulin a-coated odoribacter splanchnicus in responders to fecal microbiota transplantation for ulcerative colitis limits colonic inflammation. *Gastroenterology*. 162(1), 166–78. doi: 10.1053/j.gastro.2021.09.061

Li, W., Sun, Y., Dai, L., Chen, H., Yi, B., Niu, J., et al. (2021b). Ecological and network analyses identify four microbial species with potential significance for the diagnosis/treatment of ulcerative colitis (UC). *BMC Microbiol.* 21 (1), 138. doi: 10.1186/s12866-021-02201-6

Liu, Y., Fan, L., Cheng, Z., Yu, L., Cong, S., Hu, Y., et al. (2021). Fecal transplantation alleviates acute liver injury in mice through regulating Treg/Th17 cytokines balance. *Sci. Rep.* 11(1), 1611. doi: 10.1038/s41598-021-81263-y

Liu, S., Tun, H. M., Leung, F. C., Bennett, D. C., Zhang, H., and Cheng, K. M. (2018). Interaction of genotype and diet on small intestine microbiota of Japanese quail fed a cholesterol enriched diet. *Sci. Rep.* 8 (1), 2381. doi: 10.1038/s41598-018-20508-9

Liu, J., Xue, C., Sun, D., Zhu, W., and Mao, S. (2019). Impact of high-grain diet feeding on mucosa-associated bacterial community and gene expression of tight junction proteins in the small intestine of goats. *Microbiologyopen* 8 (6), e00745. doi: 10.1002/mbo3.745

Liu, S., Zhao, W., Lan, P., and Mou, X. (2021). The microbiome in inflammatory bowel diseases:from pathogenesis to therapy. *Protein Cell* 12 (5), 15. doi: 10.1007/s13238-020-00745-3

Li, S. S., Zhu, A., Benes, V., Costea, P. I., Hercog, R., Hildebrand, F., et al. (2016). Durable coexistence of donor and recipient strains after fecal microbiota transplantation. *Science* 352 (6285), 586–589. doi: 10.1126/science.aad8852

Machiels, K., Pozuelo Del Río, M., Martinez-De la Torre, A., Xie, Z., Pascal Andreu, V., Sabino, J., et al. (2020). Early postoperative endoscopic recurrence in crohn's disease is characterised by distinct microbiota recolonisation. *J. Crohns Colitis* 14 (11), 1535–1546. doi: 10.1093/ecco-jcc/jjaa081

McOrist, A. L., Miller, R. B., Bird, A. R., Keogh, J. B., Noakes, M., Topping, D. L., et al. (2011). Fecal butyrate levels vary widely among individuals but are usually increased by a diet high in resistant starch. *J. Nutr.* 141 (5), 883–889. doi: 10.3945/jn.110.128504

Mirsepasi-Lauridsen, H. C. (2022). Therapy used to promote disease remission targeting gut dysbiosis, in UC patients with active disease. *J. Clin. Med.* 11 (24), 7472. doi: 10.3390/jcm11247472

Moayyedi, P., Surette, M. G., Kim, P. T., Libertucci, J., Wolfe, M., Onischi, C., et al. (2015). Microbiota transplantation induces remission in patients with active ulcerative colitis in a randomized controlled trial. *Gastroenterology*. 149(1), 102–109. doi: 10.1053/j.gastro.2015.04.001

Nagayama, M., Yano, T., Atarashi, K., Tanoue, T., Sekiya, M., Kobayashi, Y., et al. (2020). TH1 cell-inducing Escherichia coli strain identified from the small intestinal mucosa of patients with Crohn's disease. *Gut microbes* 12(1), 1788898. doi: 10.1080/19490976.2020.1788898

Ni, J., Wu, G. D., Albenberg, L., and Tomov, V. T. (2017). Gut microbiota and IBD: causation or correlation? *Nat. Rev. Gastroenterol. Hepatol.* 14 (10), 573–584. doi: 10.1038/nrgastro.2017.88

Pushalkar, S., Hundeyin, M., Daley, D., Zambirinis, C. P., Kurz, E., Mishra, A., et al. (2018). The pancreatic cancer microbiome promotes oncogenesis by induction of innate and adaptive immune suppression. *Cancer Discovery* 8 (4), 403–416. doi: 10.1158/2159-8290.Cd-17-1134

Que, Y., Cao, M., He, J., Zhang, Q., Chen, Q., Yan, C., et al. (2021). Gut bacterial characteristics of patients with type 2 diabetes mellitus and the application potential. *Front. Immunol.* 12. doi: 10.3389/fimmu.2021.722206

Rabbani, G. H., Albert, M. J., Rahman, H., and Chowdhury, A. K. (1999). Short-chain fatty acids inhibit fluid and electrolyte loss induced by cholera toxin in proximal colon of rabbit in vivo. *Dig Dis. Sci.* 44, 1547–1553. doi: 10.1023/A:1026650624193

Rangan, P., Choi, I., Wei, M., Navarrete, G., Guen, E., Brandhorst, S., et al. (2019). Fasting-mimicking diet modulates microbiota and promotes intestinal regeneration to reduce inflammatory bowel disease pathology. *Cell Rep.* 26(10), 2704–2719. doi: 10.1016/j.celrep.2019.02.019

Reuter, J. A., Spacek, D. V., and Snyder, M. P. (2015). High-throughput sequencing technologies. *Mol. Cell* 58 (4), 586–597. doi: 10.1016/j.molcel.2015.05.004

Rinaldi, E., Consonni, A., Cordiglieri, C., Sacco, G., Crasà, C., Fontana, A., et al. (2019). Therapeutic effect of bifidobacterium administration on experimental autoimmune myasthenia gravis in Lewis rats. *Front. Immunol.* 10. doi: 10.3389/fimmu.2019.02949

Rossen, N. G., Fuentes, S., van der Spek, M., Tijssen, J. G., Hartman, J. H., Duflou, A., et al. (2015). Findings from a randomized controlled trial of fecal transplantation for patients with ulcerative colitis. *Gastroenterology*. 149(1), 110–118. doi: 10.1053/j.gastro.2015.03.045

Rubin, S. J. S., Bai, L., Haileselassie, Y., Garay, G., Yun, C., Becker, L., et al. (2019). Mass cytometry reveals systemic and local immune signatures that distinguish inflammatory bowel diseases. *Nat. Commun.* 10 (1), 2686. doi: 10.1038/s41467-019-10387-7

Seekatz, A. M., Aas, J., Gessert, C. E., Rubin, T. A., Saman, D. M., Bakken, J. S., et al. (2014). Recovery of the gut microbiome following fecal microbiota transplantation. *MBio* 5 (3), e00893–e00814. doi: 10.1128/mBio.00893-14

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., et al. (2011). Metagenomic biomarker discovery and explanation. *Genome Biol.* 12 (6), R60. doi: 10.1186/gb-2011-12-6-r60

Sepúlveda Cisternas, I., Salazar, J. C., and García-Angulo, V. A. (2018). Overview on the bacterial iron-riboflavin metabolic axis. *Front. Microbiol.* 9, 1478. doi: 10.3389/fmicb.2018.01478

Sheehan, D., Moran, C., and Shanahan, F. (2015). The microbiota in inflammatory bowel disease. *J. Gastroenterol.* 50 (5), 495–507. doi: 10.1007/s00535-015-1064-1

Smillie, C. S., Sauk, J., Gevers, D., Friedman, J., Sung, J., Youngster, I., et al. (2018). Strain tracking reveals the determinants of bacterial engraftment in the human gut following fecal microbiota transplantation. *Cell Host Microbe* 23 (2), 229–240.e225. doi: 10.1016/j.chom.2018.01.003

Tian, Y., Li, M., Song, W., Jiang, R., and Li, Y. Q. (2019). Effects of probiotics on chemotherapy in patients with lung cancer. *Oncol. Lett.* 17 (3), 2836–2848. doi: 10.3892/ol.2019.9906

Wang, Q. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* 73 (16), 5261–5267. doi: 10.1128/AEM.00062-07

Weingarden, A. R., and Vaughn, B. P. (2017). Intestinal microbiota, fecal microbiota transplantation, and inflammatory bowel disease. *Gut Microbes* 8 (3), 238–252. doi: 10.1080/19490976.2017.1290757

Weisshof, R., Jurdi, K. E., Zmeter, N., and Rubin, D. T. (2018). Emerging therapies for inflammatory bowel disease. *Adv. Ther.* 35(11), 1746–1762. doi: 10.1007/s12325-018-0795-9

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. New York: Springer-Verlag. Available at: https://ggplot2.tidyverse.org.

Xie, F., Li, S., Fan, Y., Li, W., Lv, Q., Sun, X., et al. (2022). Efficacy and safety of bifidobacterium quadruple viable bacteria combined with mesalamine against UC management: A systematic review and meta-analysis. *Oxid. Med. Cell Longev* 2022, 8272371. doi: 10.1155/2022/8272371

Yang, B., Yue, Y., Chen, Y., Ding, M., Li, B., Wang, L., et al. (2021). Lactobacillus plantarum CCFM1143 alleviates chronic diarrhea *via* inflammation regulation and gut microbiota modulation: A double-blind, randomized, placebo-controlled study. *Front. Immunol.* 12. doi: 10.3389/fimmu.2021.746585

Yun, H. F., Liu, R., Han, D., Zhao, X., Guo, J. W., Yan, F. J., et al. (2020). Pingkui enema alleviates TNBS-induced ulcerative colitis by regulation of inflammatory factors, gut bifidobacterium, and intestinal mucosal barrier in rats. *Evid Based Complement Alternat Med.* 2020, 3896948. doi: 10.1155/2020/3896948

Zemb, O., Achard, C. S., Hamelin, J., De Almeida, M. L., Gabinaud, B., Cauquil, L., et al. (2020). Absolute quantitation of microbes using 16S rRNA gene metabarcoding: A rapid normalization of relative abundances by quantitative PCR targeting a 16S rRNA gene spike-in standard. *MicrobiologyOpen* 9 (3), e977. doi: 10.1002/mbo3.977

Zhang, T., Lu, G., Zhao, Z., Liu, Y., Shen, Q., Li, P., et al. (2020). Washed microbiota transplantation vs. manual fecal microbiota transplantation: clinical findings, animal studies and *in vitro* screening. *Protein Cell* 11 (4), 16. doi: 10.1007/s13238-019-00684-8

Zhang, W. Q., Quan, K. Y., Feng, C. J., Zhang, T., He, Q. W., Kwok, L. Y., et al. (2022). The lactobacillus gasseri G098 strain mitigates symptoms of DSS-induced inflammatory bowel disease in mice. *Nutrients* 14 (18). doi: 10.3390/nu14183745

Zhang, B., Xu, S., Xu, W., Chen, Q., Chen, Z., Yan, C., et al. (2019). Leveraging fecal bacterial survey data to predict colorectal tumors. *Front. Genet.* 10 (447). doi: 10.3389/fgene.2019.00447

Zhang, B., Yang, L., Ning, H., Cao, M., Chen, Z., Chen, Q., et al. (2022). A matching strategy to guide donor selection for ulcerative colitis in fecal microbiota transplantation: Meta-analysis and analytic hierarchy process. *Microbiol. Spectr.*, e0215921. doi: 10.1128/spectrum.02159-21

frontiers | Frontiers in Cellular and Infection Microbiology

TYPE Review
PUBLISHED 13 March 2023
DOI 10.3389/fcimb.2023.1139800

OPEN ACCESS

EDITED BY
Xiangtian Yu,
Shanghai Jiao Tong University, China

REVIEWED BY
Zhangran Chen,
Xiamen University, China
Cecilia Ximenez,
Faculty of Medicine, National Autonomous University of Mexico, Mexico

*CORRESPONDENCE
Lin Lin
1550770732@qq.com

SPECIALTY SECTION
This article was submitted to Intestinal Microbiome, a section of the journal Frontiers in Cellular and Infection Microbiology

RECEIVED 07 January 2023
ACCEPTED 28 February 2023
PUBLISHED 13 March 2023

CITATION
Fan S, Chen S and Lin L (2023) Research progress of gut microbiota and obesity caused by high-fat diet.
Front. Cell. Infect. Microbiol. 13:1139800.
doi: 10.3389/fcimb.2023.1139800

Research progress of gut microbiota and obesity caused by high-fat diet

Shuyi Fan[1,2], Suyun Chen[2] and Lin Lin[1]*

[1]Scientific Research Department, Brain Hospital of Hunan Province, Second People's Hospital of Hunan Province, Changsha, Hunan, China, [2]Department of Clinical Medicine, Xiamen Medical College, Xiamen, Fujian, China

Obesity, a chronic metabolic disorder caused by an energy imbalance, has been increasingly prevalent and poses a global health concern. The multifactorial etiology of obesity includes genetics factors, high-fat diet, gut microbiota, and other factors. Among these factors, the implication of gut microbiota in the pathogenesis of obesity has been prominently acknowledged. This study endeavors to investigate the potential contribution of gut microbiota to the development of high-fat diet induced obesity, as well as the current state of probiotic intervention therapy research, in order to provide novel insights for the prevention and management of obesity.

KEYWORDS

gut microbiota, high-fat diet, obesity, probiotics, treatment

1 Introduction

Obesity is a chronic and recurring condition that results from excessive or inappropriate fat accumulation (Obesity: preventing and managing the global epidemic. Report of a WHO consultation, 2000; Bray et al., 2017). The global incidence of obesity among adults has increased by 1.5-fold since 2000, with over 1.9 billion overweight adults in 2016. Children and adolescents have also experienced a rise in the prevalence of obesity, with an increase from 2.9% to 6.8% in the population aged 5 to 19 years (Abarca-Gómez et al., 2017). Obesity has serious implications for health, including an elevated risk of mortality, type 2 diabetes, and cardiovascular disease. The etiology of obesity is multifactorial, with contributing factors including genetics, a high-fat diet (HFD), and gut microbiota. The gut microbiota, which is composed mainly of anaerobic bacteria, facultative anaerobic bacteria, and aerobic bacteria, is a dynamic ecosystem that coevolves with its host (Wu et al., 2022). The gut microbiota plays a crucial role in maintaining the health of the host through vitamin production, nutrient absorption, and the secretion of small molecules involved in immune regulation, angiogenesis, and nerve function (Dominguez-Bello et al., 2019; Robertson et al., 2019). The human gut contains approximately 10^{14} microorganisms (Gill et al., 2006), predominantly composed of Firmicutes and Bacteroidetes species (Bolam and van den Berg, 2018). Different bacterial species occupy distinct sections of the intestine; for instance, Firmicutes often predominate

at the top of the gut crypto-villous unit while *Proteus predominates* at the bottom (Sommer and Backhed, 2016). The functional consistency of each bacterial genus is quite high (Costea et al., 2018) and is not affected by the host's age, sex, BMI, or nationality (Sebastian Domingo and Sanchez Sanchez, 2018).

2 Gut microbiota and obesity

2.1 Animal studies demonstrate a link between gut microbiota and obesity

The present study shows that the manifestation of obesity and its metabolic dysfunctions were absent in germ-free mice. Notably, the transplantation of cecal or fecal samples from obese mice into germ-free mice resulted in the development of similar symptoms, indicating that the gut microbiota plays a critical role in the pathogenesis of obesity (Ridaura et al., 2013). Furthermore, it was observed that the transfer of gut microbiota could also transmit the obesity phenotype (Kapoor et al., 2021; Romani-Perez et al., 2021). In mice fed the same HFD, some developed obesity and some were resistant to it, and differences in gut microbiota composition may be the most important factor in both outcomes. In addition, intestinal barrier function, intestinal inflammation and neurotrophic factors also play an important role in diet-induced obesity (Zhang et al., 2019b). A growing body of evidence from animal studies suggests a link between diet, gut microbiota and obesity, as well as in humans. But studies have not reached a consistent conclusion on exactly what microbial composition is at work. Moreover, an interesting study found that transfer of the whole microbiota may not reduce diabetes incidence despite a major change in gut microbiota of the non-obese diabetes (NOD) mice model. NOD mouse models can be divided into two colonies (high or low diabetes incidence), transplanting intestinal flora from low-incidence NOD mice to high-incidence NOD mice did not change the incidence of diabetes, but transplantation of *A. muciniphila* to high-incidence NOD mice can promote mucogenesis, increase the expression of antimicrobial peptide Reg3γ, inhibit the growth of rumen contortus, reduce the level of serum endotoxin, reduce the expression of TLR in pancreatic islets, promote regulatory immunity, and delay the development of diabetes (Hanninen et al., 2018). It shows that some single species of bacteria, rather than the entire intestinal flora, may play a major role in inducing or resisting metabolic diseases under certain conditions.

2.2 Research on demographics has discovered variations in the distribution of gut microbiota in obese people

As per conventional understanding, the establishment of gut microbiota occurs after birth, while the mother's uterus remains free of microorganisms. Various factors, such as delivery mode, feeding type, and medication administration (including antibiotics), impact the diversity of gut microbiota, as stated in the literature (Theis et al., 2019; Akagawa et al., 2021). By age 3, the gut microbiota progresses towards a complex and stable state similar to that of adults (Derrien et al., 2019), which then remains mostly consistent throughout adulthood. According to a population-based study, the obese population demonstrates significant differences in gut microbiota composition compared to the general population (Cuevas-Sierra et al., 2019). A few studies propose that the "enterotype of the fertility microbiota" is characterized by a higher abundance of Firmicutes/Bacteroidetes (Kim et al., 2021). Nevertheless, the distribution of this distinct microbiota is still subject to debate due to variation in sample size, individual clinical and anthropometric traits (age, sex, microbiota distribution, and degree of obesity), and microbiota analysis techniques (qPCR, 16S rRNA gene sequencing, and Fluorescence *in situ* hybridization) (Zeng et al., 2019; Assmann et al., 2020).

3 A high-fat diet alters gut permeability and gut microbiota in ways associated to obesity

The human gut microbiota is highly responsive to changes in food intake and the physiological state of the digestive system (Turnbaugh et al., 2009; Qin et al., 2020), with alterations observed within a period as short as 24 hours (David et al., 2014). A HFD has been found to significantly reduce the diversity of gut microbiota (Wan et al., 2019), resulting in a decrease in the number of bacteria that are responsible for maintaining the integrity of the gut mucosal barrier and an increase in the number of bacteria that breach it (Monk et al., 2019; Zhang et al., 2019a). This alteration in gut microbiota is characterized by a reduction in the relative abundance of Bacteroides and an increase in the relative abundance of Firmicutes (An et al., 2022). Moreover, the concentration of lipopolysaccharide (LPS) has been found to increase with the number of *Actinomycetes* while the number of *Bifidobacteria* declines as *Vibrio desulfonate* increased. Excess sulfate is converted to hydrogen sulfide, which further compromises the gut barrier and promotes inflammation (Chen et al., 2019). Additionally, the gut barrier is disrupted by *Akkermansia muciniphila (A. muciniphila)*, a member of phylum Verrucomicrobia that degrades mucins and has anti-inflammatory and protective effects on the intestinal mucosal barrier (Hanninen et al., 2018).

3.1 Gut permeability is increased by HFD

Previous research has provided evidence that a HFD can lead to obesity, inflammation, and enhance gut epithelial cell permeability (Lemons and Liu, 2022). The mechanism through which HFD induces increased gut permeability involves several processes (Figure 1).

In the HFD, intestinal epithelial cells in the lower intestine actively ingest a significant amount of fat, which leads to the simultaneous generation of reactive oxygen species (ROS), iron, copper, aldehydes, lipid peroxidation, as well as ATP by the

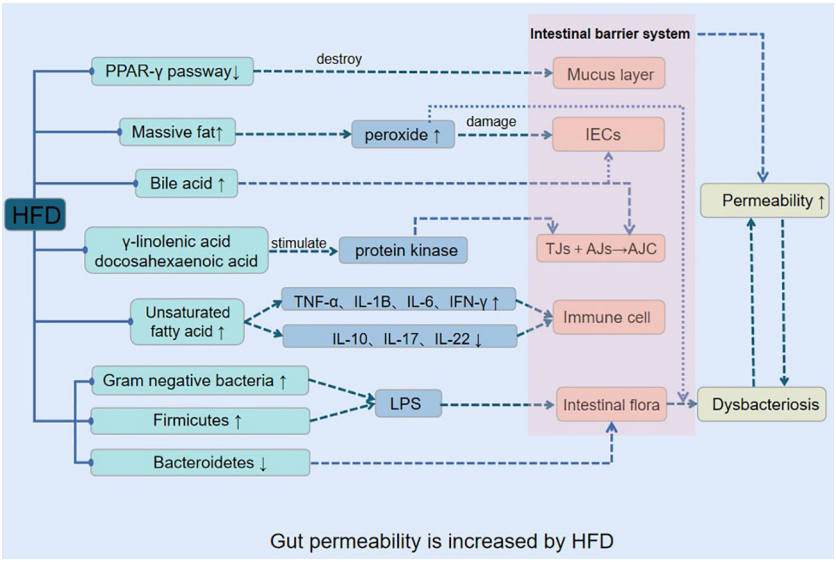


FIGURE 1
Gut permeability is increased by HFD.

mitochondrial respiratory chain (Spinelli and Haigis, 2018). The ROS generated under the influence of the HFD cause increased gut epithelial cell permeability (Ballard and Towarnicki, 2020), ultimately leading to the destruction of the gut barrier function and the proliferation of harmful bacteria like *Salmonella* and *Escherichia coli* in the gut cavity. Furthermore, the hydrogen sulfide generated by the HFD inhibits the mitochondrial respiratory chain, which makes it easier for pathogenic bacteria to infect more cells (Mottawea et al., 2016). The production of iron, copper, aldehydes, and lipid peroxidation during the digestion and absorption of high dietary fats leads to an increase in oxidative stress in gut tissues, destroying the microbiota's living environment, resulting in an imbalance of gut microbiota.

The HFD contains a large amount of polyunsaturated fatty acids that are prone to oxidation of their double bonds (Mariamenatu and Abdu, 2021). The free fatty acids generated under the influence of the HFD impact the gut immune system directly (Tanaka et al., 2020) raising the levels of barrier-damaging cytokines such as TNF-α, IL-1β, IL-6, IFN-γ, while decreasing barrier-protective cytokines such as IL-10, IL-17, IL-22, ultimately leading to an increase in gut permeability (Bartoszek et al., 2020; Stoeva et al., 2021). The resulting pathological changes, including low-grade inflammation, decreased expression of antimicrobial peptides, mucus secretion, and expression of tight junction protein, impact multiple system functions and lead to obesity and its metabolic complications (insulin resistance, hyperglycemia, systemic inflammation, and dyslipidemia) (Araújo et al., 2017; Jiang et al., 2020; Kumar et al., 2021).

The gut barrier system comprises mucus layers, gut epithelial cells (IECs), tight junctions (TJS), immune cells, and gut microbiota (Rohr et al., 2020). The apical junctional complex (AJC) is composed of the membrane proteins TJS and adhere junctions (AJS) (Capaldo et al., 2017). The AJC's integrity is critical for the selective passage of nutrients while obstructing the entry of toxins and antigens, leading to high permeability of the gut. Dietary fat has the potential to directly impact the integrity of the AJC (Netto Candido et al., 2018; Tsukita et al., 2019; Otani and Furuse, 2020). In long-term HFD, gut occlusion zone-1 (ZO-1) and occludin gene expression are decreased, which leads to an increase in gut permeability (Oliveira et al., 2019; Nascimento et al., 2021). The HFD's abundance of docosahexaenoic acid and γ-linolenic acid triggers protein kinase activation, actin and TJ protein redistribution, and increased gut permeability (Usami et al., 2003). Additionally, part of the eicosapentaenoic acids in HFD can be converted into bioactive metabolites to increase gut permeability (Usami et al., 2001).

Dietary fat consumption and bile acid secretion exhibit a positive correlation (Ocvirk and O'Keefe, 2021), and IECs possess the ability to resist bile acid degradation under normal physiological conditions. However, HFD induces long-term and high-level secretion of bile acids, resulting in the release of numerous hydrophobic bile acids, such as cholic acid and deoxycholic acid (Iwamoto et al., 2021). These bile acids promote occludin protein dephosphorylation, leading to the dissociation of the adhesive junction complex and ultimately causing an increase in gut permeability. In addition, they can cause harm to the gut mucosal barrier and induce oxidative stress and cell apoptosis in IECs (Raimondi et al., 2008; Di Ciaula et al., 2017; Sarathy et al., 2017; Gupta et al., 2020).

Furthermore, HFD inhibits the peroxisome proliferator-activated receptor-γ (PPAR-γ) pathway in mice, disrupting the gut mucus layer, decreasing electrolyte secretion, and impairing mucosal immune defense. However, a week of treatment with a specific PPAR-γ agonist, rosiglitazone, or a return to a normal diet can reverse the increased gut epithelial permeability caused by HFD (Lee et al., 2020), resulting in the disruption of the gut mucus layer, reduced electrolyte secretion, and decreased mucosal immune defense. Following a week of therapy with rosiglitazone, a

particular PPAR-agonist, or returning to the usual diet, this increase in gut epithelial permeability was reversed.

3.2 Obesity and other related metabolic diseases are mediated by increased gut permeability, which also encourages gut dysbacteriosis

The consumption of a HFD has been observed to enhance the permeability of gut epithelial cells and disrupt the interplay between the local intestine mucosal immune system and the gut microbiota, leading to an imbalance in the microbiota composition. This imbalance is characterized by a rise in the number of gram-negative bacteria, and the resultant LPS produced by these bacteria interact with the CD14/Toll-like receptor 4 (TLR4) complexes of gut epithelial cells, leading to the activation of the innate immune system. This activation causes local and systemic persistent low-level inflammation, which leads to further destruction of the mucous layer and increased permeability of IEC. The heightened permeability of IECs facilitates the entry of gut microbiota metabolites into the bloodstream, resulting in a vicious cycle of inflammation and dysbacteriosis. The ongoing activation of the LPS/TLR4 signal pathway is believed to be a major contributor to the development of obesity and related metabolic disorders (Kasselman et al., 2018; Giordano et al., 2020; Mohammad and Thiemermann, 2020) (Figure 2).

4 Obesity is a result of gut microbiota's involvement in the regulation of the human metabolism

4.1 Gut microbiota is directly involved in the expression and regulation of host metabolism-related genes

The modulation of host gene promoters related to lipid metabolism, obesity, and inflammatory responses by the dominant Firmicutes within the gut microbiota has been reported through recent investigations (Cuevas-Sierra et al., 2019; Amabebe et al., 2020). However, the examination of gut microbiota-obesity association at a population level presents a significant challenge, given the inadequate sample sizes and inadequate representation of individual subjects in existing gut microbiota studies (Stanislawski et al., 2019). This shortcoming necessitates further research efforts towards resolving these limitations.

4.2 Gut microbiota intervenes host glycometabolism through metabolic intermediates

The gut microbiota is responsible for the production of short-chain fatty acids (SCFAs) which impact the host's ability to absorb and store energy from the diet (Blaak et al., 2020). Production of SCFAs, including acetate, butyrate, and propionate, occurs through fermentation of soluble dietary fiber and resistant starch by gut microbiota (den Besten et al., 2013). The SCFAs bind to the G-protein-coupled receptors GPR41 and GPR43 (Kim et al., 2018; Carretta et al., 2021; Moniri and Farah, 2021), and regulate molecular signaling pathways that indirectly affect gene expression, such as increasing the expression of glucagon-like peptide 1 (GLP-1) and peptide YY (PYY) in the gut (Tanaka et al., 2020). Both GLP-1 and PYY have been found to inhibit appetite (Stubbs et al., 2018), reduce body weight, and improve insulin resistance in obese mice (McNabney and Henagan, 2017; Blanco, 2020). However, in the absence of GPR41 signaling, PYY levels in plasma decrease, causing an increase in gut motility and a decrease in the amount of energy gained from meals (Samuel et al., 2008). Moreover, acetate has been found to positively influence appetite, insulin and ghrelin release, and obesity and its associated complications by influencing the parasympathetic neural system (Hernandez et al., 2019). On the other hand, propionate has been shown to produce insulin resistance and hyperinsulinemia, increases glucagon and fatty acid-binding protein production, activates the sympathetic nervous system, and promotes obesity and metabolic abnormalities (Tirosh et al., 2019). Therefore, further research is needed to explore the relationship between changes in the types and quantity of SCFAs and obesity as it appears that SCFAs act as mediators between diet, gut microbiota, and body physiology.

4.3 Gut microbiota interferes with the host lipid metabolism by altering enzyme activity

Bäckhed et al. have proposed potential pathways that contribute to the development of obesity (Backhed et al., 2005). One such pathway involves the gut microbiota promoting the absorption of monosaccharide in the gut, thereby increasing triglyceride synthesis in the liver. Furthermore, gut microbiota has been identified as the primary regulator of lipid metabolism, with both promoting and inhibitory effects. Fasting-induced adipocyte factor (FIAF, also known as PPAR-Angiopoietin Related Protein, which is a cell signal glycoprotein hormone) is known to increase adipocytes' lipoprotein lipase (LPL) activity and fatty acid accumulation (Backhed et al., 2005). Notably, FIAF is produced by various tissues, including white adipose tissue (WAT), the colon, the liver, the heart, and the skeletal muscle (Baek et al., 2021; Montaigne et al., 2021). Studies have shown that *A. muciniphila* fermentation products, such as SCFAs, promote FIAF expression in gut cells through PPAR-γ (Carvalho and Saad, 2013), inhibit LPL and stimulate WAT lipolysis (Thyagarajan and Foster, 2017). In contrast, *Bacteroides thetaiotaomicron* can stimulate lipogenesis by inhibiting FIAF expression (Backhed et al., 2007). Therefore, FIAF may serve as a gut microbiota modulator, influencing lipid metabolism and contributing to obesity. Additionally, the endogenous

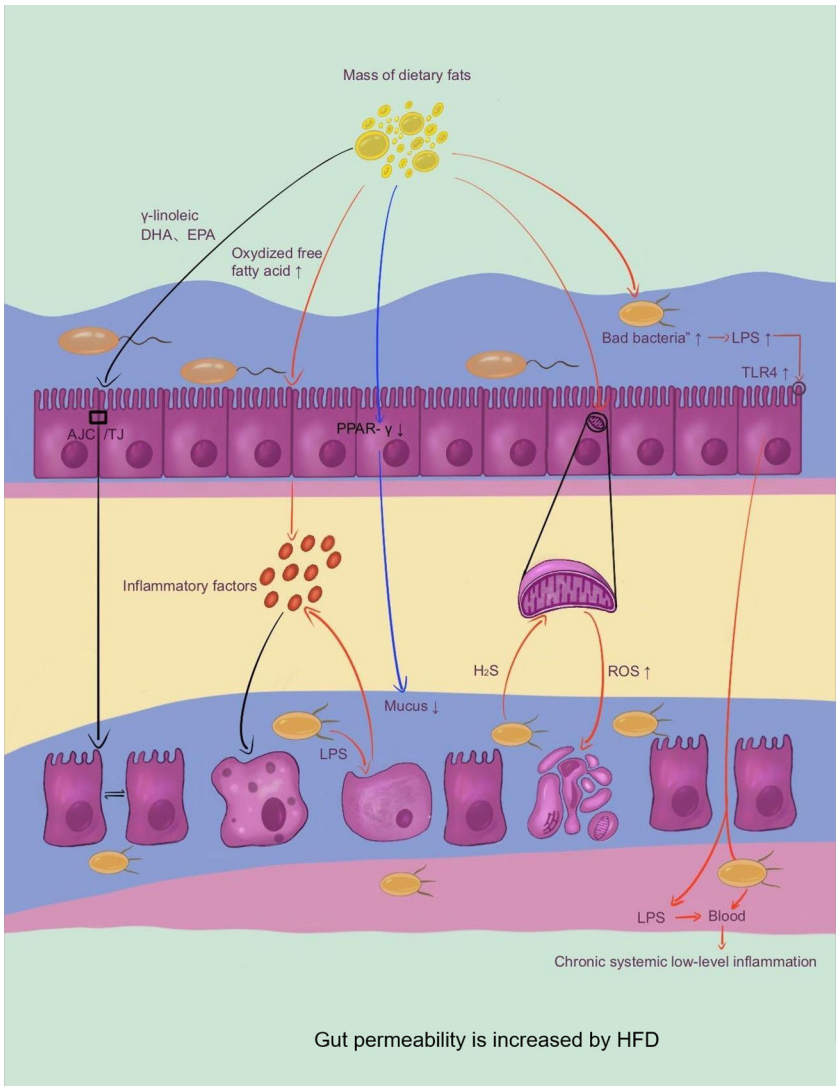


FIGURE 2
Gut permeability is increased by HFD.

cannabinoid system (EC) has been implicated in regulating blood lipid and glucose metabolism, with over-activation posing a significant risk for obesity. Specific gut microbiota, such as *A. muciniphila*, can interfere with fat metabolism *in vivo* by blocking EC-driven lipogenesis, promoting adipocyte proliferation, and increasing fat accumulation in adipocytes (Geurts et al., 2011; Forte et al., 2020; Jansma et al., 2021).

5 Probiotics are promising to be a new strategy for treating hfd obesity

Currently, clinical approaches to treating obesity involve reducing caloric intake, increasing exercise consumption, using appetite suppressants, and gastrectomy (Blundell et al., 2017; El Moussaoui et al., 2021; Fanti et al., 2021). Nevertheless, these methods exhibit certain limitations such as limited therapeutic efficacy, drug abuse, and a high incidence of complications (Sarwer et al., 2019; Paccosi et al., 2020; Bray and Ryan, 2021). As a result, innovative treatments are necessary.

Probiotics are living strains that are considered beneficial to the host's health when consumed in adequate amounts. These microorganisms aid in nutrient digestion and absorption, maintain the digestive system, and improve key metabolic disease risk variables such body mass index, fasting blood glucose, alanine and aspartate transaminase (Jager et al., 2018; Kijmanawat et al., 2019). Utilizing probiotics to regulate gut microbiota has emerged as a promising approach for treating obesity, particularly in cases of HFD obesity (Bianchi et al., 2018; Kong et al., 2019). Numerous animal studies and clinical trials have confirmed the efficacy of probiotics, particularly those from the *Bifidobacterium* and *Lacto bacillus*strains, as well as some members of *Bacillus* and *Propioni bacterium*in treating obesity and overweight by controlling gut microbiota function, bile acid metabolism, and gene expression associated with calorie homeostasis and fat formation (summarized in Supplementary Tables 1, 2). Obese animals treated with multiple

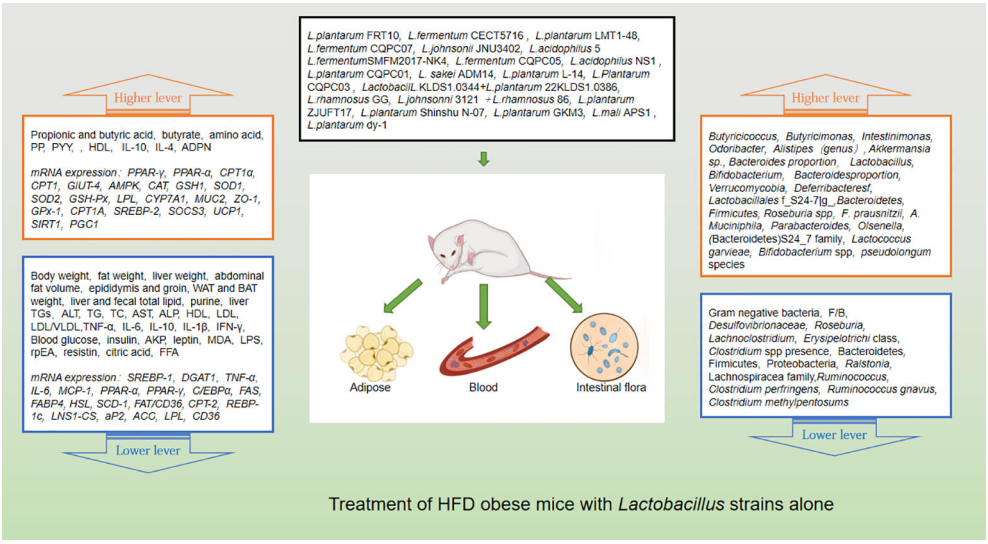


FIGURE 3
Treatment of HFD obese mice with *Lactobacillus* strains alone.

Lactobacillus strains alone (Figure 3) or in combination with *Bifido bacterium*s trains (Figure 4) exhibited lower body weight and fat mass, improved dyslipidemia and insulin resistance, and lessened liver damage and chronic low-grade inflammation. Clinical trials using probiotics to treat obesity and overweight have also successfully observed weight loss and improved metabolic markers in subjects, probiotics's increased presence has negative associations with obesity and diabetes while positively impacting gut health (Figure 5). Although data from current human testing studies are limited and urgently need further research and detailed documentation, intestinal bacterial transplantation has emerged in the treatment of HFD obesity and related metabolic issues following successful applications in diseases such as *Clostridium difficile* infection, providing a new option for the prevention and treatment of human HFD obesity.

The gut microbiota of healthy adults and children typically contains 1%-4% of the probiotic *A. muciniphila* (Derrien et al., 2008). *A. muciniphila* has special survival advantages due to its ability to utilize mucin, the primary growth and metabolic substrate produced by goblet cells in the host gastrogut tissue. Its unique structure enables *A. muciniphila* to modulate gut barrier integrity, enhance gut permeability, and thicken the mucus layer in HFD mice (Chelakkot et al., 2018; Liu et al., 2019). Furthermore, the Type IV pili of *A. muciniphila*are able to directly signal to host immune receptors, regulate the expression of genes involved in fat synthesis and inflammation in the liver, and maintain gut immune system

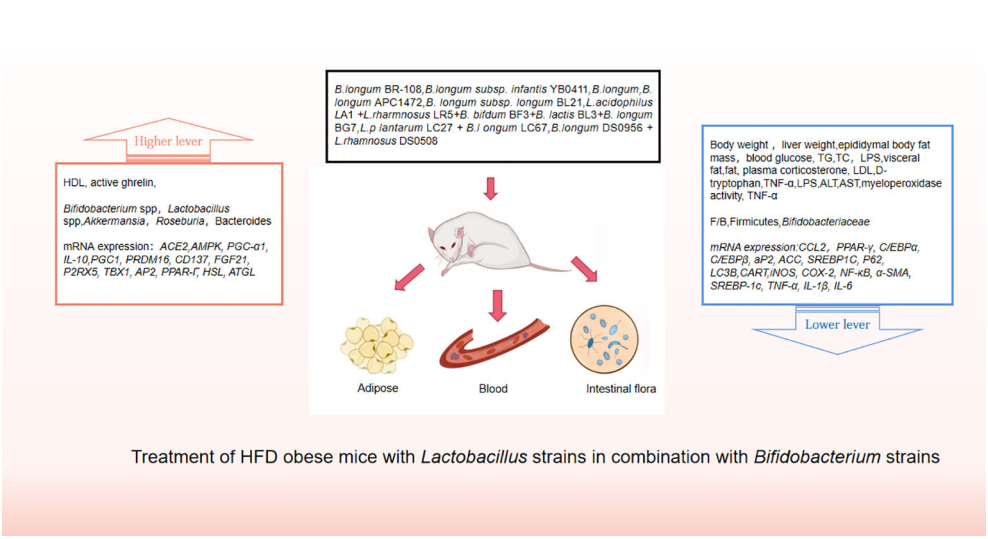


FIGURE 4
Treatment of HFD obese mice with *Lactobacillus* strains in combination with *Bifidobacterium* strains.

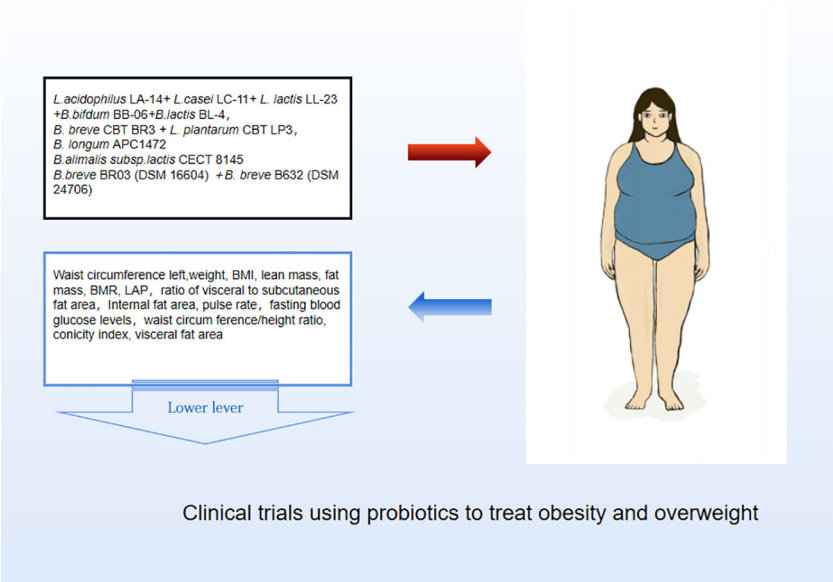


FIGURE 5
Clinical trials using probiotics to treat obesity and overweight.

homeostasis (Zhou and Zhang, 2019; Kim et al., 2020; Yang et al., 2020; Xiang et al., 2021). *A. muciniphila* is also capable of secreting oligosaccharides and SCFA, which act as growth substrates for other beneficial bacteria and promote the abundance of microbiota associated with a reduced risk of obesity (Belzer and de Vos, 2012; Clarke et al., 2014; Keshavarz Azizi Raftar et al., 2021). Long-term supplementation with *A. muciniphila* can increase the thickness of the mucus layer of the gut barrier and attenuate the expression of genes and pathways associated with inflammation (Dao et al., 2016; van der Lugt et al., 2019), thus making it a promising candidate for the treatment of HFD obesity and a potential new generation of probiotics.

6 Summary and prospect

Recent studies have shown that there is a distinct distribution of gut bacteria in obese individuals compared to those with a normal weight. This suggests that gut microbiota may play a significant role in the development of obesity and related metabolic disorders, as it is involved in energy metabolism through processes such as acquiring energy from the diet, controlling fat storage, controlling fat creation, and controlling fatty acid oxidation. In light of these findings, new therapeutic approaches such as improving high-fat diet obesity, reducing systemic inflammation, and participating in weight control through targeting gut bacteria have been explored with some success. However, human gut microbiota is a complex research area with various influencing factors, including nutrition, exercise, medications, country, and gender. Some of these variables are beyond our control. Understanding the intricate interaction between billions of distinct bacterial populations, thousands of host cell types, and chemical mediators requires developing well-designed and suitable experimental models. Probiotics have emerged as a safe and effective option for treating HFD-induced obesity in animals, with few adverse effects and good tolerance, making them ideal for long-term administration (Liu et al., 2017), and the combination of *Lactobacillus* and Bifidobacterium has been shown to significantly alter gut microbiota composition and improve insulin sensitivity in HFD mice. In clinical trials, the use of synbiotic bacteria (*Bifidobacterium* and *Lactobacillus*) supplements increased the number of potential probiotics[148], however, it was discovered that the species and quantity of lactic acid bacteria were much higher in obese individuals than in the control group (Armougom et al., 2009), leading to the hypothesis that obese patients may exhibit "resistance" to lactic acid bacteria, which may due to the widespread usage of *Lactobacillus* as a growth stimulant in agriculture. In 2011, MetaHIT team proposed the concept of enterotypes, which divided gut microbiota into three categories: B, P and F. This has potential research and clinical value, but it is controversial. According to different tests, algorithms and analysis methods, different people think that the gut microbiota should be divided into 2, 4 enterotypes or even continuous undivided types. In order to unify the understanding and guide the practice, 29 mainstream microflora scientists in the world jointly proposed a new intestinal type classifier and open comparison database. The new scheme makes full use of and verifies the database such as HMP, comprehensively considers the function, ecology and clinical needs of the flora, and can better indicate the flora types of disease and health status, however, the consensus is significant but still limited, the treatment of obesity still cannot "model" the use of probiotics according to the existing enterotypes classification, and use of personalized probiotics based on precise analysis of each patient's gut bacteria composition is not yet feasible.

Probiotics therapy may be a novel option for treating HDF-induced obesity, and recent research has shown that using synbiotic supplements and isolating new probiotic strains could increase the potential benefits of probiotic therapy. Nevertheless, it is important to note that a brief course of probiotics may not undo the long-term

effects of a physiological disorder, and more research is required to fully understand the role of probiotics in appetite control (Liang et al., 2021).

Although intestinal bacteria transplantation has shown potential for disease prevention and treatment in both animal and human experiments, there are still great controversies over enterotypes, the selection of specific transplant strains and the combination of prebiotics. Since its establishment, microbiology has been limited by axenic culture, but the emergence of mixed culture mode opens up another way for understanding microorganisms and application development, and also has a profound impact on microbial ecology, symbiosis, pathology and other fields. The transition from pure culture to hybrid culture depends on three advances: microfluidic technology, next-generation 3D bioprinting, and single-cell metabolomics. The progress of these technologies is expected to lead to systematic large-scale symbiotic culture studies involving three or more microorganisms in the future. On the basis of in-depth understanding of the correlation between specific enterotypes and metabolic diseases, mixed culture will greatly accelerate the clinical transformation of intestinal bacteria transplantation research. As microbiota science and analytical technology continue to advance, targeted gut microbiota intervention presents potential therapeutic options towards promoting host health in the future.

Author contributions

SF is the first author, responsible for consulting the literature and forming the first draft, and LL is the corresponding author, responsible for revising the draft. All authors contributed to the article and approved the submitted version.

Funding

This article is funded by Innovation and entrepreneurship training program for college students of Xiamen Medical 12 College, Fujian Province (202012631050).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2023.1139800/full#supplementary-material

References

Abarca-Gómez, L., Abdeen, Z. A., Hamid, Z. A., Abu-Rmeileh, N. M., Acosta-Cazares, B., Acuin, C., et al. (2017). Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128· 9 million children, adolescents, and adults. *Lancet* 390 (10113), 2627–2642. doi: 10.1016/S0140-6736(17)32129-3

Akagawa, S., Akagawa, Y., Yamanouchi, S., Kimata, T., Tsuji, S., and Kaneko, K. (2021). Development of the gut microbiota and dysbiosis in children. *Biosci. Microbio. Food Health* 40 (1), 12–18. doi: 10.12938/bmfh.2020-034

Amabebe, E., Robert, F. O., Agbalalah, T., and Orubu, E. S. F. (2020). Microbial dysbiosis-induced obesity: role of gut microbiota in homoeostasis of energy metabolism. *Br. J. Nutr.* 123 (10), 1127–1137. doi: 10.1017/S0007114520000380

An, J., Wang, Q., Yi, S., Liu, X., Jin, H., Xu, J., et al. (2022). The source of the fat significantly affects the results of high-fat diet intervention. *Sci. Rep.* 12 (1), 1–11. doi: 10.1038/s41598-022-08249-2

Araújo, J. R., Tomas, J., Brenner, C., and Sansonetti, P. J. (2017). Impact of high-fat diet on the intestinal microbiota and small intestinal physiology before and after the onset of obesity. *Biochimie* 141, 97–106. doi: 10.1016/j.biochi.2017.05.019

Armougom, F., Henry, M., Vialettes, B., Raccah, D., and Raoult, D. (2009). Monitoring bacterial community of human gut microbiota reveals an increase in lactobacillus in obese patients and methanogens in anorexic patients. *PloS One* 4 (9), e7125. doi: 10.1371/journal.pone.0007125

Assmann, T. S., Cuevas-Sierra, A., Riezu-Boj, J. I., Milagro, F. I., and Martinez, J. A. (2020). Comprehensive analysis reveals novel interactions between circulating MicroRNAs and gut microbiota composition in human obesity. *Int. J. Mol. Sci.* 21 (24), 9509. doi: 10.3390/ijms21249509

Backhed, F., Ley, R. E., Sonnenburg, J. L., Peterson, D. A., and Gordon, J. I. (2005). Host-bacterial mutualism in the human intestine. *Science* 307 (5717), 1915–1920. doi: 10.1126/science.1104816

Backhed, F., Manchester, J. K., Semenkovich, C. F., and Gordon, J. I. (2007). Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proc. Natl. Acad. Sci. U.S.A.* 104 (3), 979–984. doi: 10.1073/pnas.0605374104

Baek, J. H., Kim, D. H., Lee, J., Kim, S. J., and Chun, K. H. (2021). Galectin-1 accelerates high-fat diet-induced obesity by activation of peroxisome proliferator-activated receptor gamma (PPARgamma) in mice. *Cell Death Dis.* 12 (1), 66. doi: 10.1038/s41419-020-03367-z

Ballard, J. W. O., and Towarnicki, S. G. (2020). Mitochondria, the gut microbiome and ROS. *Cell Signal* 75, 109737. doi: 10.1016/j.cellsig.2020.109737

Bartoszek, A., Makaro, A., Bartoszek, A., Kordek, R., Fichna, J., and Salaga, M. (2020). Walnut oil alleviates intestinal inflammation and restores intestinal barrier function in mice. *Nutrients* 12 (5), 1302. doi: 10.3390/nu12051302

Belzer, C., and de Vos, W. M. (2012). Microbes inside–from diversity to function: the case of akkermansia. *ISME. J.* 6 (8), 1449–1458. doi: 10.1038/ismej.2012.6

Bianchi, F., Larsen, N., de Mello Tieghi, T., Adorno, M. A. T., Kot, W., Saad, S. M. I., et al. (2018). Modulation of gut microbiota from obese individuals by *in vitro* fermentation of citrus pectin in combination with bifidobacterium longum BB-46. *Appl. Microbiol. Biotechnol.* 102 (20), 8827–8840. doi: 10.1007/s00253-018-9234-8

Blaak, E. E., Canfora, E. E., Theis, S., Frost, G., Groen, A. K., Mithieux, G., et al. (2020). Short chain fatty acids in human gut and metabolic health. *Benef. Microbes* 11 (5), 411–455. doi: 10.3920/BM2020.0057

Blanco, C. (2020). The influence of the gut microbiome on obesity. *J. Am. Assoc. Nurse. Pract.* 32 (7), 504–510. doi: 10.1097/JXX.0000000000000480

Blundell, J., Finlayson, G., Axelsen, M., Flint, A., Gibbons, C., Kvist, T., et al. (2017). Effects of once-weekly semaglutide on appetite, energy intake, control of eating, food preference and body weight in subjects with obesity. *Diabetes Obes. Metab.* 19 (9), 1242–1251. doi: 10.1111/dom.12932

Bolam, D. N., and van den Berg, B. (2018). TonB-dependent transport by the gut microbiota: novel aspects of an old problem. *Curr. Opin. Struct. Biol.* 51, 35–43. doi: 10.1016/j.sbi.2018.03.001

Bray, G. A., Kim, K. K., Wilding, J. P. H., and World Obesity, F. (2017). Obesity: a chronic relapsing progressive disease process. a position statement of the world obesity federation. *Obes. Rev.* 18 (7), 715–723. doi: 10.1111/obr.12551

Bray, G. A., and Ryan, D. H. (2021). Evidence-based weight loss interventions: Individualized treatment options to maximize patient outcomes. *Diabetes Obes. Metab.* 23 Suppl 1, 50–62. doi: 10.1111/dom.14200

Capaldo, C. T., Powell, D. N., and Kalman, D. (2017). Layered defense: how mucus and tight junctions seal the intestinal barrier. *J. Mol. Med. (Berl).* 95 (9), 927–934. doi: 10.1007/s00109-017-1557-x

Carretta, M. D., Quiroga, J., Lopez, R., Hidalgo, M. A., and Burgos, R. A. (2021). Participation of short-chain fatty acids and their receptors in gut inflammation and colon cancer. *Front. Physiol.* 12. doi: 10.3389/fphys.2021.662739

Carvalho, B. M., and Saad, M. J. (2013). Influence of gut microbiota on subclinical inflammation and insulin resistance. *Mediators Inflammation* 2013, 986734. doi: 10.1155/2013/986734

Chelakkot, C., Choi, Y., Kim, D. K., Park, H. T., Ghim, J., Kwon, Y., et al. (2018). Akkermansia muciniphila-derived extracellular vesicles influence gut permeability through the regulation of tight junctions. *Exp. Mol. Med.* 50 (2), e450. doi: 10.1038/emm.2017.282

Chen, S., Zuo, S., Zhu, J., Yue, T., Bu, D., Wang, X., et al. (2019). Decreased expression of cystathionine beta-synthase exacerbates intestinal barrier injury in ulcerative colitis. *J. Crohns. Colitis.* 13 (8), 1067–1080. doi: 10.1093/ecco-jcc/jjz027

Clarke, S. F., Murphy, E. F., O'Sullivan, O., Lucey, A. J., Humphreys, M., Hogan, A., et al. (2014). Exercise and associated dietary extremes impact on gut microbial diversity. *Gut* 63 (12), 1913–1920. doi: 10.1136/gutjnl-2013-306541

Costea, P. I., Hildebrand, F., Arumugam, M., Backhed, F., Blaser, M. J., Bushman, F. D., et al. (2018). Publisher correction: Enterotypes in the landscape of gut microbial community composition. *Nat. Microbiol.* 3 (3), 388. doi: 10.1038/s41564-018-0114-x

Cuevas-Sierra, A., Ramos-Lopez, O., Riezu-Boj, J. I., Milagro, F. I., and Martinez, J. A. (2019). Diet, gut microbiota, and obesity: Links with host genetics and epigenetics and potential applications. *Adv. Nutr.* 10 (suppl_1), S17–S30. doi: 10.1093/advances/nmy078

Dao, M. C., Everard, A., Aron-Wisnewsky, J., Sokolovska, N., Prifti, E., Verger, E. O., et al. (2016). Akkermansia muciniphila and improved metabolic health during a dietary intervention in obesity: relationship with gut microbiome richness and ecology. *Gut* 65 (3), 426–436. doi: 10.1136/gutjnl-2014-308778

David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., et al. (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 505 (7484), 559–563. doi: 10.1038/nature12820

den Besten, G., van Eunen, K., Groen, A. K., Venema, K., Reijngoud, D. J., and Bakker, B. M. (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J. Lipid Res.* 54 (9), 2325–2340. doi: 10.1194/jlr.R036012

Derrien, M., Alvarez, A. S., and de Vos, W. M. (2019). The gut microbiota in the first decade of life. *Trends Microbiol.* 27 (12), 997–1010. doi: 10.1016/j.tim.2019.08.001

Derrien, M., Collado, M. C., Ben-Amor, K., Salminen, S., and de Vos, W. M. (2008). The mucin degrader akkermansia muciniphila is an abundant resident of the human intestinal tract. *Appl. Environ. Microbiol.* 74 (5), 1646–1648. doi: 10.1128/AEM.01226-07

Di Ciaula, A., Wang, D. Q., Molina-Molina, E., Lunardi Baccetto, R., Calamita, G., Palmieri, V. O., et al. (2017). Bile acids and cancer: Direct and environmental-dependent effects. *Ann. Hepatol.* 16 Suppl 1, S87–S105. doi: 10.5604/01.3001.0010.5501

Dominguez-Bello, M. G., Godoy-Vitorino, F., Knight, R., and Blaser, M. J. (2019). Role of the microbiome in human development. *Gut* 68 (6), 1108–1114. doi: 10.1136/gutjnl-2018-317503

El Moussaoui, I., Van Vyve, E., Johanet, H., Dabrowski, A., Piquard, A., Delaunay, T., et al. (2021). Laparoscopic sleeve gastrectomy for morbid obesity in a Belgian-French prospective multicenter study: outcomes and predictors weight loss failure. *Acta Chir. Belg.* 121 (6), 413–419. doi: 10.1080/00015458.2020.1841485

Fanti, M., Mishra, A., Longo, V. D., and Brandhorst, S. (2021). Time-restricted eating, intermittent fasting, and fasting-mimicking diets in weight loss. *Curr. Obes. Rep.* 10 (2), 70–80. doi: 10.1007/s13679-021-00424-2

Forte, N., Fernandez-Rilo, A. C., Palomba, L., Di Marzo, V., and Cristino, L. (2020). Obesity affects the microbiota-Gut-Brain axis and the regulation thereof by endocannabinoids and related mediators. *Int. J. Mol. Sci.* 21 (5), 1554. doi: 10.3390/ijms21051554

Geurts, L., Lazarevic, V., Derrien, M., Everard, A., Van Roye, M., Knauf, C., et al. (2011). Altered gut microbiota and endocannabinoid system tone in obese and diabetic leptin-resistant mice: impact on apelin regulation in adipose tissue. *Front. Microbiol.* 2. doi: 10.3389/fmicb.2011.00149

Gill, S. R., Pop, M., Deboy, R. T., Eckburg, P. B., Turnbaugh, P. J., Samuel, B. S., et al. (2006). Metagenomic analysis of the human distal gut microbiome. *Science* 312 (5778), 1355–1359. doi: 10.1126/science.1124234

Giordano, N. P., Cian, M. B., and Dalebroux, Z. D. (2020). Outer membrane lipid secretion and the innate immune response to gram-negative bacteria. *Infect. Immun.* 88 (7), e00920-19. doi: 10.1128/IAI.00920-19

Gupta, B., Liu, Y., Chopyk, D. M., Rai, R. P., Desai, C., Kumar, P., et al. (2020). Western Diet-induced increase in colonic bile acids compromises epithelial barrier in nonalcoholic steatohepatitis. *FASEB J.* 34 (5), 7089–7102. doi: 10.1096/fj.201902687R

Hanninen, A., Toivonen, R., Poysti, S., Belzer, C., Plovier, H., Ouwerkerk, J. P., et al. (2018). Akkermansia muciniphila induces gut microbiota remodelling and controls islet autoimmunity in NOD mice. *Gut* 67 (8), 1445–1453. doi: 10.1136/gutjnl-2017-314508

Hernandez, M. A. G., Canfora, E. E., Jocken, J. W. E., and Blaak, E. E. (2019). The short-chain fatty acid acetate in body weight control and insulin sensitivity. *Nutrients* 11 (8), 1943. doi: 10.3390/nu11081943

Iwamoto, J., Honda, A., Miyazaki, T., Monma, T., Ueda, H., Morishita, Y., et al. (2021). Western Diet changes gut microbiota and ameliorates liver injury in a mouse model with human-like bile acid composition. *Hepatol. Commun.* 5 (12), 2052–2067. doi: 10.1002/hep4.1778

Jager, R., Purpura, M., Farmer, S., Cash, H. A., and Keller, D. (2018). Probiotic bacillus coagulans GBI-30, 6086 improves protein absorption and utilization. *Probio. Antimicrob. Proteins* 10 (4), 611–615. doi: 10.1007/s12602-017-9354-y

Jansma, J., Brinkman, F., van Hemert, S., and El Aidy, S. (2021). Targeting the endocannabinoid system with microbial interventions to improve gut integrity. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 106, 110169. doi: 10.1016/j.pnpbp.2020.110169

Jiang, C., Zhang, S., Li, D., Chen, L., Zhao, Y., Mei, G., et al. (2020). Impaired ferritinophagy flux induced by high fat diet mediates hepatic insulin resistance *via* endoplasmic reticulum stress. *Food Chem. Toxicol.* 140, 111329. doi: 10.1016/j.fct.2020.111329

Kapoor, N., Kota, S., and Kalra, S. (2021). Obesity a communicable disease - a new age paradigm. *J. Pak. Med. Assoc.* 71 (8), 2100–2102.

Kasselman, L. J., Vernice, N. A., DeLeon, J., and Reiss, A. B. (2018). The gut microbiome and elevated cardiovascular risk in obesity and autoimmunity. *Atherosclerosis* 271, 203–213. doi: 10.1016/j.atherosclerosis.2018.02.036

Keshavarz Azizi Raftar, S., Ashrafian, F., Yadegar, A., Lari, A., Moradi, H. R., Shahriary, A., et al. (2021). The protective effects of live and pasteurized akkermansia muciniphila and its extracellular vesicles against HFD/CCl4-induced liver injury. *Microbiol. Spectr.* 9 (2), e0048421. doi: 10.1128/Spectrum.00484-21

Kijmanawat, A., Panburana, P., Reutrakul, S., and Tangshewinsirikul, C. (2019). Effects of probiotic supplements on insulin resistance in gestational diabetes mellitus: A double-blind randomized controlled trial. *J. Diabetes Investig.* 10 (1), 163–170. doi: 10.1111/jdi.12863

Kim, S., Choi, S., Dutta, M., Asubonteng, J. O., Polunas, M., Goedken, M., et al. (2021). Pregnane X receptor exacerbates nonalcoholic fatty liver disease accompanied by obesity- and inflammation-prone gut microbiome signature. *Biochem. Pharmacol.* 193, 114698. doi: 10.1016/j.bcp.2021.114698

Kim, M., Friesen, L., Park, J., Kim, H. M., and Kim, C. H. (2018). Microbial metabolites, short-chain fatty acids, restrain tissue bacterial load, chronic inflammation, and associated cancer in the colon of mice. *Eur. J. Immunol.* 48 (7), 1235–1247. doi: 10.1002/eji.201747122

Kim, S., Lee, Y., Kim, Y., Seo, Y., Lee, H., Ha, J., et al. (2020). Akkermansia muciniphila prevents fatty liver disease, decreases serum triglycerides, and maintains gut homeostasis. *Appl. Environ. Microbiol.* 86 (7), e03004-19. doi: 10.1128/AEM.03004-19

Kong, C., Gao, R., Yan, X., Huang, L., and Qin, H. (2019). Probiotics improve gut microbiota dysbiosis in obese mice fed a high-fat or high-sucrose diet. *Nutrition* 60, 175–184. doi: 10.1016/j.nut.2018.10.002

Kumar, A., Sundaram, K., Mu, J., Dryden, G. W., Sriwastva, M. K., Lei, C., et al. (2021). High-fat diet-induced upregulation of exosomal phosphatidylcholine contributes to insulin resistance. *Nat. Commun.* 12 (1), 213. doi: 10.1038/s41467-020-20500-w

Lee, J.-Y., Cevallos, S. A., Byndloss, M. X., Tiffany, C. R., Olsan, E. E., Butler, B. P., et al. (2020). High-fat diet and antibiotics cooperatively impair mitochondrial bioenergetics to trigger dysbiosis that exacerbates pre-inflammatory bowel disease. *Cell Host Microbe* 28 (2), 273–284.e276. doi: 10.1016/j.chom.2020.06.001

Lemons, J. M. S., and Liu, L. (2022). Chewing the fat with microbes: Lipid crosstalk in the gut. *Nutrients* 14 (3), 573. doi: 10.3390/nu14030573

Liang, C., Zhou, X. H., Jiao, Y. H., Guo, M. J., Meng, L., Gong, P. M., et al. (2021). Ligilactobacillus salivarius LCK11 prevents obesity by promoting PYY secretion to inhibit appetite and regulating gut microbiota in C57BL/6J mice. *Mol. Nutr. Food Res.* 65 (17), e2100136. doi: 10.1002/mnfr.202100136

Liu, R., Hong, J., Xu, X., Feng, Q., Zhang, D., Gu, Y., et al. (2017). Gut microbiome and serum metabolome alterations in obesity and after weight-loss intervention. *Nat. Med.* 23 (7), 859–868. doi: 10.1038/nm.4358

Liu, H. Y., Walden, T. B., Ahl, D., Nyman, M., Bertilsson, S., Phillipson, M., et al. (2019). High-fat diet enriched with bilberry modifies colonic mucus dynamics and restores marked alterations of gut microbiome in rats. *Mol. Nutr. Food Res.* 63 (20), e1900117. doi: 10.1002/mnfr.201900117

Mariamenatu, A. H., and Abdu, E. M. (2021). Overconsumption of omega-6 polyunsaturated fatty acids (PUFAs) versus deficiency of omega-3 PUFAs in

modern-day diets: The disturbing factor for their "Balanced antagonistic metabolic functions" in the human body. *J. Lipids* 2021, 8848161. doi: 10.1155/2021/8848161

McNabney, S. M., and Henagan, T. M. (2017). Short chain fatty acids in the colon and peripheral tissues: A focus on butyrate, colon cancer, obesity and insulin resistance. *Nutrients* 9 (12), 1348. doi: 10.3390/nu9121348

Mohammad, S., and Thiemermann, C. (2020). Role of metabolic endotoxemia in systemic inflammation and potential interventions. *Front. Immunol.* 11. doi: 10.3389/fimmu.2020.594150

Moniri, N. H., and Farah, Q. (2021). Short-chain free-fatty acid G protein-coupled receptors in colon cancer. *Biochem. Pharmacol.* 186, 114483. doi: 10.1016/j.bcp.2021.114483

Monk, J. M., Wu, W., Lepp, D., Wellings, H. R., Hutchinson, A. L., Liddle, D. M., et al. (2019). Navy bean supplemented high-fat diet improves intestinal health, epithelial barrier integrity and critical aspects of the obese inflammatory phenotype. *J. Nutr. Biochem.* 70, 91–104. doi: 10.1016/j.jnutbio.2019.04.009

Montaigne, D., Butruille, L., and Staels, B. (2021). PPAR control of metabolism and cardiovascular functions. *Nat. Rev. Cardiol.* 18 (12), 809–823. doi: 10.1038/s41569-021-00569-6

Mottawea, W., Chiang, C. K., Muhlbauer, M., Starr, A. E., Butcher, J., Abujamel, T., et al. (2016). Altered intestinal microbiota-host mitochondria crosstalk in new onset crohn's disease. *Nat. Commun.* 7, 13419. doi: 10.1038/ncomms13419

Nascimento, J. C., Matheus, V. A., Oliveira, R. B., Tada, S. F. S., and Collares-Buzato, C. B. (2021). High-fat diet induces disruption of the tight junction-mediated paracellular barrier in the proximal small intestine before the onset of type 2 diabetes and endotoxemia. *Dig. Dis. Sci.* 66 (10), 3359–3374. doi: 10.1007/s10620-020-06664-x

Netto Candido, T. L., Bressan, J., and Alfenas, R. C. G. (2018). Dysbiosis and metabolic endotoxemia induced by high-fat diet. *Nutr. Hosp.* 35 (6), 1432–1440. doi: 10.20960/nh.1792

Obesity: preventing and managing the global epidemic. (2000) *Report of a WHO consultation*. World Health Organization technical report series, 849, i–253.

Ocvirk, S., and O'Keefe, S. J. D. (2021). Dietary fat, bile acid metabolism and colorectal cancer. *Semin. Cancer Biol.* 73, 347–355. doi: 10.1016/j.semcancer.2020.10.003

Oliveira, R. B., Canuto, L. P., and Collares-Buzato, C. B. (2019). Intestinal luminal content from high-fat-fed prediabetic mice changes epithelial barrier function *in vitro*. *Life Sci.* 216, 10–21. doi: 10.1016/j.lfs.2018.11.012

Otani, T., and Furuse, M. (2020). Tight junction structure and function revisited: (Trends in cell biology 30, 805-817, 2020). *Trends Cell Biol.* 30 (12), 1014. doi: 10.1016/j.tcb.2020.10.001

Paccosi, S., Cresci, B., Pala, L., Rotella, C. M., and Parenti, A. (2020). Obesity therapy: How and why? *Curr. Med. Chem.* 27 (2), 174–186. doi: 10.2174/0929867326666190124121725

Qin, N., Song, G., Ren, X., Zhang, L., Gao, J., Xia, X., et al. (2020). Fish oil extracted from coregonus peled improves obese phenotype and changes gut microbiota in a high-fat diet-induced mouse model of recurrent obesity. *Food Funct.* 11 (7), 6158–6169. doi: 10.1039/d0fo00911c

Raimondi, F., Santoro, P., Barone, M. V., Pappacoda, S., Barretta, M. L., Nanayakkara, M., et al. (2008). Bile acids modulate tight junction structure and barrier function of caco-2 monolayers *via* EGFR activation. *Am. J. Physiol. Gastroint. Liver. Physiol.* 294 (4), G906–G913. doi: 10.1152/ajpgi.00043.2007

Ridaura, V. K., Faith, J. J., Rey, F. E., Cheng, J., Duncan, A. E., Kau, A. L., et al. (2013). Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science* 341 (6150), 1241214. doi: 10.1126/science.1241214

Robertson, R. C., Manges, A. R., Finlay, B. B., and Prendergast, A. J. (2019). The human microbiome and child growth - first 1000 days and beyond. *Trends Microbiol.* 27 (2), 131–147. doi: 10.1016/j.tim.2018.09.008

Rohr, M. W., Narasimhulu, C. A., Rudeski-Rohr, T. A., and Parthasarathy, S. (2020). Negative effects of a high-fat diet on intestinal permeability: A review. *Adv. Nutr.* 11 (1), 77–91. doi: 10.1093/advances/nmz061

Romani-Perez, M., Bullich-Vilarrubias, C., Lopez-Almela, I., Liebana-Garcia, R., Olivares, M., and Sanz, Y. (2021). The microbiota and the gut-brain axis in controlling food intake and energy homeostasis. *Int. J. Mol. Sci.* 22 (11), 5830. doi: 10.3390/ijms22115830

Samuel, B. S., Shaito, A., Motoike, T., Rey, F. E., Backhed, F., Manchester, J. K., et al. (2008). Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. *Proc. Natl. Acad. Sci. U.S.A.* 105 (43), 16767–16772. doi: 10.1073/pnas.0808567105

Sarathy, J., Detloff, S. J., Ao, M., Khan, N., French, S., Sirajuddin, H., et al. (2017). The yin and yang of bile acid action on tight junctions in a model colonic epithelium. *Physiol. Rep.* 5 (10), e13294. doi: 10.14814/phy2.13294

Sarwer, D. B., Allison, K. C., Wadden, T. A., Ashare, R., Spitzer, J. C., McCuen-Wurst, C., et al. (2019). Psychopathology, disordered eating, and impulsivity as predictors of outcomes of bariatric surgery. *Surg. Obes. Relat. Dis.* 15 (4), 650–655. doi: 10.1016/j.soard.2019.01.029

Sebastian Domingo, J. J., and Sanchez Sanchez, C. (2018). From the intestinal flora to the microbiome. *Rev. Esp. Enferm. Dig.* 110 (1), 51–56. doi: 10.17235/reed.2017.4947/2017

Sommer, F., and Backhed, F. (2016). Know your neighbor: Microbiota and host epithelial cells interact locally to control intestinal function and physiology. *Bioessays* 38 (5), 455–464. doi: 10.1002/bies.201500151

Spinelli, J. B., and Haigis, M. C. (2018). The multifaceted contributions of mitochondria to cellular metabolism. *Nat. Cell Biol.* 20 (7), 745–754. doi: 10.1038/s41556-018-0124-1

Stanislawski, M. A., Dabelea, D., Lange, L. A., Wagner, B. D., and Lozupone, C. A. (2019). Gut microbiota phenotypes of obesity. *NPJ Biofilms. Microbiomes.* 5 (1), 18. doi: 10.1038/s41522-019-0091-8

Stoeva, M. K., Garcia-So, J., Justice, N., Myers, J., Tyagi, S., Nemchek, M., et al. (2021). Butyrate-producing human gut symbiont, clostridium butyricum, and its role in health and disease. *Gut. Microbes* 13 (1), 1–28. doi: 10.1080/19490976.2021.1907272

Stubbs, B. J., Cox, P. J., Evans, R. D., Cyranka, M., Clarke, K., and de Wet, H. (2018). A ketone ester drink lowers human ghrelin and appetite. *Obes. (Silver. Spring).* 26 (2), 269–273. doi: 10.1002/oby.22051

Tanaka, S., Nemoto, Y., Takei, Y., Morikawa, R., Oshima, S., Nagaishi, T., et al. (2020). High-fat diet-derived free fatty acids impair the intestinal immune system and increase sensitivity to intestinal epithelial damage. *Biochem. Biophys. Res. Commun.* 522 (4), 971–977. doi: 10.1016/j.bbrc.2019.11.158

Theis, K. R., Romero, R., Winters, A. D., Greenberg, J. M., Gomez-Lopez, N., Alhousseini, A., et al. (2019). Does the human placenta delivered at term have a microbiota? results of cultivation, quantitative real-time PCR, 16S rRNA gene sequencing, and metagenomics. *Am. J. obstet. gynecol.* 220(3), 267.e261–267.e239 doi: 10.1016/j.ajog.2018.10.018

Thyagarajan, B., and Foster, M. T. (2017). Beiging of white adipose tissue as a therapeutic strategy for weight loss in humans. *Horm. Mol. Biol. Clin. Investig.* 31 (2). doi: 10.1515/hmbci-2017-0016

Tirosh, A., Calay, E. S., Tuncman, G., Claiborn, K. C., Inouye, K. E., Eguchi, K., et al. (2019). The short-chain fatty acid propionate increases glucagon and FABP4 production, impairing insulin action in mice and humans. *Sci. Transl. Med.* 11 (489), eaav0120. doi: 10.1126/scitranslmed.aav0120

Tsukita, K., Yano, T., Tamura, A., and Tsukita, S. (2019). Reciprocal association between the apical junctional complex and AMPK: A promising therapeutic target for Epithelial/Endothelial barrier function? *Int. J. Mol. Sci.* 20 (23), 6012. doi: 10.3390/ijms20236012

Turnbaugh, P. J., Ridaura, V. K., Faith, J. J., Rey, F. E., Knight, R., and Gordon, J. I. (2009). The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. *Sci. Transl. Med.* 1 (6), 6ra14. doi: 10.1126/scitranslmed.3000322

Usami, M., Komurasaki, T., Hanada, A., Kinoshita, K., and Ohata, A. (2003). Effect of gamma-linolenic acid or docosahexaenoic acid on tight junction permeability in intestinal monolayer cells and their mechanism by protein kinase c activation and/or eicosanoid formation. *Nutrition* 19 (2), 150–156. doi: 10.1016/s0899-9007(02)00927-9

Usami, M., Muraki, K., Iwamoto, M., Ohata, A., Matsushita, E., and Miki, A. (2001). Effect of eicosapentaenoic acid (EPA) on tight junction permeability in intestinal monolayer cells. *Clin. Nutr.* 20 (4), 351–359. doi: 10.1054/clnu.2001.0430

van der Lugt, B., van Beek, A. A., Aalvink, S., Meijer, B., Sovran, B., Vermeij, W. P., et al. (2019). Akkermansia muciniphila ameliorates the age-related decline in colonic mucus thickness and attenuates immune activation in accelerated aging Ercc1 (-/Delta7) mice. *Immun. Ageing* 16, 6. doi: 10.1186/s12979-019-0145-z

Wan, Y., Wang, F., Yuan, J., Li, J., Jiang, D., Zhang, J., et al. (2019). Effects of dietary fat on gut microbiota and faecal metabolites, and their relationship with cardiometabolic risk factors: a 6-month randomised controlled-feeding trial. *Gut* 68 (8), 1417–1429. doi: 10.1136/gutjnl-2018-317609

Wu, X., Wei, Q., Wang, X., Shang, Y., and Zhang, H. (2022). Evolutionary and dietary relationships of wild mammals based on the gut microbiome. *Gene* 808, 145999. doi: 10.1016/j.gene.2021.145999

Xiang, R., Wang, J., Xu, W., Zhang, M., and Wang, M. (2021). Amuc_1102 from akkermansia muciniphila adopts an immunoglobulin-like fold related to archaeal type IV pilus. *Biochem. Biophys. Res. Commun.* 547, 59–64. doi: 10.1016/j.bbrc.2021.02.022

Yang, M., Bose, S., Lim, S., Seo, J., Shin, J., Lee, D., et al. (2020). Beneficial effects of newly isolated akkermansia muciniphila strains from the human gut on obesity and metabolic dysregulation. *Microorganisms* 8 (9), 1413. doi: 10.3390/microorganisms8091413

Zeng, Q., Li, D., He, Y., Li, Y., Yang, Z., Zhao, X., et al. (2019). Discrepant gut microbiota markers for the classification of obesity-related metabolic abnormalities. *Sci. Rep.* 9 (1), 13424. doi: 10.1038/s41598-019-49462-w

Zhang, H.-l., Wu, Q.-x., and Qin, X.-m. (2019a). Camellia nitidissima chi flower extracts inhibit α-amylase and α-glucosidase: In vitro by analysis of optimization of addition methods, inhibitory kinetics and mechanisms. *Process. Biochem.* 86, 177–185. doi: 10.1016/j.procbio.2019.07.009

Zhang, P., Yu, Y., Qin, Y., Zhou, Y., Tang, R., Wang, Q., et al. (2019b). Alterations to the microbiota-colon-brain axis in high-fat-diet-induced obese mice compared to diet-resistant mice. *J. Nutr. Biochem.* 65, 54–65. doi: 10.1016/j.jnutbio.2018.08.016

Zhou, J. C., and Zhang, X. W. (2019). Akkermansia muciniphila: a promising target for the therapy of metabolic syndrome and related diseases. *Chin. J. Nat. Med.* 17 (11), 835–841. doi: 10.1016/S1875-5364(19)30101-3

frontiers | Frontiers in Cellular and Infection Microbiology

TYPE Original Research
PUBLISHED 26 April 2023
DOI 10.3389/fcimb.2023.1067367

OPEN ACCESS

EDITED BY
Yufang Wang,
Sichuan University, China

REVIEWED BY
Ashraf Kariminik,
Islamic Azad University Kerman, Iran
Abdoul-Razak Tidjani,
Grenoble (TIMC-IMAG), France

*CORRESPONDENCE
Fei Wei
bzmcxwk@bzmc.edu.cn
Shoufang Jiang
jiangshoufang@163.com
Bo Cui
iamcuib@sina.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Intestinal Microbiome, a section of the journal Frontiers in Cellular and Infection Microbiology

RECEIVED 11 October 2022
ACCEPTED 12 April 2023
PUBLISHED 26 April 2023

CITATION
Li X, Zheng P, Cao W, Cao Y, She X, Yang H, Ma K, Wu F, Gao X, Fu Y, Yin J, Wei F, Jiang S and Cui B (2023) *Lactobacillus rhamnosus* GG ameliorates noise-induced cognitive deficits and systemic inflammation in rats by modulating the gut-brain axis. *Front. Cell. Infect. Microbiol.* 13:1067367. doi: 10.3389/fcimb.2023.1067367

Lactobacillus rhamnosus GG ameliorates noise-induced cognitive deficits and systemic inflammation in rats by modulating the gut-brain axis

Xiaofang Li[1,2†], Pengfang Zheng[1,3†], Wa Cao[1,4†], Yang Cao[1], Xiaojun She[1], Honglian Yang[1], Kefeng Ma[1], Fangshan Wu[1,3], Xiujie Gao[1], Yu Fu[1,3], Jiayi Yin[1,5], Fei Wei[2*], Shoufang Jiang[4*] and Bo Cui[1,2,3,5*]

[1]Tianjin Institute of Environmental and Operational Medicine, Tianjin, China, [2]School of Public Health and Management, Binzhou Medical University, Yantai, China, [3]School of Public Health and Management, Weifang Medical University, Weifang, China, [4]School of Public Health, North China University of Science and Technology, Tangshan, China, [5]Shandong Academy of Occupational Health and Occupational Medicine, Shandong First Medical University, Jinan, China

Background: Environmental noise exposure is linked to neuroinflammation and imbalance of the gut microbiota. Promoting gut microbiota homeostasis may be a key factor in relieving the deleterious non-auditory effects of noise. This study aimed to investigate the effect of *Lactobacillus rhamnosus* GG (LGG) intervention on noise-induced cognitive deficits and systemic inflammation in rats.

Methods: Learning and memory were assessed using the Morris water maze, while 16S rRNA sequencing and gas chromatography-mass spectrometry were used to analyze the gut microbiota and short-chain fatty acid (SCFA) content. Endothelial tight junction proteins and serum inflammatory mediators were assessed to explore the underlying pathological mechanisms.

Results: The results indicated that *Lactobacillus rhamnosus* GG intervention ameliorated noise-induced memory deterioration, promoted the proliferation of beneficial bacteria, inhibited the growth of harmful bacteria, improved dysregulation of SCFA-producing bacteria, and regulated SCFA levels. Mechanistically, noise exposure led to a decrease in tight junction proteins in the gut and hippocampus and an increase in serum inflammatory mediators, which were significantly alleviated by *Lactobacillus rhamnosus* GG intervention.

Conclusion: Taken together, *Lactobacillus rhamnosus* GG intervention reduced gut bacterial translocation, restored gut and blood-brain barrier functions, and improved gut bacterial balance in rats exposed to chronic noise, thereby protecting against cognitive deficits and systemic inflammation by modulating the gut-brain axis.

KEYWORDS

noise, *Lactobacillus rhamnosus* GG, cognition, inflammation, gut microbiota, gut-brain axis

1 Introduction

The intestine has the most abundant and diverse bacterial community in the body (Sender et al., 2016). The gut microbiota, known as the "second brain", affects normal physiology, synaptic, immune, and barrier functions, and host behavior, including cognition, through the microbiome-gut-brain axis (Shukla et al., 2021). Dysbiosis of the gut microbiota frequently leads to brain (Hill-Burns et al., 2017; Pulikkan et al., 2018; Singhrao and Harding, 2020) and gut diseases, such as inflammatory gut disease (Al Bander et al., 2020). Our previous study demonstrated that noise intervention changed the gut microbiota, increased gut and brain endothelial barrier dysfunction, and accelerated neurochemical and inflammatory dysregulation in an Alzheimer's disease (AD) mouse model (Cui et al., 2018). Moreover, noise exposure leads to changes in the relative abundance of species belonging to the family *Lactobacillaceae* (including the genus *Lactobacillus*) (Chi et al., 2021), although other factors may also cause *Lactobacillus* disorders. For instance, the abundance of *Lactobacillus* is reduced in mice with high-fat diet induced steatohepatitis (Zhou et al., 2017).

Lactobacillus Rhamnosus GG (LGG) is a probiotic originally isolated from the human gut. In recent years, studies have shown that LGG can tolerate the environment of the digestive tract and colonize the gut, participating in the regulation of intestinal microbiota homeostasis (Ritze et al., 2014; Chen et al., 2019; Zhao et al., 2020).Sanborn et al. (Sanborn et al., 2020) reported that cognitive performance improved in older adults after supplementation with probiotic LGG, suggesting that LGG intervention may delay aging-related cognitive decline. Additionally, supplementation with *Lactobacillus* and *Bifidobacterium* reportedly improved spatial learning, memory deficits, and oxidative stress in AD rats (Athari Nik Azm et al., 2018). Moreover, LGG plays a key role in protecting against inflammatory injury and intestinal barrier dysfunction (Donato et al., 2010). For example, LGG reportedly regulates the expression of tight junction proteins Occludin and zonula occludens-1 to alleviate impaired barrier function (Han et al., 2019). Notably, one study demonstrated that LGG colonization in early life reduced inflammation, increased the abundance of SCFA-producing bacteria, and promoted the production of SCFAs in young mice, with the beneficial effects persisting up to eight months (Liu et al., 2021).

The above studies suggest potential beneficial effects of LGG on cognitive function, systemic inflammation, intestinal barrier function, and gut metabolism. Thus, the purpose of this research was to investigate whether intervention with LGG may improve noise-induced cognitive deficits and systemic inflammation by modulating the gut-brain axis in rats exposed to chronic noise.

Abbreviations: Aβ, β-amyloid peptides; AD, Alzheimer's disease; ANOSIM analysis, analysis of similarity; D-LA, D-lactic acid; IL, interleukin; LGG, *Lactobacillus Rhamnosus* GG; LDA, linear discriminant analysis; LEfSe, linear discriminant analysis effect size; LPS, lipopolysaccharide; MWM, Morris water maze; NF-κB, nuclear factor-kappa B; OTU, operational taxonomic unit; PCoA, principal coordinate analysis; SCFA, short-chain fatty acid; SPL, sound pressure level; UPGMA, unweighted pair group method with arithmetic mean.

2 Materials and methods

2.1 LGG culture

0.5 mL of sterile water was injected into LGG lyophilized powder (BeiNa Chuanglian Biotechnology Co., LTD.), gently blown, and fully dissolved into the bacterial suspension. The bacterial suspension was absorbed and 200 μL was added to two MRS solid mediums (Solarbio Science and Technology Co., Ltd. Beijing), evenly spread, and incubated at 37°C for 24 h in an anaerobic station with a gas-pak. LGG single colonies were then selected and added to MRS liquid medium (pH 6.2 ± 0.2, autoclaved at 121°C for 15 min) and incubated at 37°C for 24 h in an anaerobic station with a gas-pak. Subsequently, the bacteria were centrifuged at 4000 × *g* for 15 min at 4°C and the bacterial pellet was collected. The bacteria were resuspended at a concentration of 1×10^8 CFU/mL after the precipitate was washed with saline solution 3 times. The bacterial cultures were stored at 4°C for a short time (within 30 min) during the treatment of the samples.

2.2 Animals and experimental groups

A total of 48 healthy, male, six-week-old Wistar rats were provided by Beijing Viton Lihua Laboratory Animal Technology Co., Ltd. (Beijing, China) (animal quality certificate: 2016-0006). The rats were maintained under standard housing conditions with an ambient temperature of 23 ± 2°C and 50%–60% humidity. The rats were acclimated for five days prior to the experiment. Rats were fed a standard laboratory rodent diet and had free access to water (diet and water were autoclaved prior to ingestion). The rats were randomly assigned to the following groups: Control, LGG, Noise, and Noise + LGG. The Control and Noise groups received 1 mL saline solution by gavage daily. The LGG and Noise + LGG groups received 1 mL of LGG suspension daily by gavage. For 56 days, the Noise and Noise + LGG groups were exposed to 88 dB sound pressure level (SPL) white noise for 4 h/day, whereas the Control and LGG groups were exposed to background noise (< 40 dB SPL). All animal experimental protocols were approved by the Tianjin Institute of Environmental and Operational Medicine Animal Use and Research Committee (approval number: LACUC of AMMS-04-2020-063).

2.3 Noise exposure set-up

Noise was produced by a noise generator (BK 3560 C, Brüel & Kjær Instruments, Nærum, Denmark), which was then amplified by a power amplifier and broadcast *via* a loudspeaker. The frequency range of the generator's noise signal was 20-20,000 Hz. In a reverberation chamber, the rats were housed in wire mesh cages in the center of the sound field and exposed to the noise *via* the loudspeaker hung above the cage.

2.4 Morris water maze testing

The Morris water maze test adapted from a previous study (Chi et al., 2021), using hidden platform training (spatial learning) and a

probe trial (spatial memory). During the platform training phase, the rats sought a hidden platform 2 cm below the water surface. The rats were set in one of the four quadrants of the pool, facing the wall (alternating clockwise in each trial), and were allowed to stay on the platform for 10 s after finding it. If the rats did not find the platform within 60 s, they were manually placed on the platform for 10 s. For four days in a row, the rats finished four trials per day. On day 5 during the probe trial session, the platform was removed, and each rat was allowed to swim freely for 60 s. Video cameras were used to record the movements of the rat and to obtain the target quadrant distance/total distance, number of crossings over the target quadrant, time spent in the target quadrant during training sessions, and escape latency.

2.5 Sample collection

After noise exposure, the rats were weighed and anesthetized by intraperitoneal injection of 10% chloral hydrate at a dose of 0.3 mL/100 g. After the abdominal aorta blood collection, the rats were immediately executed by decapitation. Colon tissue was cut with sterile scissors, and colon feces were collected with sterile tweezers in sterile cryopreserved tube, hippocampus and colon tissues were collected and stored in sterile tubes, which were immediately frozen with liquid nitrogen and stored in a −80°C refrigerator for later use.

2.6 Transmission electron microscopy and hematoxylin-eosin staining of colon tissue

A sample of colon tissue (1–2 mm^3) was excised from each rat, to which 2.5% glutaraldehyde was added, and stored at 4°C. Tissues were fixed with osmium acid, dehydrated using an alcohol gradient, permeabilized with encapsulant epoxy, polymerized at 60°C for 48 h, and then stored at room temperature for approximately 20 days. Ultrathin sections (50 nm) were sliced, double stained with uranyl acetate and lead citrate at room temperature for 15 min, dried at room temperature overnight, and observed using transmission electron microscopy.

Additionally, a sample of colon tissue was fixed with 4% paraformaldehyde solution, dehydrated using an ethanol gradient, rendered transparent with xylene, embedded in paraffin, cooled to -20°C, and sliced into sections (5 μm). Subsequently, sections were heated at 45°C in a water bath, gently flattened, and dried at 60°C. After staining with HE (Fan et al., 2019), the sections were rendered transparent with xylene for 10 min, sealed with neutral resin, and observed under an optical microscope.

2.7 Western blot analysis

Total proteins of colonic and hippocampal tissues were extracted with RIPA buffer containing protease inhibitors (Solarbio, Beijing, China). The colon and hippocampus were treated with ultrasound for 1 min in a high throughput tissue grinder. The tissue lapping solution was centrifuged at 4°C at 12,000 rpm for 10 min, and the obtained supernatant was used for further analysis. Western blotting analysis was conducted using standard procedures, employing rabbit anti-Occludin (1:1000, Bioworld, USA), rabbit anti-CLDN1 (1:2000, Bioworld, USA), and rat anti-GAPDH (1:10,000, Bioworld, USA) antibodies, and GADPH as an internal reference standard.

2.8 Enzyme-linked immunosorbent assay

Serum was obtained by centrifuging abdominal aorta blood for 10 min at 3000 × *g*. The serum was stored at −80°C until analysis *via* ELISA. The following ELISA kits were used: Rat β-amyloid peptides (Aβ) 1-40 ELISA kit (Jiancheng Bioengineering, China), Rat Aβ1-42 ELISA kit (Jiancheng Bioengineering, China), Rat nuclear factor-kappa B (NF-κB) ELISA kit (Thermo Fisher Scientific, USA), Rat interleukin (IL)-10 ELISA kit (Thermo Fisher Scientific, USA), Rat IL-17 ELISA kit (Thermo Fisher Scientific, USA), Rat D-lactic acid (D-LA) ELISA kit (Thermo Fisher Scientific, USA), and Rat lipopolysaccharide (LPS) ELISA kit (Thermo Fisher Scientific, USA).

2.9 Sequencing of 16S ribosomal RNA genes in microbiota

Amplicon sequencing was employed to sequence the 16S rRNA genes of the microbiota, as previously described (Chi et al., 2021). Briefly, microbial DNA was isolated from the colon contents using the cetyltrimethylammonium bromide (CTAB) method. An appropriate amount of DNA was diluted to 1 ng/μL in sterile water. Next, the 16S rRNA V4 region were amplified using specific primers with the barcode (16S V4: 515F-806R; 515F 5'-GTGCCAGCMGCCGCGGTAA-3', 806R 5'-GGACTACHVGGGTWTCTAAT-3'). PCR reactions were performed using Phusion® High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, USA). PCR products were detected by electrophoresis with 2% agarose gel. Additionally, PCR products were mixed in equidensity ratios and purified with the Qiagen Gel Extraction Kit (Qiagen, Germany). The TruSeq® DNA PCR-free Sample Preparation Kit was then employed to construct the library, which was quantified by Qubit and Q-PCR. NovaSeq6000 was used for on-machine sequencing. Paired-end reads were assigned to samples based on their unique barcodes and were truncated by removing the barcode and primer sequences. FLASH (V1.2.7, http://ccb.jhu.edu/software/FLASH/) was then used to merge paired-end reads. This program merges paired-end reads when a portion of the reads overlap with the read generated from the opposite end of the same DNA fragment. The spliced sequences were designated raw tags, which were quality filtered under specific filtering conditions to obtain high-quality clean tags according to the QIIME (V1.9.1, http://qiime.org/scripts/split_libraries_fastq.html) quality control process. Chimera sequences were then detected, and subsequently removed, by comparing the tags with the reference database (Silva database, https://www.arb-silva.de/) using the UCHIME algorithm

(UCHIME Algorithm, http://www.drive5.com/usearch/manual/uchime_algo.html). The effective tags were obtained using Uparse software (Uparse v7.0.1001, http://www.drive5.com/uparse/); sequences with ≥ 97% similarity were assigned to the same operational taxonomic unit (OTU). Representative OTU sequences were selected for further annotation.

Alpha and beta diversity analyses were conducted using the QIIME software (Version 1.9.1) and R software (Version 2.15.3), whereas the principal coordinate analysis (PCoA) analysis was performed using weighted UniFrac. Additionally, linear discriminant analysis effect size (LEfSe) analysis was completed with the LEfSe software using a linear discriminant analysis (LDA) score cut off of > 4.0, $p < 0.05$. Analysis of similarity (ANOSIM) was performed with the Rvegan's Anosim function. Spearman correlation analysis was used to assess the correlation between intestinal microflora and fecal SCFAs. Analysis was performed using Novogene Bioinformatics Technology Co., Ltd. (Beijing, China).

2.10 Gas chromatography-mass spectrometer analysis

A total of 50 mg of the fecal sample was homogenized with 50 µL of 15% phosphoric acid, 125 µg/mL of internal standard (isohexanoic acid) solution, 100 µL and 400 µL of ether for 1 min and centrifuged at 12,000 rpm at 4°C for 10 min. The supernatant was then collected for testing. GC-MS was performed using a TRACE™ 1310 ISQ LT GC-MS (Thermo Fisher Scientific, Waltham, MA, USA) with an HP-INNOWAX GC column (30 m × 0.25 mm ID × 0.25 µm; Agilent Technologies, Santa Clara, CA, USA) under the following conditions: sample volume, 1 µL; split ratio, 10:1; inlet temperature, 250°C; ion source temperature, 230°C; transmission line temperature, 250°C; quadrupole temperature, 150°C. The initial oven temperature was 90°C, which was increased to 120°C with a ramp rate of 10°C/min, then to 150°C with a ramp rate of 5°C/min, and finally increased to 250°C with a ramp rate of 25°C/min, and was held at 250°C for 2 min. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. We used Thermo Chromeleon 7.2.10 (Novogene Bioinformatics Technology Co., Ltd.) to process the raw data coming out of the machine. The SCFA concentration was calculated from the respective peak areas of the sample and the internal standard, isohexanoic acid. The construction range and related information of calibration curves for GC-MS are provided in Supplementary Table 1.

2.11 Statistical analysis

The data are presented as the mean ± standard deviation. Statistical analysis was performed using IBM SPSS Statistics v.22.0 and GraphPad Prism v.7 software. Data were analyzed using one-way analysis of variance (ANOVA), and differences were considered statistically significant when $p < 0.05$. The Kruskal–Wallis test was used when data were not normally distributed. For two-way comparisons between groups, the least significant difference test was used if the variances were equal, and the Games-Howell test was used if they were not. Correlations were identified using Spearman's rank correlation analysis.

3 Results

3.1 Effect of LGG intervention on cognitive impairment after noise exposure

To assess changes in cognitive ability, rats underwent Morris water maze testing after noise exposure for 56 days. The escape latency of rats in the Noise group was prolonged on the second day of training compared to that of rats in the Control group ($p < 0.05$). Rats in the Noise + LGG group showed a statistically significant ($p < 0.05$) decrease in escape latency on the first day of training compared with rats in the Noise group (Figure 1A). Additionally, the time spent in the target quadrant, number of platform crossings, and target quadrant distance/total distance were reduced in the Noise group (Figures 1B–D), and the number of traverses in the target quadrant and time spent in the target quadrant were increased in the Noise + LGG group (Figures 1C, D). These results showed that LGG intervention had a protective effect against noise-induced cognitive impairment in rats.

3.2 Effect of LGG intervention on gut microbiota after noise exposure

The composition of the gut microbiota in each treatment group was detected *via* 16S rRNA high-throughput sequencing, and gut microbiota species richness (α-diversity) was reflected by Chao1 and ACE indices. The Chao1 and ACE indices were elevated in the Noise group (Supplementary Figures S1A, B), indicating that species richness of the gut microbiota in rats was disordered after noise exposure. Comparatively, the Chao1 and ACE indices were reduced in the Noise + LGG group, indicating that LGG intervention could regulate the gut microbiota in rats towards homeostasis. β diversity was obviously decreased after noise exposure compared with the control group (Tukey's test, $p < 0.05$; Supplementary Figure S1C). However, β diversity in the Noise + LGG group was significantly increased ($p < 0.05$) compared with the Noise group, indicating that LGG intervention could regulate both the richness and variety of the gut microbiota in rats.

The variation, confirmed by PCoA and ANOSIM analyses, was higher between groups than within groups (Supplementary Figures S1D, F, G). The distribution of the microbiota of the LGG group was relatively concentrated, while that of the Noise group was relatively dispersed, indicating that noise exposure and LGG intervention affected the abundance and structure of the gut microbiota. Based on the species annotation results, the top 10 most abundant bacteria of each treatment group were selected for clustering analysis based on the weighted UniFrac distance matrix (unweighted pair group method with arithmetic mean; UPGMA). Subsequently, the UPGMA results were combined with the relative abundance of the

10.3389/fcimb.2023.1067367

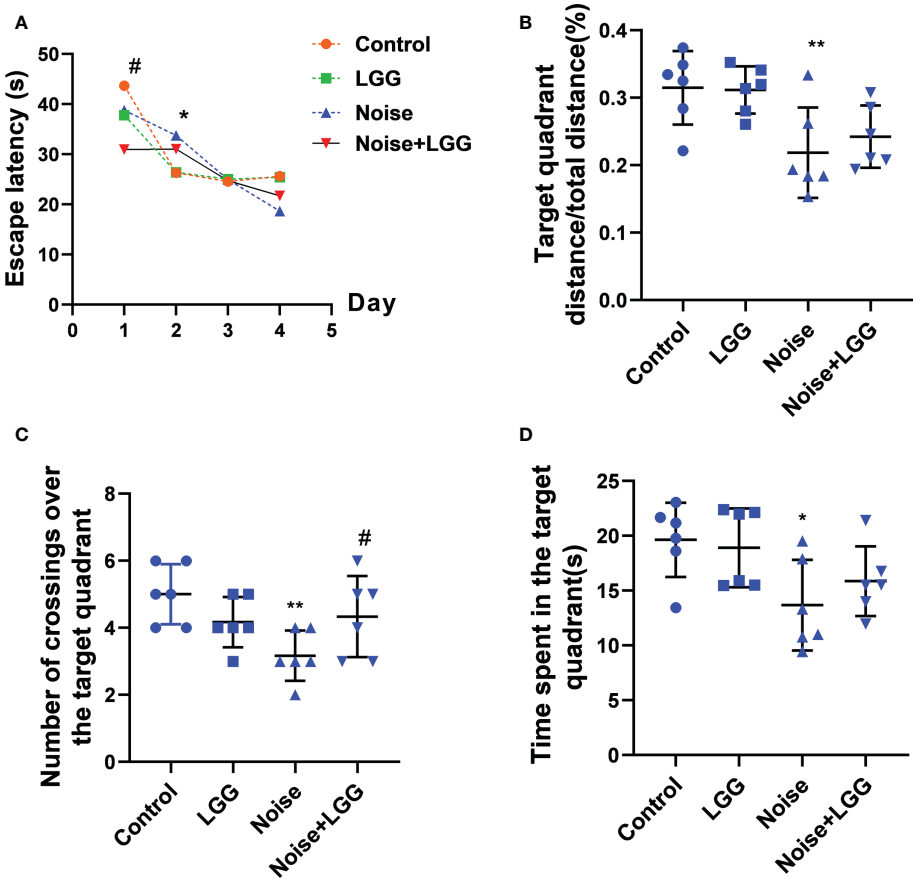


FIGURE 1
Effects of LGG intervention improved learning and memory abilities in rats. **(A)** Effects of noise exposure on escape latency in the training phase; **(B)** Effects of noise exposure on target quadrant distance/total distance in the probe trial; **(C)** Effects of noise exposure on number of crossings over the target quadrant in the probe trial; **(D)** Effects of noise exposure on time spent in the target quadrant in the probe trial. $n = 6$; *$p < 0.05$ and **$p < 0.01$ represent the comparison between Control and Noise group; #$p < 0.05$ represent the comparison between Noise and Noise + LGG group.

gut microbiota in each treatment group (Supplementary Figure S1E). *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* were the most abundant phyla in all treatment groups. Compared to the Control group, the relative abundance of *Bacteroidetes* was increased while that of *Firmicutes* was decreased in the Noise group. Meanwhile, the abundance of *Firmicutes* was increased while that of *Bacteroidetes* and *Proteobacteria* was decreased after LGG intervention compared with the Noise group.

In order to further identify differences in the gut microbiota between the four treatment groups, changes in the composition of the gut microbiota were further assessed using the LEfSe test, and the dominant flora in each group were represented by cladograms (Figures 2A, B). The taxa with the greatest differences from phylum to genus were identified using LDA scoring (Figures 2C, D). The dominant species in the Control group belonged to the *Bacteroidaceae* and *Gammaproteobacteria*, while those in the Noise group belonged to the *Gammaproteobacteria*. The dominant species in the LGG group belonged to the *Muribaculaceae*, *Erysipelotrichaceae*, and *Burkholderiacea*, and the genera *Allobaculum* and *Parasutterella*, while those in the Noise + LGG group included *Lactobacillus gasseri* and species belonging to the *Ruminococcaceae* and *Alloprevotella*. Furthermore, at the class and family levels, LEfSe analysis showed that the abundance of *Gammaproteobacteria* in the Noise + LGG group was decreased compared with that in the Noise group. While the species abundances of *Lactobacillus gasseri*, which belongs to *Lactobacillaceae* and *Ruminococcaceae*, increased (Supplementary Figures S2A–C).

To clearly demonstrate the effect of LGG intervention on the regulation of intestinal microbiota after noise exposure, we used T-test to analyze the differences in microbiota between Noise and Noise + LGG groups at the genus level and the species level, respectively. Compared with the Noise group, the relative abundances of *Alloprevotella*, *Lachnoclostridium*, and *Peptococcus* increased significantly in the Noise + LGG group at genus level after LGG treatment; whereas the abundances of *Bacteroides* and *Dubosiella* decreased significantly in the Noise + LGG group ($p < 0.05$; Figure 2E). Similarly, at species level, the relative abundances of *Clostridiales_bacterium_42_27* and *Oscillibacter_sp_1-3* increased more in the Noise + LGG group than Noise group, while the *Bacteroides sartorii* decreased ($p < 0.05$; Figure 2F). In addition, Tax4fun analysis was used to predict functional differences of gut microbiota between groups, and the results are supplemented in the Supplementary Figure S3.

10.3389/fcimb.2023.1067367

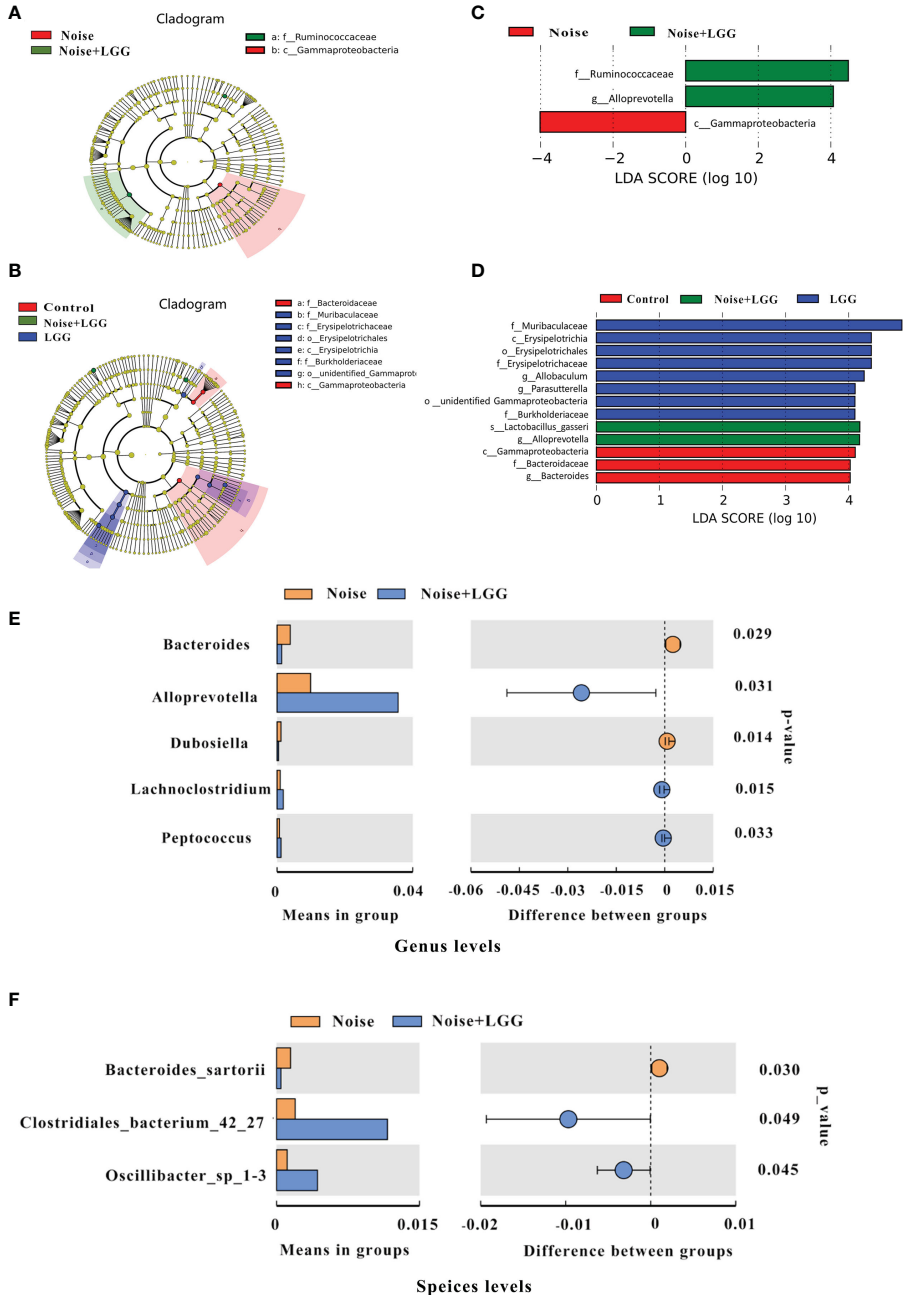


FIGURE 2
LGG regulates the composition of gut microbiota. **(A, B)** Cladogram based on linear discriminant analysis effect size (LEfSe) analysis. The central point represents the root of the tree (bacteria), and each ring represents the next lower taxonomic level (phylum to genus). The diameter of each circle represents the relative abundance of the taxon (n = 10–11). **(C, D)** The most differentially abundant taxa in each group identified by linear discriminant analysis (LDA) scores generated from the LEfSe analysis (n = 10–11). **(E, F)** Relative abundances, P-values, and confidence intervals of the gut microbial community at the genus and species level in the Noise group and Noise + LGG group (T-test analysis, $p < 0.05$, n = 10–11).

3.3 Changes of SCFA levels and metabolites in gut microbiota after LGG intervention

SCFA levels in the feces of rats in each treatment group are shown in (Figures 3A–D). Levels of propionic acid, butyric acid, isobutyric acid, and isovaleric acid were reduced in the Noise group compared to those in the Control group, whereas levels of these SCFAs were increased after LGG intervention. These results indicated that LGG intervention inhibited the noise-induced decrease in SCFAs levels in rats.

Microbial metabolites are one of the main communication channels for crosstalk between bacteria and hosts. As the main product of bacterial fermentation, changes in SCFAs content alter the acidic environment in the gut, affect the normal growth of gut microbes, and influence the composition and structure of the gut microbiota. Therefore, correlation analysis was conducted to evaluate the associations between the SCFA metabolites and genus level of gut microbiota. The correlation network

10.3389/fcimb.2023.1067367

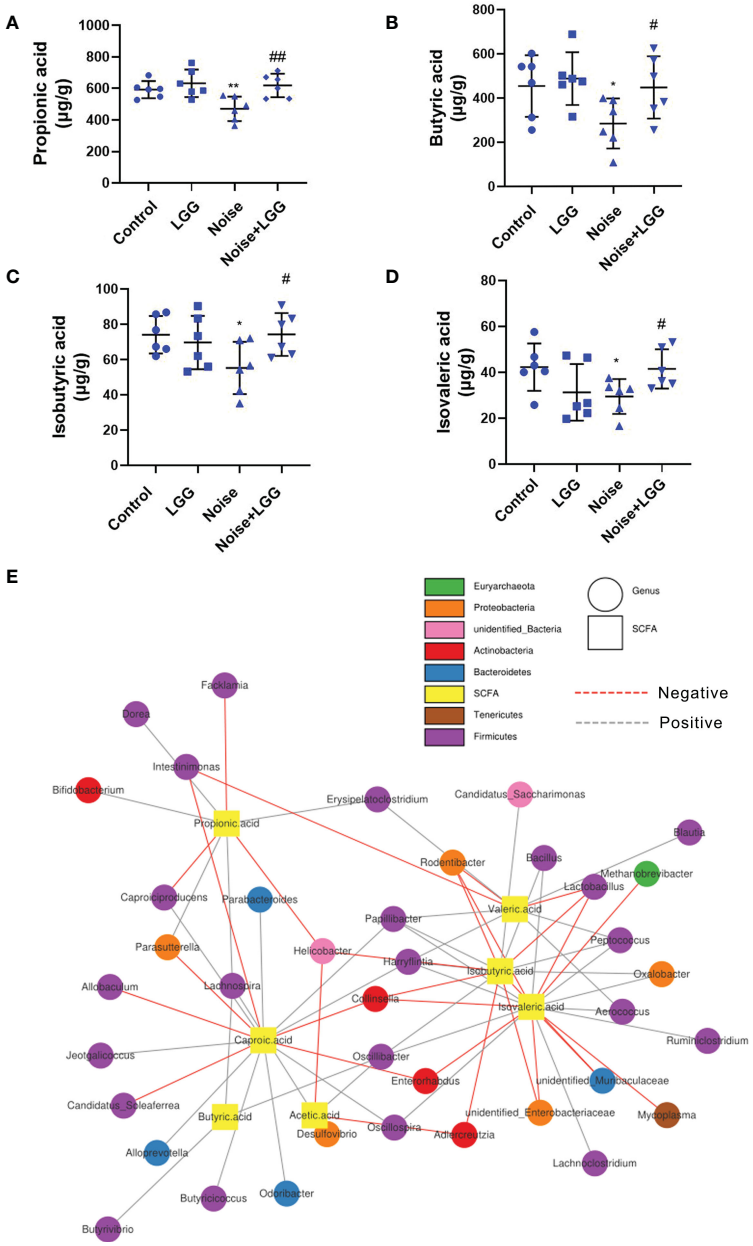


FIGURE 3
Analysis of the level of SCFAs and its correlation with gut microbiota. LGG intervention reversed the reduction in SCFAs levels caused by noise exposure **(A–D)**. **(A)** Propionic, **(B)** Butyric, **(C)** Isobutyric, and **(D)** Isovaleric acids. $n = 6$; *$p < 0.05$ and **$p < 0.01$ represent the comparison between Control and Noise group; #$p < 0.05$ and ##$p < 0.01$ represent the comparison between Noise and Noise + LGG group. **(E)** Network Diagram was used to show the associations between the SCFAs metabolites and genus levels of gut microbiota.

diagram provided the visualization of the relationship (Figure 3E). For example, butyric acid was positively correlated with *Butyrivibrio* (r = 0.393, p = 0.011); propionic acid was positively correlated with *Bifidobacterium* (r = 0.333, p = 0.033), *Lachnospira* (r = 0.306, p = 0.051), and *Dorea* (r = 0.331, p = 0.035); isobutyric acid was positively correlated with *Oscillibacter* (r = 0.331, p = 0.034), *Peptococcus* (r = 0.439, p = 0.004), and *Bacillus* (r = 0.312, p = 0.047), while negatively correlated with *Unidentified_Enterobacteriaceae* (r = -0.368, p = 0.018); isovaleric acid had positive correlations with *Oscillibacter* (r = 0.366, p = 0.019), while being significantly negatively correlated with *Enterorhabdus* (r = -0.321, p = 0.041).

3.4 Changes in β-amyloid peptides and serum inflammatory cytokines after LGG intervention

Serum levels of Aβ1-40 and Aβ1-42 were higher after noise exposure than those in the Control group (Figures 4A, B). However, serum levels of Aβ1-40 and Aβ1-42 in the Noise + LGG group were notably lower ($p < 0.05$) than those in the Noise group. These results indicated that LGG intervention can regulate abnormal increases in Aβ serum levels. Serum inflammatory cytokine and mediator levels in each treatment group are shown in Figures 4C–E. IL-17 and

10.3389/fcimb.2023.1067367

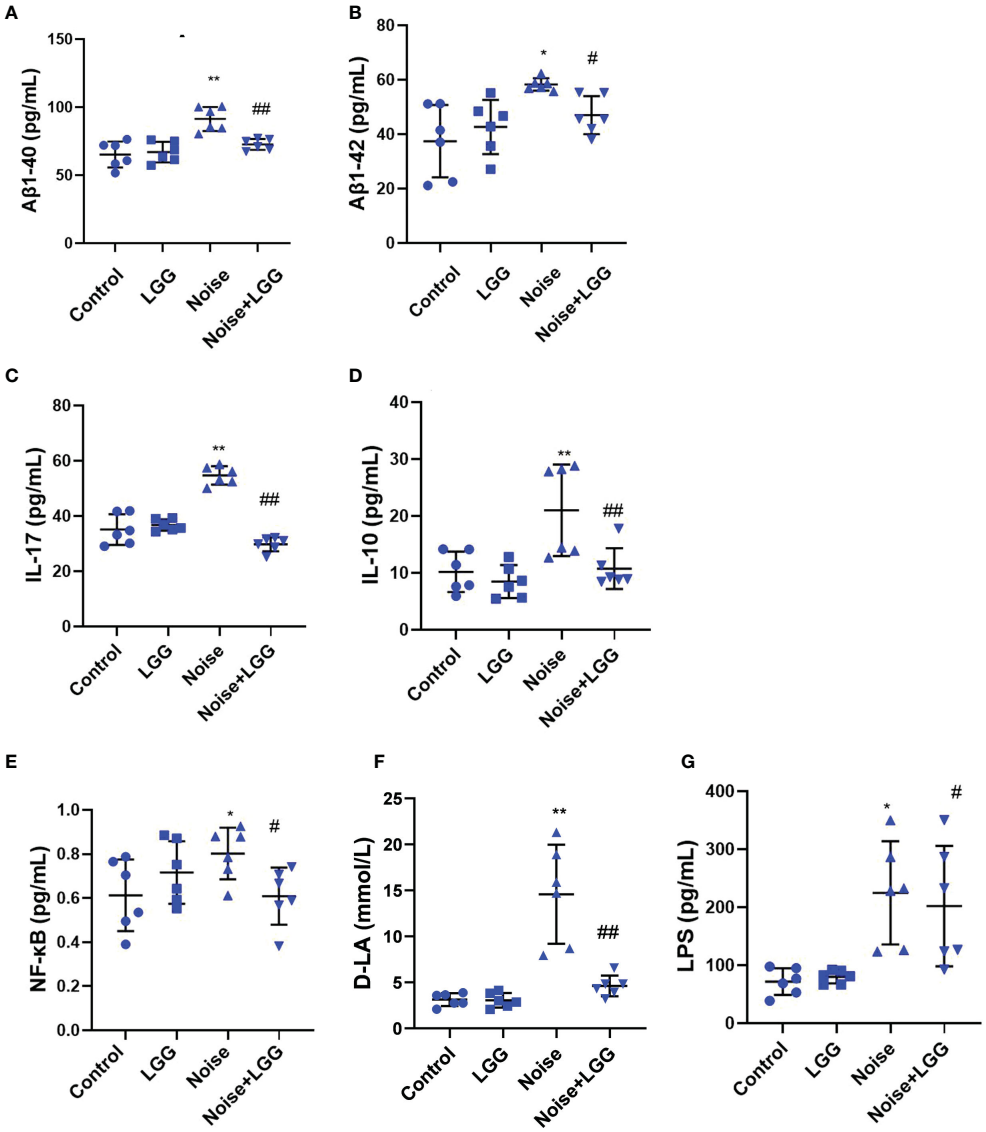


FIGURE 4
LGG intervention reduces serum inflammatory cytokines and inflammatory markers. **(A, B)** Changes in serum Aβ1-40 and Aβ1-42 in each group. **(C–E)** Changes in serum IL-17, IL-10, and NF-κB in each group. **(F)** Changes in serum D-LA in each group. **(G)** Changes in serum LPS in each group. $n = 6$. *$p < 0.05$ and **$p < 0.01$ represent the comparison between Control and Noise group; #$p < 0.05$ and ##$p < 0.01$ represent the comparison between Noise and Noise + LGG group.

IL-10 are important inflammatory cytokines, and NF-κB is a main regulator of natural immunity and inflammation that can be activated by inflammatory cytokines. Serum levels of IL-17, NF-κB, and IL-10 were higher in the Noise group than in the Control group, but lower in the Noise + LGG group than in the Noise group. Serum levels of inflammation markers D-LA and LPS, which are related to gut mucosal damage, were remarkably higher in the Noise group than those in the Control group (Figures 4F, G), suggesting injury to the gut mucosa. However, serum levels of D-LA and LPS were significantly reduced after noise and LGG intervention (Figures 4F, G). These results showed that LGG intervention may inhibit the release of inflammatory cytokines into the blood and reverse the inflammation caused by noise exposure in rats, thus playing a protective role.

3.5 Effect of LGG intervention on epithelial barrier function after noise exposure

Histopathological analysis (Figure 5A) of colon tissues from each treatment group revealed complete mucosal structure, orderly arrangement of epithelial cells, and tightly arranged lamina propria glands in the Control and LGG groups. In the Noise group, the mucosal structure of the colon tissue was incomplete, the epithelium was exfoliated, and the lamina propria glands were short and arranged loosely and irregularly. The mucosal structure of the colon tissue in the Noise + LGG group was incomplete and the lamina propria glands were arranged irregularly, but the pathological changes were reduced compared to those in rats exposed to noise alone. The electron microscopy results of colon tissues in each group

10.3389/fcimb.2023.1067367

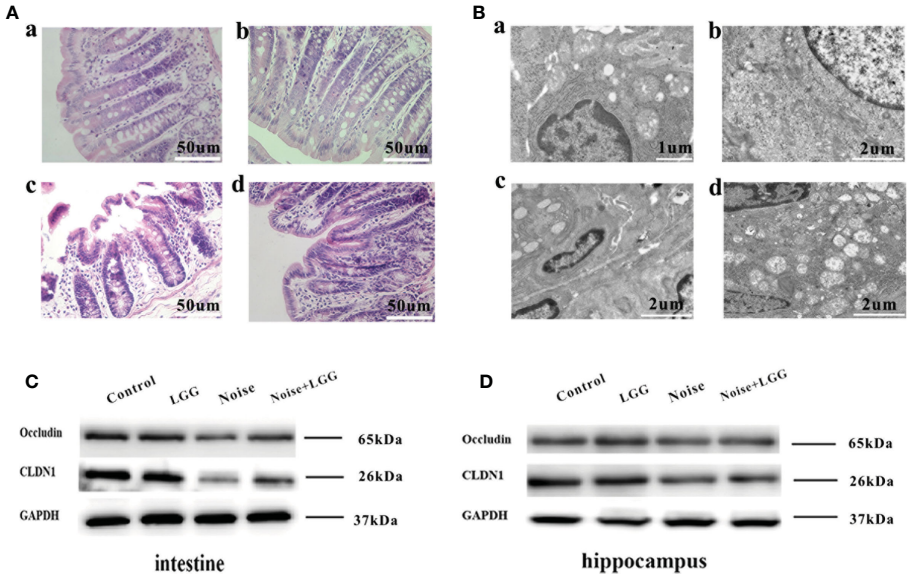


FIGURE 5
Protective effect of LGG intervention on gut and blood-brain barrier in rats. **(A)** Histopathological changes in HE-stained rat colon sections (magnification ×40). (a) Control, (b) LGG, (c) Noise, and (d) Noise + LGG groups. **(B)** The ultrastructural colon changes were observed with transmission electron microscopy (magnification: a: × 7000, b, c, d: × 5000). (a) Control, (b) LGG, (c) Noise, and (d) Noise + LGG groups. **(C)** The protein levels of occludin and CLDN1 in the colon tissues of rats. **(D)** The protein levels of Occludin and CLDN1 in the hippocampal tissues of rats. GADPH served as an internal reference standard for normalization.

(Figure 5B) showed that the colon epithelial cells in the Control group and the LGG group were normal, with more mitochondria and clear structure. In the Noise group, the junctions of epithelial cells were significantly widened and there were fewer mitochondria, with some vacuolated. However, colonic epithelial cells in Noise + LGG group were relatively normal, with clear mitochondrial structure and some vacuolated.

Analysis of tight junction proteins in the colon (Figure 5C) and hippocampus (Figure 5D) of each group revealed that the expression levels of Occludin and CLDN1 were decreased in the colon and hippocampus of the Noise group compared with the control group (Figures 5C, D), while they were increased in Noise + LGG group when compared with the Noise group. These results suggested that noise exposure led to impaired gut and blood-brain barrier functions in rats, which was ameliorated with LGG intervention.

4 Discussion

Our previous studies showed that noise exposure alters the gut microbiota (Cui et al., 2016), inducing oxidative inflammation and AD-like neuropathy (Chi et al., 2021). The results of the current study confirmed that chronic low-intensity noise exposure could induce numerous microbiome-gut-brain axis events. Moreover, the results indicated that LGG intervention could ameliorate noise-induced gut microbiota disturbance, gut and blood-brain barrier dysfunction, cognitive impairment, and systemic inflammation, which may provide new insights into treating the neurological effects of environmental noise exposure (Figure 6).

In our previous studies (Cui et al., 2018; Confer et al., 2021) long-term high-intensity noise exposure negatively impacted spatial learning and memory and caused cognitive impairment in rats. Gut microbiota dysbiosis, increased systemic inflammation, and reduced integrity of gut and blood-brain barriers may also be factors in noise-induced impairment of cognitive function *via* the microbiome-gut-brain axis. The results of the current research support that low-intensity noise exposure leads to cognitive decline and concur with two recent models of low-intensity noise exposure (Sun G. et al., 2021; Zhang Y. et al., 2021). Our results indicated that after LGG intervention, the behavioral performance of rats improved, which was consistent with the normal control group, suggesting that cognitive deficits were improved by LGG intervention. Therefore, we hypothesize that the microbiome-gut-brain axis may play a key role in the amelioration of cognitive impairment caused by noise exposure.

Additionally, the results of the current study indicate that noise exposure leads to abnormal changes in the gut microbiota and disrupts normal metabolism of SCFAs, which in turn damages the intestinal barrier, creating a vicious cycle (Lee et al., 2020). LGG intervention can ameliorate gut microbiota imbalance and SCFAs metabolism abnormalities caused by noise. *Gammaproteobacteria* are related to increased intestinal permeability, inflammatory cell proliferation, and secretion of inflammatory mediators, leading to activation of the immune inflammatory response (Shin et al., 2015; Panasevich et al., 2018). In addition to providing energy to the host (Huang et al., 2015), *Ruminococcaceae* are known to have beneficial effects on intestinal barrier function (Leclercq et al., 2014). Further, *Lactobacillus-gasseri*, a symbiotic lactic acid bacterium, can inhibit the NF-κB signaling pathway and increase intestinal barrier

10.3389/fcimb.2023.1067367

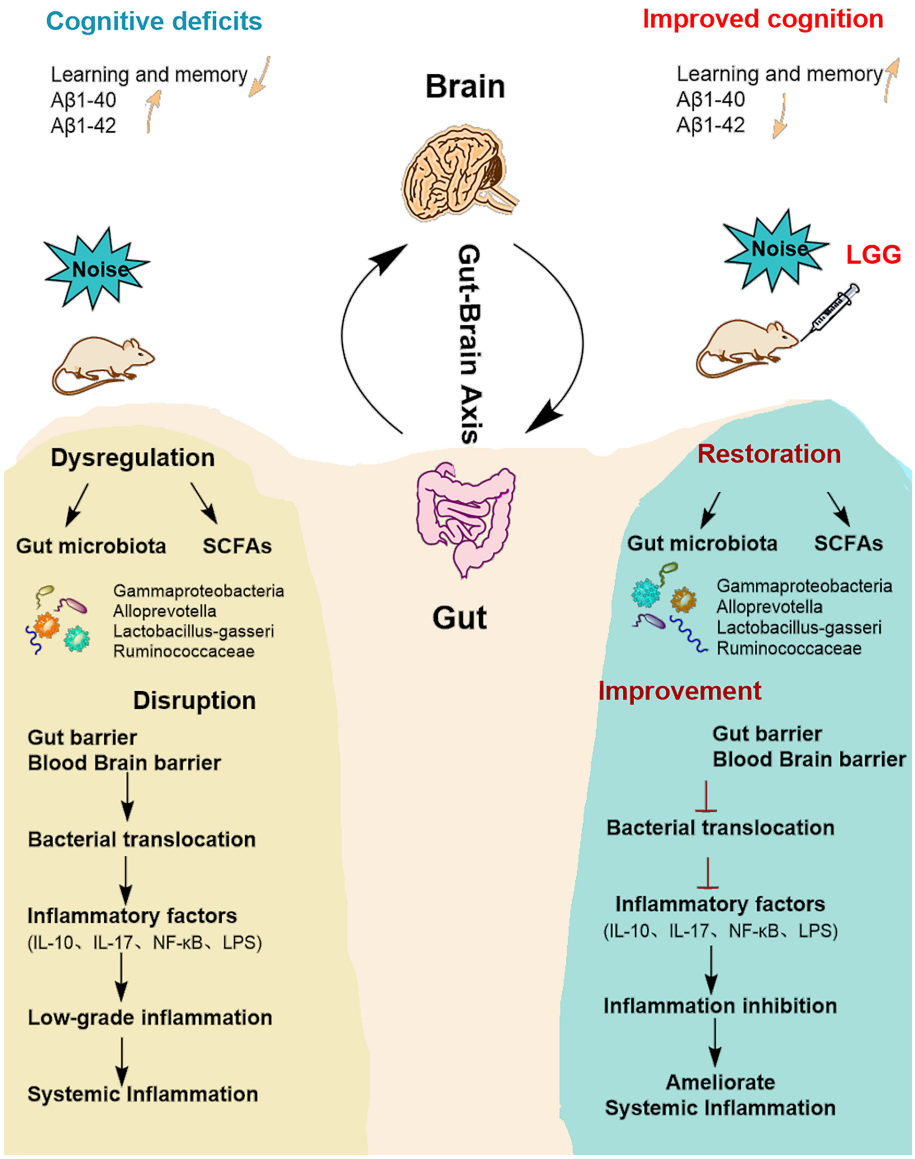


FIGURE 6
Summarizing central scheme: LGG intervention ameliorates systemic inflammation and cognitive impairment. LGG intervention significantly increased the abundance of beneficial bacteria (*Ruminococcaceae*, *Lactobacillus-gasseri*, and *Alloprevotella*) and decreased harmful strains (*Gammaproteobacteria*). LGG colonization increased the abundance of SCFA-producing bacteria (*Ruminococcaceae*, *Alloprevotella*) and the content of SCFAs. In addition, LGG intervention also improved cognitive function and reduced systemic inflammation through the microbial-gut-brain axis by repairing gut-blood-brain barrier damage, thus avoiding bacterial translocation, reducing inflammatory cytokine levels, and improving learning and memory abilities in rats. LGG, *Lactobacillus rhamnosus* GG; SCFAs, short-chain fatty acids; IL-10, interleukin 10; IL-17, interleukin 17; LPS, lipopolysaccharide.

integrity (Shiraishi et al., 2021). *Lactobacillus-gasseri* can also regulate gut bacterial dysbiosis, alleviate colonic inflammation, and improve cognitive dysfunction in mice (Yun et al., 2020). Moreover, the relative abundance of *Alloprevotella* has been negatively associated with inflammation (Wang et al., 2020) and may also enhance antioxidant capacity (Wang et al., 2021). In this study, LGG intervention increased the abundance of *Alloprevotella* and probiotic bacteria belonging to the *Firmicutes*, such as *Ruminococcaceae* and *Lactobacillus-gasseri*, which are known to produce SCFAs (Kong et al., 2019; Xu et al., 2021).

We found that LGG intervention could improve *Oscillibacter_SP_1-3* and *Clostridiales_bacterium_42_27*, which belong to *Ruminococcaceae* in the Noise group. The elevation of blood LPS concentration had a strong association with *Bacteroides* (Ding et al., 2020). After LGG intervention, the abundance of *Bacteroides* and *Bacteroides-sartorii* decreased in the Noise + LGG group, which is also consistent with reduced levels of LPS in the serum. The beneficial bacteria *Lachnoclostridium* can inhibit the growth of pathogenic bacteria and regulate intestinal homeostasis (Zhang et al., 2020) and *Peptococcus* can promote butyric acid production. Our results also showed that isobutyric

acid was positively correlated with *Peptococcus* (Zhang et al., 2022). In addition, the abundance of species belonging to the *Enterobacteriaceae* (*unidentified_Enterobacteriaceae*) in the *Gammaproteobacteria* was negatively correlated with isobutyric acid. LGG decreased the abundance of harmful *Gammaproteobacteria* caused by noise, and the increase of these harmful bacteria led to the higher abundance of gut microbiota in the noise group. These results show that LGG can mitigate the negative effects of noise by increasing the abundance of *Firmicutes* and reversing the *Firmicutes*/*Bacteroidetes* ratio, which is considered an important indicator of gut microbiota health (Li and Ma, 2020). These variations were followed by beneficial changes in gut microbiota diversity and improved SCFAs levels. The study findings demonstrate that LGG intervention can reshape the gut microbiota structure, increase the SCFAs content, and maintain the normal intestinal microenvironment by enriching beneficial bacteria and inhibiting pathogenic bacteria.

The results of the present research indicate that noise exposure increases gut and blood-brain barrier permeability and systemic inflammatory responses in rats, which is consistent with our previous research (Chi et al., 2021). D-LA is used as a serum marker of intestinal permeability (Zhang H. et al., 2021) and LPS can be used as an indicator of gut microbiota translocation (Luchetti et al., 2021). Gut mucosal inflammation is positively correlated with D-LA and LPS levels, which are also associated with increased gut mucosal permeability (Vitetta et al., 2017). Consistently, noise exposure significantly increased serum levels of LPS and D-LA, pro-inflammatory cytokine IL-17, and inflammatory mediators NF-κB, Aβ1-40, and Aβ1-42. Interestingly, noise exposure led to elevated levels of anti-inflammatory cytokine IL-10. NF-κB can drive the differentiation of monocytes into macrophages, with the M1 type producing pro-inflammatory cytokines and the M2 type producing anti-inflammatory cytokines, such as IL-10 (Sun S. et al., 2021). We hypothesize that gut microbiota dysbiosis after noise exposure and excessive release of LPS activates NF-κB, which in turn drives the differentiation of monocytes into macrophages, with the M1 phenotype leading to a massive release of IL-17, thus causing an inflammatory response. Subsequently, the M2 phenotype produces IL-10 to resist the body's exposure to inflammation.

LGG can act on the gut epithelium to form a barrier and block the invasion of harmful bacteria in the intestine (Rao and Samak, 2013), regulate the NF-κB signaling pathway, and exert certain anti-inflammatory effects (Hou et al., 2019). Therefore, serum levels of D-LA, LPS, and NF-κB decreased after LGG intervention. The reduced levels of IL-17 and IL-10 may be due to less LPS being released after improvement of intestinal damage, thus downregulating NF-κB expression, inhibiting macrophage differentiation, and balancing inflammatory and anti-inflammatory cytokines. In addition, LGG intervention reduced serum levels of Aβ, preventing abnormal entry of Aβ into the brain through the damaged blood-brain barrier, which can damage neurons, exacerbate neuroinflammation, and lead to cognitive dysfunction (Deane, 2012; Long et al., 2019). These results further suggest that LGG regulates gut microbiota homeostasis and SCFAs levels, inhibits the release of inflammatory cytokines, and protects the intestinal barrier. SCFA can not only enhance the intestinal barrier and local anti-inflammatory effect (Luo et al., 2020), but also directly cross the blood-brain barrier and enter the brain tissue (Den et al., 2020). Intake of SCFA can reduce hippocampal neuroinflammation and neuronal apoptosis by inhibiting NF-κB, and improve cognitive performance (Xiao et al., 2022). Therefore, LGG intervention may prevent local inflammation in the gut, avoiding systemic inflammation induced *via* the gut-brain axis pathway that could impair neurological function and increased SCFA may be involved in the improvement of cognitive function.

This study had two major limitations. First, based on animal ethics and principles of the experiment, partial experiments randomly selected only six samples per group for statistical analysis. Second, due to the limited samples, western blotting results were only assessed to observe trends in expression levels, without statistical analyses being performed. Therefore, it is necessary to increase the sample size and adopt a variety of detection methods in the future experimental design and detection.

5 Conclusions

Taken together, the study findings indicate that noise exposure disturbs intestinal microbiota homeostasis, SCFAs metabolism, and upregulates systemic low-grade inflammation, which may be the cause of intestinal and brain epithelial barrier deficiencies. LGG intervention can ameliorate cognitive deficits and systemic inflammation in rats exposed to noise, possibly through linked changes in the microbiome-gut-brain axis. This research advances the current knowledge regarding the etiological signaling pathways participating in negative non-auditory effects of environmental noise.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI SRA, PRJNA890543.

Ethics statement

The animal study was reviewed and approved by Institutional Animal Use and Care Committee of the Tianjin Institute of Environmental and Occupational Medicine.

Author contributions

Conceptualization, BC, SJ and FW. XL, PZ and WC shared first authors. Methodology, XL, PZ, WC, YC and XS, software, WC, YC. Validation, SJ and FSW. Formal analysis, HY. Investigation, BC. Resources, KM and FW. Data curation, JY and YF. Writing original draft preparation, WC. Writing review and editing, XL and PZ.

Visualization, PZ and XL. Supervision, XG. Project administration, XS. Funding acquisition, BC. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by National Natural Science Foundation of China (No. 81673136) and Scientific Research Projects (No. JK20202A020453, 20WQ042) awarded to BC.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2023.1067367/full#supplementary-material

References

Al Bander, Z., Nitert, M. D., Mousa, A., and Naderpoor, N. (2020). The gut microbiota and inflammation: an overview. *Int. J. Environ. Res. Public Health* 17, (20). doi: 10.3390/ijerph17207618

Athari Nik Azm, S., Djazayeri, A., Safa, M., Azami, K., Ahmadvand, B., Sabbaghziarani, F., et al. (2018). Lactobacilli and bifidobacteria ameliorate memory and learning deficits and oxidative stress in β-amyloid (1-42) injected rats. *Appl. Physiol. Nutr. Metab.* 43 (7), 718–726. doi: 10.1139/apnm-2017-0648

Chen, L., Li, H., Li, J., Chen, Y., and Yang, Y. (2019). Lactobacillus rhamnosus GG treatment improves intestinal permeability and modulates microbiota dysbiosis in an experimental model of sepsis. *Int. J. Mol. Med.* 43 (3), 1139–1148. doi: 10.3892/ijmm.2019.4050

Chi, H., Cao, W., Zhang, M., Su, D., Yang, H., Li, Z., et al. (2021). Environmental noise stress disturbs commensal microbiota homeostasis and induces oxi-inflammmation and AD-like neuropathology through epithelial barrier disruption in the EOAD mouse model. *J. Neuroinflamm.* 18 (1), 9. doi: 10.1186/s12974-020-02053-3

Confer, M. P., Holcombe, B. M., Foes, A. G., Holmquist, J. M., Walker, S. C., Deb, S., et al. (2021). Label-free infrared spectroscopic imaging reveals heterogeneity of β-sheet aggregates in alzheimer's disease. *J. Phys. Chem. Lett.* 12 (39), 9662–9671. doi: 10.1021/acs.jpclett.1c02306

Cui, B., Gai, Z., She, X., Wang, R., and Xi, Z. (2016). Effects of chronic noise on glucose metabolism and gut microbiota-host inflammatory homeostasis in rats. *Sci. Rep.* 6, 36693. doi: 10.1038/srep36693

Cui, B., Su, D., Li, W., She, X., Zhang, M., Wang, R., et al. (2018). Effects of chronic noise exposure on the microbiome-gut-brain axis in senescence-accelerated prone mice: implications for alzheimer's disease. *J. Neuroinflamm.* 15 (1), 190. doi: 10.1186/s12974-018-1223-4

Deane, R. J. (2012). Is RAGE still a therapeutic target for alzheimer's disease? *Future Med. Chem.* 4 (7), 915–925. doi: 10.4155/fmc.12.51

Den, H., Dong, X., Chen, M., and Zou, Z. (2020). Efficacy of probiotics on cognition, and biomarkers of inflammation and oxidative stress in adults with alzheimer's disease or mild cognitive impairment - a meta-analysis of randomized controlled trials. *Aging (Albany NY)* 12 (4), 4010–4039. doi: 10.18632/aging.102810

Ding, N., Zhang, X., Zhang, X. D., Jing, J., Liu, S. S., Mu, Y. P., et al. (2020). Impairment of spermatogenesis and sperm motility by the high-fat diet-induced dysbiosis of gut microbes. *Gut* 69 (9), 1608–1619. doi: 10.1136/gutjnl-2019-319127

Donato, K. A., Gareau, M. G., Wang, Y. J. J., and Sherman, P. M. (2010). Lactobacillus rhamnosus GG attenuates interferon-{gamma} and tumour necrosis factor-alpha-induced barrier dysfunction and pro-inflammatory signalling. *Microbiol. (Reading). 156:(Pt* 11), 3288–3297. doi: 10.1099/mic.0.040139-0

Fan, H., Chen, Z., Lin, R., Liu, Y., Wu, X., Puthiyakunnon, S., et al. (2019). Bacteroides fragilis strain ZY-312 defense against cronobacter sakazakii-induced necrotizing enterocolitis *In vitro* and in a neonatal rat model. *mSystems.* 4 (4), e00305-19. doi: 10.1128/mSystems.00305-19

Han, X., Lee, A., Huang, S., Gao, J., Spence, J. R., and Owyang, C. (2019). Lactobacillus rhamnosus GG prevents epithelial barrier dysfunction induced by interferon-gamma and fecal supernatants from irritable bowel syndrome patients in human intestinal enteroids and colonoids. *Gut Microbes* 10 (1), 59–76. doi: 10.1080/19490976.2018.1479625

Hill-Burns, E. M., Debelius, J. W., Morton, J. T., Wissemann, W. T., Lewis, M. R., Wallen, Z. D., et al. (2017). Parkinson's disease and parkinson's disease medications have distinct signatures of the gut microbiome. *Mov Disord.* 32 (5), 739–749. doi: 10.1002/mds.26942

Hou, Y., Li, X., Liu, X., Zhang, Y., Zhang, W., Man, C., et al. (2019). Transcriptomic responses of caco-2 cells to lactobacillus rhamnosus GG and lactobacillus plantarum J26 against oxidative stress. *J. Dairy Sci.* 102 (9), 7684–7696. doi: 10.3168/jds.2019-16332

Huang, C., Song, P., Fan, P., Hou, C., Thacker, P., and Ma, X. (2015). Dietary sodium butyrate decreases postweaning diarrhea by modulating intestinal permeability and changing the bacterial communities in weaned piglets. *J. Nutr.* 145 (12), 2774–2780. doi: 10.3945/jn.115.217406

Kong, C., Gao, R., Yan, X., Huang, L., and Qin, H. (2019). Probiotics improve gut microbiota dysbiosis in obese mice fed a high-fat or high-sucrose diet. *Nutrition.* 60, 175–184. doi: 10.1016/j.nut.2018.10.002

Leclercq, S., Matamoros, S., Cani, P. D., Neyrinck, A. M., Jamar, F., Starkel, P., et al. (2014). Intestinal permeability, gut-bacterial dysbiosis, and behavioral markers of alcohol-dependence severity. *Proc. Natl. Acad. Sci. U.S.A.* 111 (42), E4485–E4493. doi: 10.1073/pnas.1415174111

Lee, J., Venna, V. R., Durgan, D. J., Shi, H., Hudobenko, J., Putluri, N., et al. (2020). Young versus aged microbiota transplants to germ-free mice: increased short-chain fatty acids and improved cognitive performance. *Gut Microbes* 12 (1), 1–14. doi: 10.1080/19490976.2020.1814107

Li, W., and Ma, Z. S. (2020). FBA ecological guild: trio of firmicutes-bacteroidetes alliance against actinobacteria in human oral microbiome. *Sci. Rep.* 10 (1), 287. doi: 10.1038/s41598-019-56561-1

Liu, T., Song, X., An, Y., Wu, X., Zhang, W., Li, J., et al. (2021). Lactobacillus rhamnosus GG colonization in early life ameliorates inflammaging of offspring by activating SIRT1/AMPK/PGC-1α pathway. *Oxid. Med. Cell Longev.* 2021, 3328505. doi: 10.1155/2021/3328505

Long, H., Zhong, G., Wang, C., Zhang, J., Zhang, Y., Luo, J., et al. (2019). TREM2 attenuates Aβ1-42-Mediated neuroinflammation in BV-2 cells by downregulating TLR signaling. *Neurochem. Res.* 44 (8), 1830–1839. doi: 10.1007/s11064-019-02817-1

Luchetti, M. M., Ciccia, F., Avellini, C., Benfaremo, D., Rizzo, A., Spadoni, T., et al. (2021). Gut epithelial impairment, microbial translocation and immune system activation in inflammatory bowel disease-associated spondyloarthritis. *Rheumatol. (Oxford).* 60 (1), 92–102. doi: 10.1093/rheumatology/keaa164

Luo, D., Chen, K., Li, J., Fang, Z., Pang, H., Yin, Y., et al. (2020). Gut microbiota combined with metabolomics reveals the metabolic profile of the normal aging process and the anti-aging effect of FuFang zhenshu TiaoZhi(FTZ) in mice. *BioMed. Pharmacother.* 121, 109550. doi: 10.1016/j.biopha.2019.109550

Panasevich, M. R., Meers, G. M., Linden, M. A., Booth, F. W., Perfield, J. W.2nd, Fritsche, K. L., et al. (2018). High-fat, high-fructose, high-cholesterol feeding causes severe NASH and cecal microbiota dysbiosis in juvenile ossabaw swine. *Am. J. Physiol. Endocrinol. Metab.* 314 (1), E78-e92. doi: 10.1152/ajpendo.00015.2017

Pulikkan, J., Maji, A., Dhakan, D. B., Saxena, R., Mohan, B., Anto, M. M., et al. (2018). Gut microbial dysbiosis in Indian children with autism spectrum disorders. *Microb. Ecol.* 76 (4), 1102–1114. doi: 10.1007/s00248-018-1176-2

Rao, R. K., and Samak, G. (2013). Protection and restitution of gut barrier by probiotics: nutritional and clinical implications. *Curr. Nutr. Food Sci.* 9 (2), 99–107. doi: 10.2174/1573401311309020004

Ritze, Y., Bardos, G., Claus, A., Ehrmann, V., Bergheim, I., Schwiertz, A., et al. (2014). Lactobacillus rhamnosus GG protects against non-alcoholic fatty liver disease in mice. *PloS One* 9 (1), e80169. doi: 10.1371/journal.pone.0080169

Sanborn, V., Azcarate-Peril, M. A., Updegraff, J., Manderino, L., and Gunstad, J. (2020). Randomized clinical trial examining the impact of lactobacillus rhamnosus GG probiotic supplementation on cognitive functioning in middle-aged and older adults. *Neuropsychiatr. Dis. Treat.* 16, 2765–2777. doi: 10.2147/ndt.S270035

Sender, R., Fuchs, S., and Milo, R. (2016). Revised estimates for the number of human and bacteria cells in the body. *PloS Biol.* 14 (8), e1002533. doi: 10.1371/journal.pbio.1002533

Shin, N. R., Whon, T. W., and Bae, J. W. (2015). Proteobacteria: microbial signature of dysbiosis in gut microbiota. *Trends Biotechnol.* 33 (9), 496–503. doi: 10.1016/j.tibtech.2015.06.011

Shiraishi, T., Maeno, S., Kishi, S., Fujii, T., Tanno, H., Hirano, K., et al. (2021). Oligosaccharide metabolism and lipoteichoic acid production in lactobacillus gasseri and lactobacillus paragasseri. *Microorganisms.* 9, (8). doi: 10.3390/microorganisms9081590

Shukla, P. K., Delotterie, D. F., Xiao, J., Pierre, J. F., Rao, R., McDonald, M. P., et al. (2021). Alterations in the gut-Microbial-Inflammasome-Brain axis in a mouse model of alzheimer's disease. *Cells.* 10, (4). doi: 10.3390/cells10040779

Singhrao, S. K., and Harding, A. (2020). Is alzheimer's disease a polymicrobial host microbiome dysbiosis? *Expert Rev. Anti Infect. Ther.* 18 (4), 275–277. doi: 10.1080/14787210.2020.1729741

Sun, G., Lin, X., Yi, X., Zhang, P., Liu, R., Fu, B., et al. (2021). Aircraft noise, like heat stress, causes cognitive impairments *Via* similar mechanisms in male mice. *Chemosphere.* 274, 129739. doi: 10.1016/j.chemosphere.2021.129739

Sun, S., Xu, X., Liang, L., Wang, X., Bai, X., Zhu, L., et al. (2021). Lactic acid-producing probiotic saccharomyces cerevisiae attenuates ulcerative colitis *Via* suppressing macrophage pyroptosis and modulating gut microbiota. *Front. Immunol.* 12. doi: 10.3389/fimmu.2021.777665

Vitetta, L., Coulson, S., Thomsen, M., Nguyen, T., and Hall, S. (2017). Probiotics, d-lactic acidosis, oxidative stress and strain specificity. *Gut Microbes* 8 (4), 311–322. doi: 10.1080/19490976.2017.1279379

Wang, J., Wang, P., Li, D., Hu, X., and Chen, F. (2020). Beneficial effects of ginger on prevention of obesity through modulation of gut microbiota in mice. *Eur. J. Nutr.* 59 (2), 699–718. doi: 10.1007/s00394-019-01938-1

Wang, M., Zhang, S., Zhong, R., Wan, F., Chen, L., Liu, L., et al. (2021). Olive fruit extracts supplement improve antioxidant capacity *Via* altering colonic microbiota composition in mice. *Front. Nutr.* 8. doi: 10.3389/fnut.2021.645099

Xiao, W., Su, J., Gao, X., Yang, H., Weng, R., Ni, W., et al. (2022). The microbiota-gut-brain axis participates in chronic cerebral hypoperfusion by disrupting the metabolism of short-chain fatty acids. *Microbiome.* 10 (1), 62. doi: 10.1186/s40168-022-01255-6

Xu, H. M., Huang, H. L., Liu, Y. D., Zhu, J. Q., Zhou, Y. L., Chen, H. T., et al. (2021). Selection strategy of dextran sulfate sodium-induced acute or chronic colitis mouse models based on gut microbial profile. *BMC Microbiol.* 21 (1), 279. doi: 10.1186/s12866-021-02342-8

Yun, S. W., Kim, J. K., Lee, K. E., Oh, Y. J., Choi, H. J., Han, M. J., et al. (2020). A probiotic lactobacillus gasseri alleviates escherichia coli-induced cognitive impairment and depression in mice by regulating IL-1β expression and gut microbiota. *Nutrients.* 12, (11). doi: 10.3390/nu12113441

Zhang, H., Liu, Y., Fang, X., Gu, L., Luo, C., Chen, L., et al. (2021). Vitamin D(3) protects mice from diquat-induced oxidative stress through the NF-κB/Nrf2/HO-1 signaling pathway. *Oxid. Med. Cell Longev.* 2021, 6776956. doi: 10.1155/2021/6776956

Zhang, Q., Vasquez, R., Yoo, J. M., Kim, S. H., Kang, D. K., and Kim, I. H. (2022). Dietary supplementation of limosilactobacillus mucosae LM1 enhances immune functions and modulates gut microbiota without affecting the growth performance of growing pigs. *Front. Vet. Sci.* 9. doi: 10.3389/fvets.2022.918114

Zhang, Y., Zhu, M., Sun, Y., Tang, B., Zhang, G., An, P., et al. (2021). Environmental noise degrades hippocampus-related learning and memory. *Proc. Natl. Acad. Sci. U.S.A.* 118, (1). doi: 10.1073/pnas.2017841117

Zhang, W., Zou, G., Li, B., Du, X., Sun, Z., Sun, Y., et al. (2020). Fecal microbiota transplantation (FMT) alleviates experimental colitis in mice by gut microbiota regulation. *J. Microbiol. Biotechnol.* 30 (8), 1132–1141. doi: 10.4014/jmb.2002.02044

Zhao, Y., Tang, Y., Chen, L., Lv, S., Liu, S., Nie, P., et al. (2020). Restraining the TiO (2) nanoparticles-induced intestinal inflammation mediated by gut microbiota in juvenile rats *Via* ingestion of lactobacillus rhamnosus GG. *Ecotoxicol Environ. Saf.* 206, 111393. doi: 10.1016/j.ecoenv.2020.111393

Zhou, D., Pan, Q., Xin, F. Z., Zhang, R. N., He, C. X., Chen, G. Y., et al. (2017). Sodium butyrate attenuates high-fat diet-induced steatohepatitis in mice by improving gut microbiota and gastrointestinal barrier. *World J. Gastroenterol.* 23 (1), 60–75. doi: 10.3748/wjg.v23.i1.60

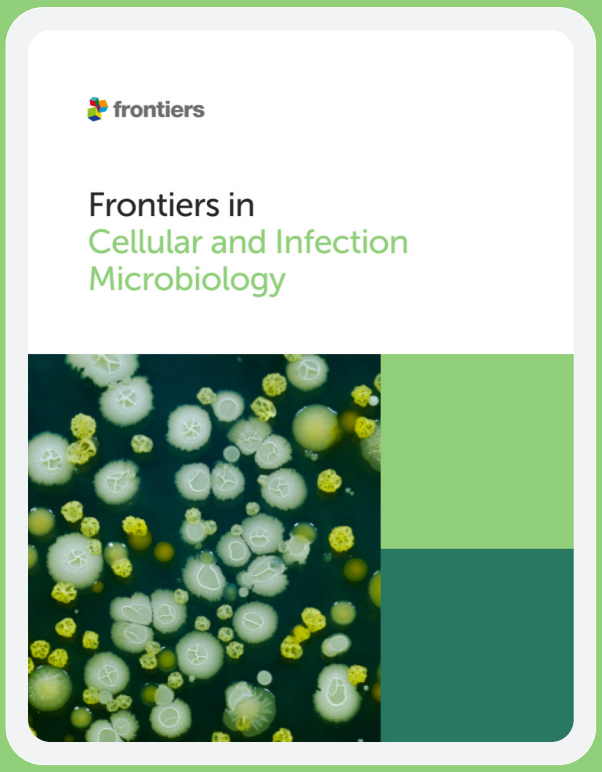
Frontiers in
Cellular and Infection Microbiology

Investigates how microorganisms interact with their hosts

Explores bacteria, fungi, parasites, viruses, endosymbionts, prions and all microbial pathogens as well as the microbiota and its effect on health and disease in various hosts.

Discover the latest Research Topics

See more →



Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact