Heliyon 9 (2023) e18995

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Characteristics of gut microbiota in male periadolescent rats with irritable bowel syndrome

Wei Lin [a,1], Dongxiao Wu [b,1], Yongbin Zeng [c,d], Yuan Liu [e], Dajie Yu [a], Jianhang Wei [a], Yanliang Cai [a], Yueli Lin [a], Bin Wu [a,**], Huanhuan Huang [a,*]

[a] Department of Pediatrics, The First Affiliated Hospital, Fujian Medical University, Fuzhou, China
[b] Department of Pediatrics, The Second Affiliated Hospital, Fujian Medical University, Quanzhou, China
[c] Department of Laboratory Medicine, Gene Diagnosis Research Center, The First Affiliated Hospital, Fujian Medical University, Fuzhou, China
[d] Fujian Key Laboratory of Laboratory Medicine, The First Affiliated Hospital, Fujian Medical University, Fuzhou, China
[e] Cancer Research Center Nantong, Affiliated Tumor Hospital of Nantong University, China

ARTICLE INFO

Keywords:
Irritable bowel syndrome
Gut microbiota
16s rDNA genes
Periadolescent rats
Visceral hypersensitivity

ABSTRACT

Irritable bowel syndrome (IBS) is a prevalent functional gastrointestinal disorder, however, its effect on gut microbiota during the periadolescent period remains unclear. In this study, our objective was to investigate the characteristics of gut microbiota in male periadolescent rats with IBS induced by neonatal maternal separation (NMS). We evaluated visceral sensitivity by electromyography (EMG), analyzed gut microbiota composition using 16S rDNA gene sequencing, and examined intestinal pathological changes between control and IBS-like groups. The IBS-like group had significantly higher discharge amplitude of the external oblique muscle of the abdomen during colorectal distension (CRD) at 40- and 60 mmHg pressures. We observed differences in gut microbiota composition, with an increase in Bacteroidetes abundance and a decrease in Firmicutes in IBS-like rats. Beta-diversity analysis revealed the gut microbiota of the IBS-like group displayed higher consistent, while that of the control group exhibited substantial variation. Linear discriminant analysis effect size (LEfSe) detected 10 bacterial taxonomic clades showing statistically significant differences (7 increased and 3 decreased) in the IBS-like group. Functional analysis revealed that aminoacyl-tRNA biosynthesis and fatty acid biosynthesis were significantly altered, leading to changes in gene expression. Our findings demonstrate a definite correlation between gut microbiota dysbiosis and IBS during the male periadolescent period, with Alloprevotella and Bacteroide positively associated with high risk of IBS. The effects of specific bacterial genera may provide new insights for the development of treatments for IBS.

1. Introduction

Functional abdominal pain disorders (FAPDs) are one of the most common disorders in adolescence, with a prevalence up to 25% of

* Corresponding author. Department of Pediatrics, The First Affiliated Hospital, Fujian Medical University, 20 Chazhong Road, Fuzhou, 350005, Fujian, China.
** Corresponding author. Department of Pediatrics, The First Affiliated Hospital, Fujian Medical University, 20 Chazhong Road, Fuzhou, 350005, Fujian, China.
E-mail addresses: wbdnch@163.com (B. Wu), fyekxsek@163.com (H. Huang).
[1] These authors have contributed equally to this work and share first authorship.

https://doi.org/10.1016/j.heliyon.2023.e18995
Received 24 May 2023; Received in revised form 26 July 2023; Accepted 4 August 2023
Available online 6 August 2023

children worldwide [1]. Akin to adults, pediatric FAPDs are subdivided into several clinically distinct entities using the Rome IV criteria, the most representative of which is irritable bowel syndrome (IBS) [2]. It is characterized by chronic abdominal pain, altered bowel habits, and bloating, leading to considerable impairment in children' quality of life [3]. Despite its high prevalence and impact, the underlying mechanisms of IBS pathogenesis and effective treatment options remain elusive.

In recent years, growing evidence has suggested a critical role of the gut microbiota in the development and progression of IBS [4,5]. The gut microbiota, a complex community of microorganisms residing in the gastrointestinal tract, has emerged as a pivotal factor in gut health and overall well-being. Since the primitive society period, trillions of microorganisms have been parasitic in human intestinal tract and along with our evolution to this day [6]. Gut microbiota includes about 150 times more genes than the number of human genes, which means gut microbiota can provide functions that human otherwise does not have [7]. Human is linked inextricably to gut microbiota [8]. Alterations in the composition and functionality of the gut microbiota have been implicated in various gastrointestinal disorders, including IBS. These alterations can disrupt the delicate balance of microbial populations, leading to intestinal dysbiosis, increased gut permeability, aberrant immune responses, and visceral hypersensitivity [9].

Understanding the intricate relationship between intestinal microbiota and IBS is crucial for elucidating the mechanisms underlying the disorder and developing targeted therapeutic interventions. Previous studies have provided preliminary evidence linking gut microbiota dysbiosis to IBS, highlighting the potential role of specific bacterial taxa in disease pathogenesis [10,11]. However, many of these investigations have focused on adult populations, while the impact of gut microbiota on IBS during the periadolescent period remains poorly understood.

Thus, it is imperative to explore the characteristics of gut microbiota in male periadolescent rats with IBS induced by neonatal maternal separation (NMS). Our study aims to fill the existing research gap by conducting comprehensive assessments of visceral sensitivity, gut microbiota composition, and intestinal pathological changes. By uncovering the specific alterations in gut microbiota associated with IBS during the periadolescent period, this research can provide valuable insights into the underlying mechanisms and potentially identify novel therapeutic targets for the treatment of IBS.

2. Materials and methods

2.1. Animals

Two Sprague-Dawley (SD) pregnant rats were obtained from the Department of the Experimental Animal Center of Fujian Medical University. We chose 12 male offspring of the two SD pregnant rats to conduct the experiment. Subsequently, a random number table method was employed to randomly assign 6 individuals as the IBS-like group. IBS-like model in rats was established using neonatal maternal separation (NMS) as the previously described [12]. Briefly, IBS-like litters were taken away from the home cage and placed in a separate, clean cage once daily from 8 to 11 a.m. during postnatal days 2–21. Then, the litters were returned to their mothers. Besides, the IBS-like litters after weaning on the 21 days old, and they were individually housed. The control group received the same procedure except NMS. The rats were accommodated in a controlled environment at a stable temperature of 24 $\pm$ 2 °C, accompanied by a relative humidity ranging from 40% to 60%. The animals were housed in a specially designated animal facility at the Experimental Animal Center of Fujian Medical University, which adhered to high cleanliness standards. Furthermore, the rats were subjected to a 12-h light/dark cycle to maintain a consistent diurnal pattern. All experiments were approved by the Animal Care and Use Committee of Fujian Medical University.

2.2. Assessment of visceral hypersensitivity

Electromyography (EMG) was carried out to assess visceral hypersensitivity according to previous studies [13]. Briefly, male rats (Postnatal Day, PND 42) were anaesthetized with isoflurane. A balloon was inserted into the anus, and the attached tube was secured to the tail of the rat. A pair of silver bipolar electrodes was inserted into the external oblique abdominal muscle. The balloon pressure was increased to 40- and 60- mmHg and held at that pressure for 10 s with an interval of 4 min. The EMG responses to different degrees of CRD were recorded using the RM6240BD system (Chengdu, China). Data were analyzed by averaging the baseline amplitudes. Values above the baseline were used to assess visceral hypersensitivity.

2.3. Sampling

On PND 42, 12 rats were euthanized by cervical dislocation following nebulized isoflurane anesthesia. The abdominal cavity was opened with hemostatic forceps and surgical scissors to separate the colon and terminal ileum.

2.4. Pathological section preparation and hematoxylin-eosin staining

Colon specimens were fixed in 10% formalin for 48 h and then rinsed with saline before being embedded in paraffin. Tissue sections of 4–6 μm thickness were prepared and subjected with HE staining [14]. Criteria: Following hematoxylin-eosin staining, morphological changes of intestinal tissues were observed under light microscopy (Nikon, Japan). Villus height, villus width and crypt depth were measured by Image Pro Plus 6.0.

2.5. DNA extraction and PCR amplification

Fresh samples of ileal contents were aseptically collected using sterile EP tubes and immediately preserved at −80 °C until utilization. The extraction of total bacterial DNA was performed utilizing the QIAamp Fast DNA Stool Mini Kit (Qiagen) in accordance with the manufacturer's guidelines. Firstly, a 30 ng DNA sample and fusion primers were used to configure the PCR reaction system. Secondly, the PCR reaction parameters for PCR amplification were set as follows: 98 °C for 30 s, 98 °C for 10 s, 54 °C for 30 s, 72 °C for 45 s. A total of 35 cycles were performed with 3 replicates for each sample. Lastly, the PCR products were purified using Agencourt AMPure XP magnetic beads, dissolved, and labeled in the eluent to construct the DNA library, which was detected by the Agilent 2100 Bioanalyzer.

2.6. Bioinformatic and statistical analysis

Sequencing of the 16S rDNA gene amplification products was performed using the Illumina HiSeq platform (Illumina) with paired-end (PE) reads of 2 × 250 base pairs (bp), following the standard protocol. For data processing and filtering, the raw sequencing data were processed using an in-house written program to obtain clean data, and then FLASH (Fast Length Adjustment of Short reads, v1.2.11) software was applied to assemble pairs of reads obtained from double-end sequencing into one sequence using overlap relationships to obtain tags of high variance regions. The above processed clean tags were clustered into OTUs, and then the species classification of OTUs was completed by annotation of OTUs. Lastly, the OTU species classification was completed by annotating the OTUs for the next step of analysis.

Alpha diversity (Simpson, Chao1 and Shannon) was used to compare the bacterial richness and diversity of different samples. Different from Alpha diversity analysis, Beta diversity using principal component analysis (PCA), non-metric multidimensional scaling (NMDS) and linear discriminant analysis (LDA) were used to compare the differences in species diversity of different ecosystems among the samples. PCA can reflect differences and distances by analyzing the composition of OTU (97% similarity) of different sample. NMDS is a spatial positioning map based on Bray-Curtis dissimilarities, which reflects the degree of difference among groups through the distance between points. LDA effect size (LEfSe) analysis was applied to identify different abundant bacterial taxa among groups. Only those taxa with an LDA score >3 were considered. Functional genes were predicted by employing phylogenetic investigation of communities (PICRUSt) through the reconstruction of unobserved states. This prediction was achieved by utilizing the Kyoto Encyclopedia of Genes and Genomes (KEGG) database with high-quality sequences as the input. The network analysis was used for visualization of significant co-occurrence (Cytoscape, version 3.6.1).

Ultimately, basic information data were expressed as the means ± SE. The EMG results were analyzed using a two-way ANOVA. Statistical analysis of intestinal microbial differences between IBS-like group and control group was performed by *Wilcoxon rank sum test,* student's *t*-test *and Kruskal-Wallis* rank sum test using R3.1.0. We also applied the *Chi-squared* and nonparametric test to compare categorical and continuous variables between groups. All *P*-values reported are two-sided, and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Characteristics of the rats

A total of 12 separate litters utilized as experimental subjects. These litters were evenly divided into two groups: the IBS-like group consisting of six litters, and the control group also comprising six litters. No differences were observed in activity ability, mental state, and hair gloss of the IBS-like and control groups during the experiment. All periadolescent rats' weight at intestinal tissue sampling ranged between 151.3 and 185.9 g (**Table S1**) and was not significantly different between groups (**Table 1**). Compared with the control group, the feces of the rats in IBS-like group were abnormal, which were more watery, hard, and lumpy.

3.2. Neonatal maternal separation induces visceral hypersensitivity

The sensitivity of visceral pain was assessed by recording response of EMG to CRD pressure in rats. Compared to the control group, the visceral sensitivity in the IBS-like group significantly increased from 93.24 ± 31.87% to 175.95 ± 55.65% at 40 mmHg CRD, and from 149.77 ± 65.65% to 246.69 ± 56.87% at 60 mmHg CRD (Fig. 1A&B, $*P < 0.05$, two-way ANOVA followed by Bonferroni's post hoc analysis). We further examined the colonic tissues of control and IBS-like rats using HE staining, and the microscopic results

Table 1
The characteristics of periadolescent rats.

	IBS-like (n = 6)	Control (n = 6)	*P* value
Male, N	6	6	1[a]
Birth weight, grams, mean (SD)	9.15 (0.3)	8.55 (0.2)	0.153[b]
Weight at intestinal tissue, grams, mean (SD)	181.0 (24.1)	181.5 (10.7)	0.966[b]

[a] Chi-square test.
[b] 2-tailed Student t-test.

showed that compared with the control group, the colonic tissues of IBS-like rats had no significant edema and ulceration (Fig. 1C). The villus height, villus width and crypt depth of colonic tissues between the two groups were no significant difference (Fig. 1D, n. s. indicates no significant, 2-tailed Student t-test).

3.3. Overview of sequencing data

Six samples were obtained from the control and IBS-like rats, respectively, and subsequently sequenced to generate V3–V4 16S rDNA gene profiles. Original reads were obtained after filtering low-quality fastq data, then further removed from chimera and short sequences, a total of 339 374, and 351 186 sequences were provided for the control and IBS-like groups, respectively. Ninety-nine percent of the sequences were distributed in 200–260 bp (Fig. S1). There was an average of 56 562.33 and 58 531 reads per sample in the control and IBS-like groups, respectively. A total of 10 188 OTUs were detected. The sparse curve of Shannon index showed that most samples had sufficient test depth. (Fig. S2). Besides, the species accumulation curve also showed a plateau phase, indicating that the sample size was sufficient represent the microbiota structure of the sample. (Fig. S3).

3.4. Alpha-diversity analysis of gut microbiota

The alpha-diversity indexes data (*Simpson, Chao1 and Shannon*) were lower in IBS-like group than that in controls, however, there was no significant difference between the two groups (Table 2).

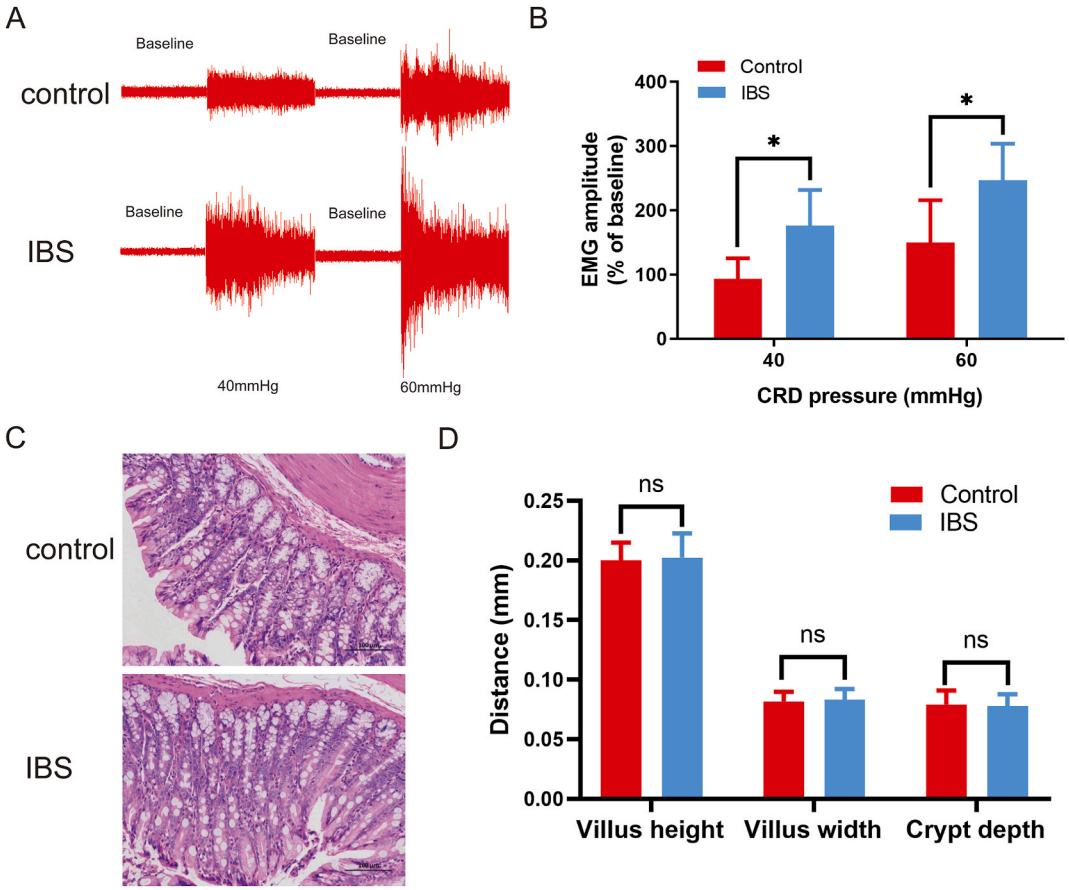


Fig. 1. Neonatal maternal separation induces visceral hypersensitivity. (**A**) Original typical recording of EMG in control and IBS-like rats at 40- and 60 mmHg CRD pressure. (**B**) The statistical chart of the percentage of EMG amplitude over baseline at 40- and 60 mmHg CRD in control and IBS-like rats. *P < 0.05, two-way ANOVA followed by Bonferroni's post hoc analysis, n = 6 for each group. (**C**) Representative photomicrographs of colon tissue histological changes in control group (up) versus IBS-like group (down). Original magnification, ×200. (**D**) The comparison of villus height and crypt depth between control and IBS-like rats. Data was expressed as the mean ± SD (n. s. indicates no significant, 2-tailed Student t-test, n = 6). EMG: electromyography; CRD: colorectal distension; IBS: irritable bowel syndrome.

Table 2
The average alpha-diversity indexes (*Simpson, Chao1 and Shannon* index) of the gut microbiota in control and IBS-like group.

	Simpson		Chao1		Shannon	
Group	Mean	SE	Mean	SE	Mean	SE
IBS-like	0.87	0.02	386.48	71.84	4.10	0.38
Control	0.88	0.03	464.05	79.16	4.40	0.39

Abbreviation: SE, standard error.

3.5. Microbial abundance and distribution of the predominant gut microbiota at different taxonomic levels

In the ileal content samples, a comprehensive diversity of microorganisms was detected, comprising a total of 18 phyla, 34 classes, 56 orders, 113 families, and 247 genera. The taxonomic distributions of the primary microbiota, with a relative abundance exceeding 1% of the total sequences, were illustrated at various hierarchical levels in Fig. 2. Significantly higher abundance of Bacteroidetes, a taxonomic group encompassing numerous pathogenic species, was observed in patients with IBS-like symptoms compared to the healthy controls. Conversely, the phylum Firmicutes exhibited a substantial reduction in the IBS-like group when compared to the controls. This finding underscores the potential association between altered gut microbiota composition and the manifestation of IBS-like symptoms in affected individuals (Fig. 2A&B, $*P < 0.05$, 2-tailed Student t-test); In addition, the abundance of Bacteroidia, Bacteroidales, Bacteroidales (S24-7group), Prevotellaceae*, Alloprevotella, Eubacterium* (*coprostanoligenes group*), *Romboutsia*, *Bacteroides* and *Bifidobacterium* were higher, while *Enterococcaceae* was lower at other taxon levels with IBS-like group than controls (Fig. 2 C-J, $*P < 0.05$, 2-tailed Student t-test). Fig. S4 displayed a heat map illustrating the significantly expressed genera. The statistical data pertaining to the predominant gut microbiota in Phylum, Class, Order, Family, and Genus across each group of samples were presented in Table S2.1-S2.5.

3.6. Comparison of the microbial community in and control IBS-like rats

3.6.1. Principal component analysis and nonmetric multidimensional scaling analysis

The β-diversity values were calculated using the weighted UniFrac method and principal coordinates analysis. The data showed a separate clustering of the control and IBS-like groups (Fig. 3), suggesting that the gut microbiota of IBS-like group was more consistent, whereas control group varied substantially.

3.6.2. Partial least squares discriminant analysis of control and IBS-like group

Partial least squares discriminant analysis (PLS-DA) based on least squares regression models, revealed differences in the gut microbiota structure between the groups (Fig. S5). These differences were assessed by calculating UniFrac distances, a phylogenetic based distance metric that, when weighted, accounts for the relative abundance of taxa.

3.7. Differential microbiota compositions between IBS-like and control

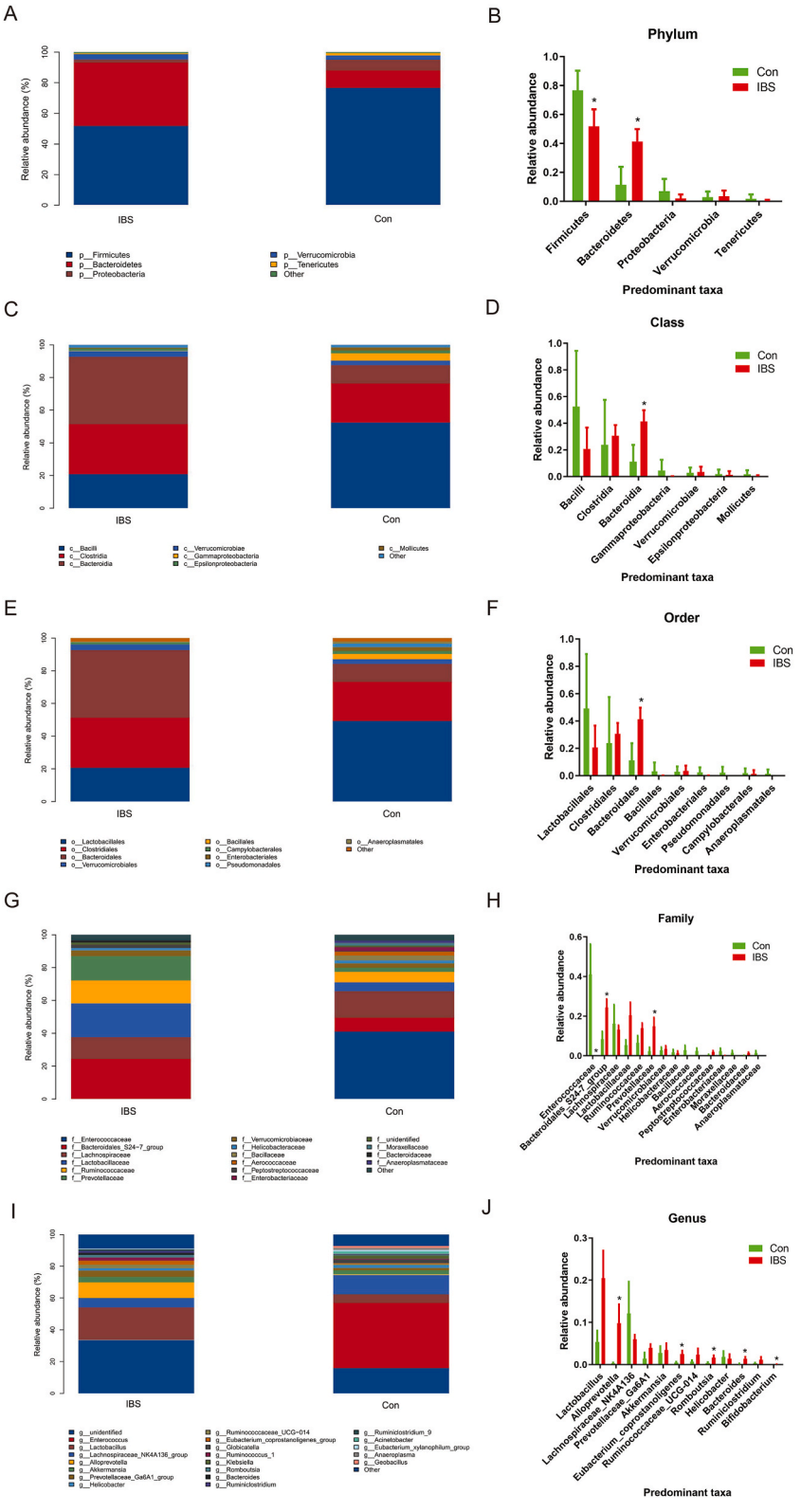
According to the results of linear discriminant analysis (LDA) effect size (LEfSe) analysis (Fig. 4), there were seven significantly different taxa. The microbiota with increased abundance in IBS-like group belonged to Bacteroidetes-Alloprevotella (LDA score = 4.65, $P = 0.04$), and the phylum with reduced abundance belonged to Firmicutes (LDA score = 5.04, $P = 0.01$). The details are shown in Table S3.

It has been reported that an increase in the ratio of Bacteroidetes to Firmicutes (B/F) is related to inflammatory diseases. In our study, the abundance of Bacteroidetes was higher while Firmicutes was lower in rats with IBS-like group than in controls. Additionally, *Lactobacillus* were significantly more abundant in IBS-like group (20.47% vs 5.38%, $Z = -1.92$, $P = 0.055$). Based on these observations it was speculated that a higher B/F and higher abundance of *Lactobacillus* could contribute in some way to FAPDs pathogenesis and that these significantly different taxa were likely to be used as potential biomarkers in the future.

3.8. Microbiota co-occurrence network and functional analysis

As shown in Fig. 5A, more than half of the biological process (BP) displayed positive associations. Of these, DNA replication, fatty acid biosynthesis, aminoacyl-tRNA biosynthesis and amino acid metabolism exhibited a high degree of linkage with others.

PICRUSt was then used as an exploratory tool to predict the microbiota functions between control and IBS-like groups. A total of 19 genetic information processing functions were predicted in all samples, with the most enrichment of KEGG orthologs (level 3) in mismatch repair (1.80%), aminoacyl-tRNA biosynthesis (1.76%), ribosome (1.68%), homologous recombination (1.59%), protein export (1.49%), and DNA replication (1.31%). We also investigated microbiota functions related with environmental information processing, including membrane transport (0.39%), signal transduction (0.45%) and signaling molecules and interaction (0.000001%). For KEGG orthologs of level 3, as indicated in Fig. 5B, there was significant difference of aminoacyl-tRNA biosynthesis, fatty acid biosynthesis, D-Glutamine and D-glutamate metabolism, one carbon pool by folate, etc. between control and IBS-like group ($P < 0.05$, 2-tailed Student t-test). Furthermore, functional predictions involving cell growth or death and translation also disclosed significant differences (Table S4.1-4.3, $P < 0.01$, 2-tailed Student t-test).



(caption on next page)

Fig. 2. Distribution of the predominant bacteria at different taxonomic levels (phylum, class, order, family, and genus). **A**, **C**, **E**, **G** and **I**, stacked bars of the phylum, class, order, family, and genus level in IBS-like and control respectively; **B**, **D**, **F**, **H** and J, bar chart [The predominant taxa (>1% relative abundance) in each level were shown] of the phylum, class, order, family, and genus levels in control and IBS-like group respectively. *$P <$ 0.05, vs control, 2-tailed Student t-test. Con: control group; IBS: IBS-like group.

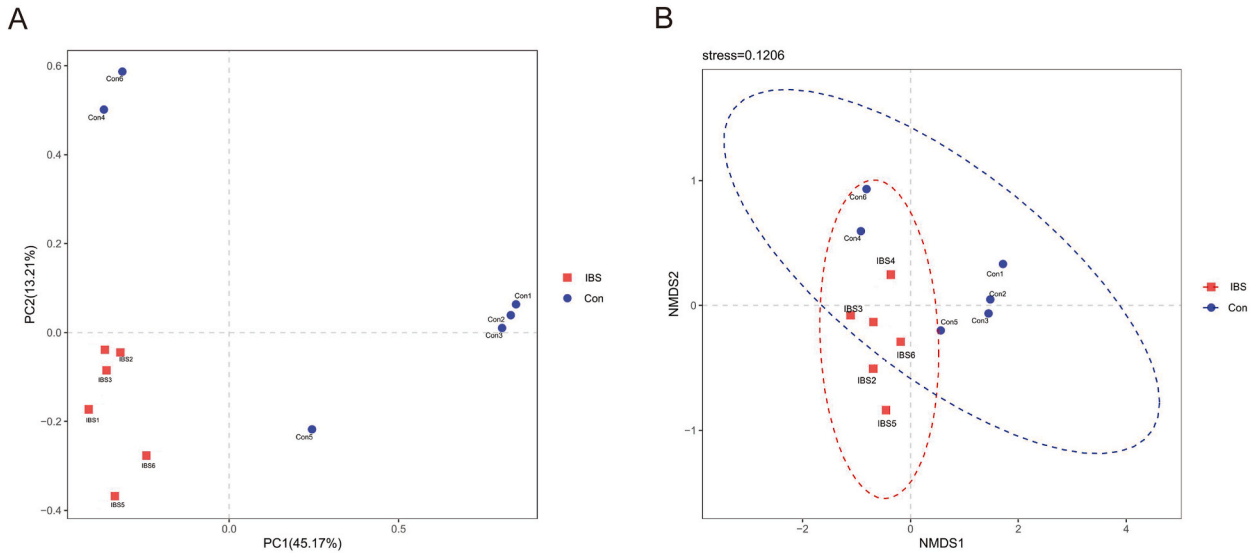


Fig. 3. The beta-diversity analysis. (**A**) Principal component analysis on the relative abundance. Each point represents a sample, plotted by the second principal component on the Y-axis and the first principal component on the X-axis, colored by group. (**B**) Comparing sample distributions belonging to different groups by using weighted nonmetric multidimensional scaling (NMDS) analysis. Each sample was represented by a dot.

The above-mentioned findings indicated that the differences in aminoacyl-tRNA biosynthesis and fatty acid biosynthesis could have considerable impacts on biological function, potentially influencing the progression of IBS.

4. Discussion

We have clarified, for the first time, the characteristics of gut microbiota in male periadolescent rats with IBS. Although growing evidence shows that there is a close relationship between the imbalance of gut microbiota and IBS [15]. Nevertheless, a significant knowledge gap exists regarding the influence of gut microbiota on IBS during the periadolescent phase, as most of the previous inquiries have predominantly concentrated on adult cohorts.

In the existing research, there was no significant difference in the alpha diversity of different groups, which was consistent with our results [16]. However, Carroll IM et al. found that the microbial diversity of healthy and diarrheal IBS (D-IBS) patients increased from mucosa to fecal samples, but the fecal microbial diversity decreased from healthy to D-IBS patients [17]. We can only speculate that these microbial differences may be related to dietary habits, past medication history, 16S rRNA gene target sequencing regions, and especially the age of the subject in these conflicting studies. Most importantly, in view of the stability of the intestinal microbiota, we chose the rats in PND 42, which can more objectively reflect the characteristics of the intestinal microbiota of IBS in children and adolescents [18,19]. The establishment and development of the human gut microbiota is a complex process, just like human life, the microbiota also undergoes development and establishment, maturity, and stability, and then eventually aging. Studies have shown that the developing intestinal microbiome undergoes three distinct phases of microbiome progression: a developmental phase (months 3–14), a transitional phase (months 15–30), and a stable phase (months 31–46) in human infants and childhood [20]. That is to say, adolescence is a period when the intestinal flora of children is relatively stable.

Previous studies in microbiota research have primarily focused on colon contents or fecal specimens [21,22], while our study collected samples from the ileum. On one hand, in terms of microbiota representation, the ileum provides a more accurate reflection of the overall gut microbiota compared to colorectal intestinal content and feces. Being in the middle section of the gastrointestinal tract, the ileum is in closer proximity to the entire gut microbiota. As a result, obtaining ileal samples allows for a more comprehensive and representative assessment of the microbiota composition and abundance within the gut. On the other hand, the ileum exhibits higher microbial diversity compared to the colon or fecal samples [23]. Given its role in food digestion and nutrient absorption, the ileum harbors a more complex and diverse microbial community. Thus, investigating the microbiota from ileal samples enables a more thorough understanding of microbial diversity within the gut.

In the control group and the IBS-like group, 12 and 19 phyla were respectively identified at the phylum level. The phyla with the highest representation comprised Firmicutes, Bacteroidetes, Proteobacteria, Verrucomicrobia, Tenericutes, and Actinobacteria,

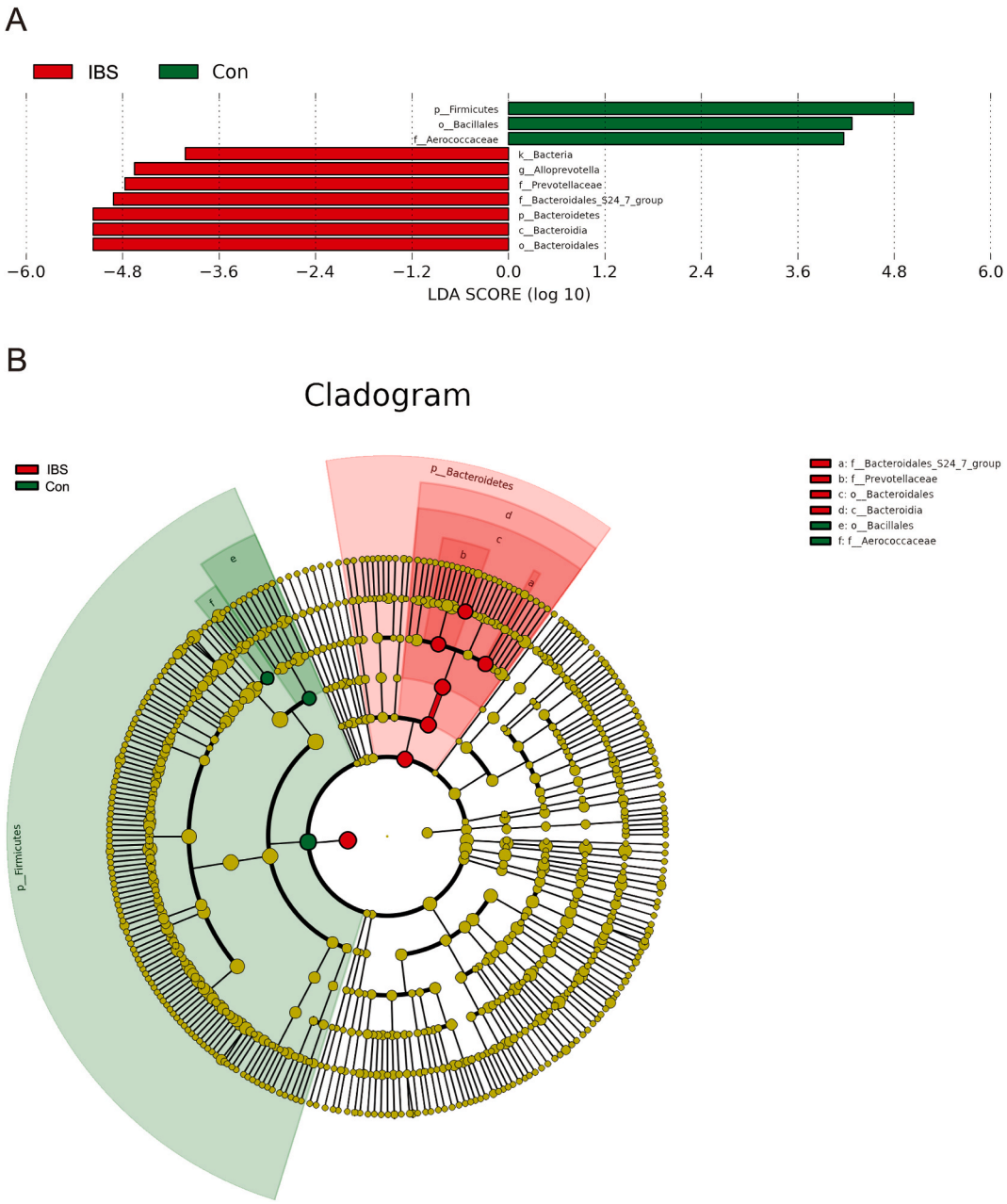


Fig. 4. Comparison of microbiota variation using the LEfSe online tool. (**A**) Histogram of the linear discriminant analysis (LDA) scores for differentially abundant genera between groups (logarithmic LDA score higher than 3 indicated a higher relative abundance in the corresponding group than in the other group). (**B**) Taxonomic cladogram obtained from LEfSe analysis of 16S rDNA gene sequences, taxonomic representation of statistically significant differences between groups. The diameter of each circle was proportional to taxon abundance.

collectively constituting 99.7% of the total sequences. Our study revealed a reduced abundance of Firmicutes and an elevated abundance of Bacteroidetes in the IBS-like group compared to the control group. This is probably due to the unique lipopolysaccharide (LPS) component of Gram-negative bacteria, which reflects the diversity of antigenic determinants on their cell surface [24]; however, most Firmicutes were gram-positive bacteria without LPS synthesis. Therefore, the higher the ratio of Bacteroidetes/Firmicutes, the greater the exposure of LPS. Current knowledge supports that increasing the number of Gram-negative bacteria in the rats' intestine can increase LPS and induce intestinal pathologies, such as inflammation, barrier dysfunction, and visceral hypersensitivity [25,26]. LPS binding protein (LBP) recognizes and binds LPS to activate the transmembrane receptor TLR4, which would initiate the downstream NF-κB signaling [27], and secretion of inflammatory cytokines [28,29]. So, does this also happen to children? A more recent study has demonstrated patients with D-IBS have increased serum LPS levels [30]. It is of great interest that several members of the

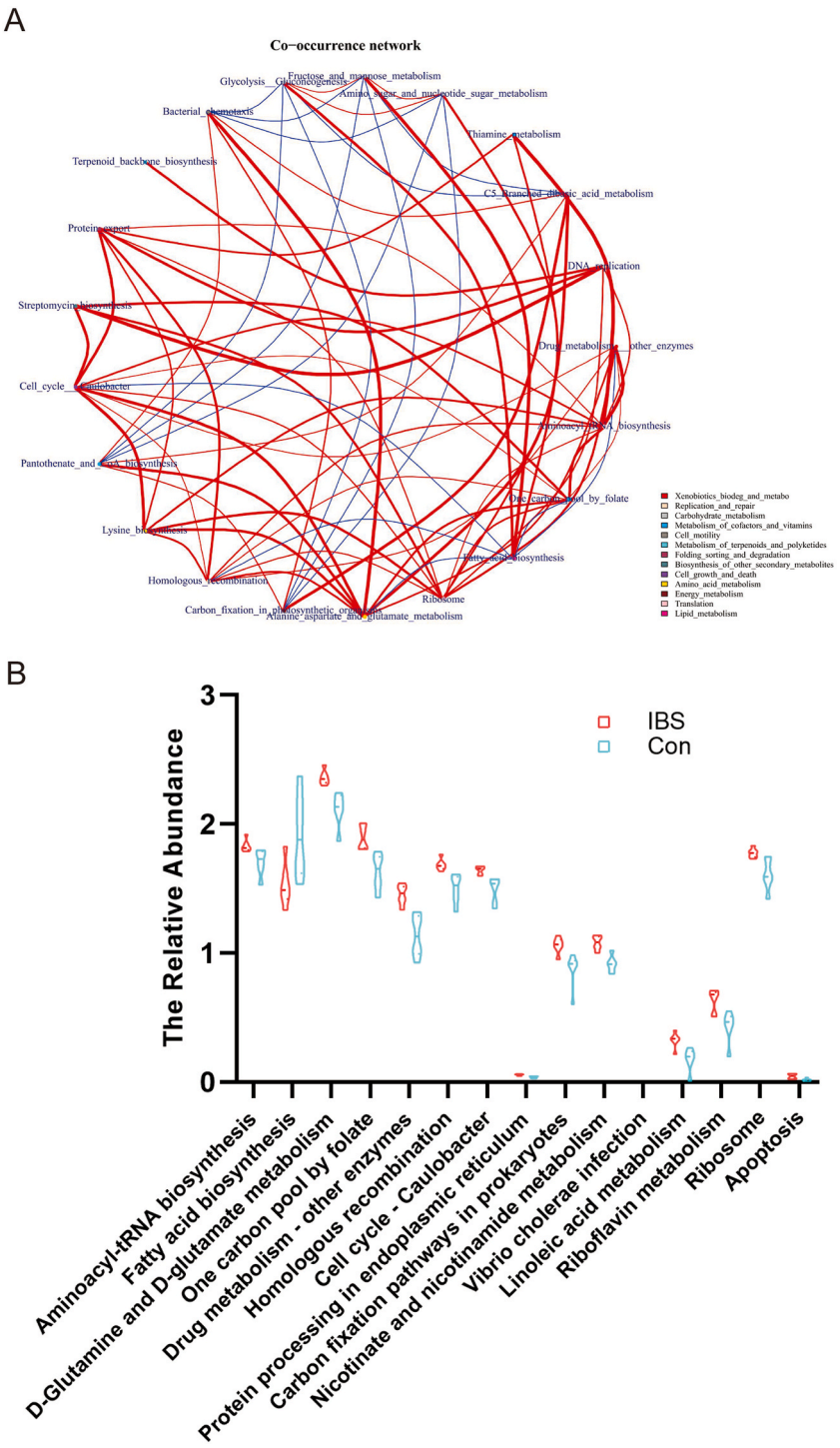


Fig. 5. (**A**) Network diagrams of biological process (BP) level results (The thickness of the line represents the size of the correlation; the color of the point represents BP, the line is red for positive correlation, and blue for negative correlation); (**B**) The violin plot of the significant difference in the level 3 of each group samples (*$P < 0.05$, 2-tailed Student t-test).

Bacteroidetes phylum in the fecal microbiota of patients with IBS-D increased by 12 times compared with healthy subjects from some clinical studies [31].

At the genus level, our results revealed that the incidence of *Romboutsia*, *Bacteroides* and *Prevotella* (potentially harmful bacteria) was significantly higher compared with the controls. *Romboutsia*, closely related to *Clostridium* clusters [32,33], was found significantly

increased in the IBS-like group compared with controls. There is a complete biosynthetic pathway for the aspartic acid, asparagine, glutamic, glutamine and cysteine, using the carbon skeleton from the central metabolite or through the conversion of other amino acids [34]. However, considering the fact that *Romboutsia* does not have genes encoding other amino acid biosynthesis enzymes [33], it could be speculated that *Romboutsia* depends on a number of exogenous amino acids, peptides and/or proteins to fuel protein synthesis and that which could contribute in some way to pathogenesis of IBS.

In addition, the *Bacteroides* family bacteria (a kind of taxon comprising many conditional pathogenic bacteria including *Fragilis*, Thetaiotaomicron, and Pyogenes, etc.) belonging to the Firmicutes phylum were found to be increased in IBS-like group. Recent studies have discovered that these bacteria secrete immunomodulatory molecules [35,36]. Moreover, high levels of Bacteroides species have been associated with inflammatory diseases and immune dysregulation in animals [37]. Notably, another kind of microbe, *Alloprevotella,* increased significantly in IBS-like cohort. *Prevotella,* traditionally not considered a pathogenic genus, has recently been found to play a key role in mucosal formation [38]. An increased abundance of *Prevotella* was recently identified as a high-risk for IBS-D [39]. Thus, it can be speculated that *Bacteroides* and *Alloprevotella* may be clinically important pathobionts that can participate the development of IBS in early life. Further case-control studies are needed to elucidate the associated markers.

By PICRUSt analysis and network diagrams of biological process (BP) level results, we observed that the genetic and environmental information processing genes were more abundant in rats with IBS-like phenotype than in controls, especially in terms of aminoacyl-tRNA biosynthesis. This may explain - at least in part - why children with IBS are more prone to dysbiosis and visceral hypersensitivity than normal people. Furthermore, fatty acid biosynthesis genes were reduced significantly in IBS-like rats compared to controls. One possible reason for this could be that fats are primarily absorbed in the intestine, and intestinal dysfunction can result in decreased absorption of substances such as fats.

This study has some limitations. First, we did not further verify how this altered gut microbiota affects IBS through the brain-gut axis or other neural pathways. Second, the reason why certain bacteria, including *Eubacterium*, increase in IBS-like rats is unclear, and more research are needed to explore the underlying mechanism. Third, the data were acquired only on PND 42, dynamic study on microbiota and hypersensitivity should be included in the future.

In conclusion, our study on male periadolescent rats with IBS induced by NMS revealed significant alterations in gut microbiota composition. The IBS-like rats exhibited increased visceral sensitivity and distinct changes in microbial abundance, with higher Bacteroidetes and lower Firmicutes levels compared to the control group. We identified specific bacterial clades associated with IBS risk, such as *Alloprevotella* , *Bacteroides*, etc. Functional analysis indicated significant changes in aminoacyl-tRNA biosynthesis and fatty acid biosynthesis pathways. These findings provide valuable insights into the relationship between gut microbiota dysbiosis and IBS, offering potential targets for future therapeutic interventions.

Author contribution statement

Wei Lin: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Dongxiao Wu: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data.

Yongbin Zeng: Analyzed and interpreted the data; Wrote the paper.

Yuan Liu: Bin Wu: Analyzed and interpreted the data.

Dajie Yu: Jianhang Wei: Yanliang Cai: Yueli Lin: Performed the experiments.

Huanhuan Huang: Conceived and designed the experiments.

Funding

The study was supported by the grant from National Natural Science Foundation of China (82172338).

Availability of data and material

The sequencing data have been uploaded to the NCBI database (SUB 11111451), and other data that supports the findings of this study are available in the supplementary material of this article.

Ethics approval and consent to participate

All animal studies were implemented in accordance with institutional regulations and approved by the Department of the Experimental Animal Center of Fujian Medical University (SCXK- 2016- 0002).

Consent for publication

Not applicable.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e18995.

References

[1] S.G. Robin, et al., Prevalence of pediatric functional gastrointestinal disorders utilizing the Rome IV criteria, J. Pediatr. 195 (2018) 134–139.
[2] D.A. Drossman, Functional gastrointestinal disorders: history, pathophysiology, clinical features and Rome IV, Gastroenterology 150 (6) (2016) 1262–1279.
[3] A.D. Sperber, et al., Worldwide prevalence and burden of functional gastrointestinal disorders, results of Rome foundation global study, Gastroenterology 160 (1) (2021) 99–114.e3.
[4] A.E.V. Quaglio, et al., Gut microbiota, inflammatory bowel disease and colorectal cancer, World J. Gastroenterol. 28 (30) (2022) 4053–4060.
[5] Z. Mamieva, et al., Antibiotics, gut microbiota, and irritable bowel syndrome: what are the relations? World J. Gastroenterol. 28 (12) (2022) 1204–1219.
[6] J.S. Hyams, et al., Functional disorders: children and adolescents, Gastroenterology 150 (6) (2016) 1456–1468.
[7] J. Qin, et al., A human gut microbial gene catalogue established by metagenomic sequencing, Nature 464 (7285) (2010) 59–65.
[8] N. Shi, et al., Interaction between the gut microbiome and mucosal immune system, Mil Med Res 4 (2017) 14.
[9] N. Thapar, et al., Paediatric functional abdominal pain disorders, Nat. Rev. Dis. Prim. 6 (1) (2020) 89.
[10] L. Putignani, et al., Gut microbiota dysbiosis as risk and premorbid factors of IBD and IBS along the childhood-adulthood transition, Inflamm. Bowel Dis. 22 (2) (2016) 487–504.
[11] A. Altomare, et al., Diarrhea predominant-irritable bowel syndrome (IBS-D): effects of different nutritional patterns on intestinal dysbiosis and symptoms, Nutrients 13 (5) (2021).
[12] Y. Tang, et al., Spinal P2X4 receptors involved in visceral hypersensitivity of neonatal maternal separation rats, Purinergic Signal. 19 (1) (2023) 113–122.
[13] Y.Z. Yang, et al., [Assessment of the young rat model of visceral hypersensitivity by measuring electrical discharge of external oblique], Zhong Guo Dang Dai Er Ke Za Zhi 10 (5) (2008) 637–641.
[14] F.S. Wang, et al., Intestinal tract and parenteral multi-organ sequential pathological injury caused by necrotizing enterocolitis, BMC Pediatr. 20 (1) (2020) 418.
[15] H.L. Dupont, Review article: evidence for the role of gut microbiota in irritable bowel syndrome and its potential influence on therapeutic targets, Aliment. Pharmacol. Ther. 39 (10) (2014) 1033–1042.
[16] C. Codling, et al., A molecular analysis of fecal and mucosal bacterial communities in irritable bowel syndrome, Dig. Dis. Sci. 55 (2) (2010) 392–397.
[17] I.M. Carroll, et al., Molecular analysis of the luminal- and mucosal-associated intestinal microbiota in diarrhea-predominant irritable bowel syndrome, Am. J. Physiol. Gastrointest. Liver Physiol. 301 (5) (2011) G799–G807.
[18] P. Sengupta, The laboratory rat: relating its age with human's, Int. J. Prev. Med. 4 (6) (2013) 624–630.
[19] P.D. Schloss, et al., Stabilization of the murine gut microbiome following weaning, Gut Microb. 3 (4) (2012) 383–393.
[20] C.J. Stewart, et al., Temporal development of the gut microbiome in early childhood from the TEDDY study, Nature 562 (7728) (2018) 583–588.
[21] P.W. O'Toole, I.B. Jeffery, Gut microbiota and aging, Science 350 (6265) (2015) 1214–1215.
[22] Y. Fang, et al., Characteristics of the gut microbiota and metabolism in patients with latent autoimmune diabetes in adults: a case-control study, Diabetes Care 44 (12) (2021) 2738–2746.
[23] J.E. Kim, et al., Microbial diversity and metabolic function in duodenum, jejunum and ileum of emu (Dromaius novaehollandiae), Sci. Rep. 13 (1) (2023) 4488.
[24] R.F. Maldonado, I. Sá-Correia, M.A. Valvano, Lipopolysaccharide modification in Gram-negative bacteria during chronic infection, FEMS Microbiol. Rev. 40 (4) (2016) 480–493.
[25] S.Y. Zhou, et al., FODMAP diet modulates visceral nociception by lipopolysaccharide-mediated intestinal inflammation and barrier dysfunction, J. Clin. Invest. 128 (1) (2018) 267–280.
[26] I. Dickson, IBS: high FODMAP diet induces LPS-derived intestinal inflammation and visceral hypersensitivity, Nat. Rev. Gastroenterol. Hepatol. 15 (2) (2018) 68.
[27] E.M. Pålsson-McDermott, L.A. O'Neill, Signal transduction by the lipopolysaccharide receptor, Toll-like receptor-4, Immunology 113 (2) (2004) 153–162.
[28] R.R. Schumann, et al., Structure and function of lipopolysaccharide binding protein, Science 249 (4975) (1990) 1429–1431.
[29] C. Lenoir, et al., MD-2 controls bacterial lipopolysaccharide hyporesponsiveness in human intestinal epithelial cells, Life Sci. 82 (9–10) (2008) 519–528.
[30] A. Dlugosz, et al., Increased serum levels of lipopolysaccharide and antiflagellin antibodies in patients with diarrhea-predominant irritable bowel syndrome, Neuro Gastroenterol. Motil. 27 (12) (2015) 1747–1754.
[31] J. Jalanka-Tuovinen, et al., Faecal microbiota composition and host-microbe cross-talk following gastroenteritis and in postinfectious irritable bowel syndrome, Gut 63 (11) (2014) 1737–1745.
[32] E. Stackebrandt, et al., Phylogenetic analysis of the genus Desulfotomaculum: evidence for the misclassification of Desulfotomaculum guttoideum and description of Desulfotomaculum orientis as Desulfosporosinus orientis gen. nov., comb. nov, Int. J. Syst. Bacteriol. 47 (4) (1997) 1134–1139.
[33] J. Gerritsen, et al., Characterization of Romboutsia ilealis gen. nov., sp. nov., isolated from the gastro-intestinal tract of a rat, and proposal for the reclassification of five closely related members of the genus Clostridium into the genera Romboutsia gen. nov., Intestinibacter gen. nov., Terrisporobacter gen. nov. and Asaccharospora gen. nov, Int. J. Syst. Evol. Microbiol. 64 (Pt 5) (2014) 1600–1616.
[34] J. Gerritsen, et al., Genomic and functional analysis of Romboutsia ilealis CRIB(T) reveals adaptation to the small intestine, PeerJ 5 (2017) e3698.
[35] K. Hiippala, et al., The potential of gut commensals in reinforcing intestinal barrier function and alleviating inflammation, Nutrients 10 (8) (2018).
[36] A. Schwiertz, et al., Microbiota and SCFA in lean and overweight healthy subjects, Obesity 18 (1) (2010) 190–195.
[37] E. Terán-Ventura, et al., Specific changes of gut commensal microbiota and TLRs during indomethacin-induced acute intestinal inflammation in rats, J. Crohns Colitis 8 (9) (2014) 1043–1054.
[38] J.M. Larsen, The immune response to Prevotella bacteria in chronic inflammatory disease, Immunology 151 (4) (2017) 363–374.
[39] T. Su, et al., Altered intestinal microbiota with increased abundance of prevotella is associated with high risk of diarrhea-predominant irritable bowel syndrome, Gastroenterol. Res. Pract. 2018 (2018), 6961783.