Parasutterella, in associated with Irritable Bowel Syndrome and intestinal chronic inflammation

Running head: Parasutterella may be related with IBS

Authors: Yan-Jie Chen¹, Hao Wu¹, Sheng-Di Wu¹, Nan Lu, Yi-Ting Wang, Hai-Ning Liu, Ling Dong, Tao-Tao Liu, Xi-Zhong Shen*

Author address: Department of Gastroenterology, Zhongshan Hospital of Fudan University, Shanghai 200032, China; Shanghai Institute of Liver Diseases, Zhongshan Hospital of Fudan University, Shanghai, China

*Correspondences: Xi-Zhong Shen, MD, PhD, Department of Gastroenterology, Zhongshan Hospital of Fudan University, 180 Fenglin Rd., Shanghai 200032, China, Tel: +86-21-64041990-2070; Fax: +86-21-64038038. E-mail: shen.xizhong@zs-hospital.sh.cn / xizhongshen@126.com

¹These authors contributed equally to this work.

Acknowledgments
The authors would like to express gratitude to the staff of Prof. Xi-Zhong Shen's laboratory for their critical discussion and reading of the manuscript. This study was supported by Youth Foundation of Zhongshan Hospital (No. 2015ZSQN08), Shanghai Sailing Program (No. 16YF1401500), Foundation of Shanghai Institute of Liver Diseases, and National Natural Science Foundation of China (No. 81101540; No. 81101637; No. 81172273; No. 81272388).

Conflict of interest statement
The authors declare that there are no conflicts of interest.
Abstract

Background and Aim: Irritable bowel syndrome (IBS) is a highly prevalent chronic functional gastrointestinal disorder. Recent studies have showed increasing important role of gut microbiota in the pathophysiological changes of IBS. Our study aims to elaborate the association between intestinal flora with the genesis and the development of IBS.

Methods: Illumina high-throughput sequencing technology was applied to investigate microbial communities of IBS patients and healthy donors. Stool specimens from the IBS-D patients were equally premixed and implanted into germ free C57B/6 mice to construct IBS animal model, and the normal group was also transplanted with normal premixed feces. The post-transplant defecation and intraepithelial lymphocyte counts were evaluated. Microbial communities were also checked by the illumina high-throughput sequencing technology.

Results: Fifteen genuses significantly differences were found expressed in the gut flora of IBS patients, and 6 genuses showed significantly different abundances between the stool specimens of mice of IBS group and normal group. Among these differences, *Parasutterella* expression was remarkably different in both screening and validation experiments, and also related to chronic intestinal inflammation, therefore, *parasutterella* expression is considered in association with the development and progression of IBS.

Conclusion: *Parasutterella* may be related with the genesis and development of IBS, and also associated with chronic intestinal inflammation in IBS patients.

Keywords: Irritable bowel syndrome (IBS); Gut microbiota; *Parasutterella*; Chronic intestinal inflammation
Introduction

Irritable bowel syndrome (IBS) is a chronic bowel disorder and always recognized as functional gastrointestinal disturbance with abdominal pain and change in stool frequency or form\(^1,2\). The prevalence of IBS is between 7% and 16% in American, and between 10% and 15% in China\(^3,4\). It is estimated that the direct treatment cost associated with IBS is more than $1 billion in America\(^5\), bringing a significant medical and economic burden. Therefore, diagnosing accurately with reduced invasive examination, figuring out the pathogenesis, and recommending effective treatment for IBS are necessary.

The pathogenesis mechanism of IBS is yet unclear. Traditionally, it has been conceptualized as a brain-gut disorder because the abdominal pain, visceral hypersensitivity, altered motility of gut and increased intestinal wall permeability are all involved in the pathophysiology of IBS\(^1\). The central nervous system (CNS) alterations lead by the genetic predisposition and environmental factors may cause alterations in intestinal epithelial cells and microbiome, bringing increased intestinal wall permeability and local inflammation, thus resulting in the symptoms of IBS\(^6\). On the other hand, infection, inflammation, food antigens and medications may cause the intestinal microflora alterations, resulting in the infiltration of inflammatory cells, immune cell function change, and cytokine release, further causes the development and occurrence of IBS and lead to changes in CNS function\(^7\).

The gut microbiota may contribute to the development of IBS according to preliminary researches\(^8-10\). Some studies found a higher ratio of *Firmicutes/Bacteroidetes*\(^9,11\), leading to lower diversity of gut microbiota in IBS patients\(^12\). Ng et al found an increased level of *Bacteroides* in intestinal mucosa of IBS patients while it was decreased significantly after the probiotic treatment\(^11\). Another study reported that the abundance of mucosa-associated *Bifidobacteria* and *Lactobacilli* is closely related to stool frequency\(^13\). Tap J and his colleagues found specific changes in intestinal flora in terms of severity IBS patients, characterized by reduced microbial diversity and a reduced prevalence of *Methanobacteriales* and *prevotella* through a retrospective study involving 110 patients with IBS\(^14\). It is also found that the
prebiotic treatment can prevent IBS in mice by improving gut microbiota and modulating immune response\textsuperscript{15}. Dietary changes can alter the microbiome rapidly and reproducibly\textsuperscript{16}, although whether this explains the benefit of dietary treatment in some IBS patients is still unclear.

In this study, we investigated the contribution of gut microbiota in IBS patients and IBS models in sterile C57BL/6 mice, and found that \textit{Parasutterella} was abundant in the stool of IBS patient and might be related to the development of irritable bowel syndrome. Spearman test also showed a significant positive correlation between the abundance of \textit{Parasutterella} and the ratio of inflammatory cells to epithelial cells in subcutaneous tissue, which suggested that \textit{Parasutterella} might be associated with chronic intestinal inflammation in patients with IBS.

\textbf{Materials and Methods}

\textbf{Patients and Specimens}

Stool specimens used in this study were obtained from 20 IBS-D patients who were treated at The Department of Gastroenterology, Zhongshan Hospital of Fudan University between 2014 to 2016. The inclusion and exclusion criteria of the patients include: (a) between the age of 18-70 years old and signed informed consent; (b) excluding the other diseases such as inflammatory bowel disease, intestinal polyp, intestinal malignant tumor, and so on; (c) having abdominal pain associated with recurrent diarrhea, and conforms to the Rome IV diagnostic standard clinically; (d) with certain diagnostic standard as IBS-D according to the Bristol stool scale; (e) with no antibiotics, probiotics or other diet treatment within 4 weeks before admission. Another 20 healthy volunteers were also enrolled in this study with the inclusion and exclusion criteria including: (a) between the age of 18-70 years old and signed informed consent; (b) excluding the other diseases such as inflammatory bowel disease, intestinal polyp, intestinal malignant tumor, and so on by previous colonoscopy or abdominal imaging; (c) with no change in stool frequency and traits; (d) no antibiotics and probiotics were given within 4
weeks before admission. Ten stool specimens of IBS-D patients and 10 stool specimens of healthy volunteers were used for high-throughput sequencing experiments directly, while the remaining samples were equivalently mixed according to the group to reduce the error between individuals within-group. Ethical approval for human subjects was obtained from the research ethics committee of Zhongshan Hospital, and informed consent was obtained from each patient.

**DNA extraction and PCR amplification**

Microbial DNA was extracted from stool samples using the TIANamp Stool DNA Kit (TIANGEN BIOTECH, Beijing, China) according to manufacturer’s protocols. The final DNA concentration and purification were determined by NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA), and DNA quality was checked by 1% agarose gel electrophoresis. The V3-V4 hypervariable regions of the bacteria 16S rRNA gene were amplified with primers 338F (5’- ACTCCTACGGGAGGCAGCAG-3’) and 806R (5’-GGACTACHVGGGTWTCTAAT-3’) by thermocycler PCR system (GeneAmp 9700, ABI, USA). The PCR reactions were conducted using the following program: 3 min of denaturation at 95 °C, 27 cycles of 30 s at 95 °C, 30s for annealing at 55 °C, and 45s for elongation at 72 °C, and a final extension at 72 °C for 10 min. PCR reactions were performed in triplicate 20 μL mixture containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase and 10 ng of template DNA. The resulted PCR products were extracted from a 2% agarose gel and further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, USA) according to the manufacturer’s protocol.

**Illumina MiSeq sequencing**

Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).
Processing of sequencing data

Raw fastq files were demultiplexed, quality-filtered by Trimmomatic and merged by FLASH with the following criteria: (i) The reads were truncated at any site receiving an average quality score <20 over a 50 bp sliding window. (ii) Primers were exactly matched allowing 2 nucleotide mismatching, and reads containing ambiguous bases were removed. (iii) Sequences whose overlap longer than 10 bp were merged according to their overlap sequence. Operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSE (version 7.1 http://drive5.com/uparse/) and chimeric sequences were identified and removed using UCHIME. The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier algorithm (http://rdp.cme.msu.edu/) against the Silva (SSU123) 16S rRNA database using confidence threshold of 70%.

Real-time quantitative PCR for germ copy number

A tenfold serial dilution of plasmid was generated to produce a standard curve. The PCR system contained 2 × ChamQ SYBR Color qPCR Master Mix (16.5 μL, containing DNA polymerase and dNTP), the Forward Primer (0.8 μL), the Reverse Primer (0.8 μL), and template DNA (2 μL). The primers used were as follows: forward primer: GGAAGTACGGTCGCAAGA (Parasutterella excrementihominis) and reverse primer: TGTCAGGTTAGGTAAGGT (Parasutterella excrementihominis); forward primer: AGGCGGTCTGGGTAAGACA (Parasutterella secunda) and reverse primer: TACGCAATTCACTGCTACACTT (Parasutterella secunda). Melting curve analyses were carried out using an ABI7500 Realtime Fluorescent Quantitative PCR instrument following these steps: a preliminary denaturation at 95 °C (5 min), 40 cycles of 95 °C (5 sec), annealing at 50 °C (30 sec), extension at 72 °C (40 sec) and thermal insulation at 4°C.

Afterwards, the target gene concentrations were measured with a spectrophotometer, and the measurements generated five-point calibration curves (Ct value versus the log of the log of the initial gene copy number). The standard curve of Parasutterella excrementihominis was \( y = -3.5356 \lg(x) + 40.285 \) (\( R^2 = 0.9998 \)) with the efficiency of 91.8%. The standard curve of Parasutterella secunda was \( y = -3.5724 \lg(x) + 39.471 \) (\( R^2 = 0.9993 \)) with the efficiency of...
90.51%. The lowest detection limit was $10^2$ copies/μL. From the calibration curves, the Ct value of the test sample was used to calculate the copy numbers.

**Animal Model**

For the xenograft experiments, six-week-old specific pathogen-free male C57BL/6 mice were divided into three groups and maintained separately in sterilized filtered cages inoculated with the mix of fresh bacterial culture of IBS patients, mix of fresh bacterial culture of healthy donors, and sterile saline. First day, 3rd day, 1st week, 2nd week, 3rd week, 4th week, and 5th week after the gavage feces, we stimulate defecation and record the fecal particles and characters. The feces were graded into 5 scales: formative and hard – dilute and watery like bowel. After 5 weeks of inoculation, the mice were weighed and then euthanized via cervical dislocation.

Following euthanasia, laparotomy was performed. Stool specimens in the colon were collected for the high-throughput sequencing experiments. Jejunum, ileum, cecum, left colon, and rectum were removed and placed in 10% buffered formalin (VWR Int, West Chester, PA) for histological analysis.

**Histopathology study**

Five-micron thick sections of formalin-fixed and paraffin-embedded TMA was performed. Hematoxylin-eosin (HE) staining of the jejunum, ileum, cecum, left colon, and rectum tissues. The severity of the lesions in the intestine was graded according to the numbers of neutrophil infiltration in the lamina propria.

**Data analysis**

The rarefaction curves and alpha diversity indices referring to community richness (Chao and Ace), community diversity (Simpson and Shannon) are based on the OTUs information. The species composition was revealed by Venn chart and colony bar diagram. Diversity among groups was showed by the phylogenetic beta diversities calculated by principal co-ordinate analysis (PCoA) with unweighted unifrac and sample grouping analysis calculated by partial least squares discriminant analysis (PLS-DA) with unweighted unifrac. ANOSIM/Adonis analysis was used to determine whether the grouping was meaningful by testing if the difference between groups was significantly greater than those within groups.
Wilcoxon rank-test was used to examine the significant differences between groups. To detect the potential biomarkers, the linear discriminant analysis (LDA) effect size (LEfSe) method based on Kruskal wallis test was used based on a normalized relative abundance matrix. A LDA threshold score of 2.0 and a significant \( \alpha \) of 0.05 were used to detect biomarkers.

The correlation between microbial classification and chronic intestinal inflammation was showed by the correlation heatmap based on the spearman test. Mann-Whitney test was used for comparison between groups. The concentration copies of germ were log treated and independent sample t-test was used to examine the significant differences between groups. \( P < 0.05 \) was considered statistically significant.

**Results**

**Diversity of the microbial community in IBS patient and normal people**

In this work, we used the MiSeq high-throughput sequencing method to analyze totally 20 stool specimens from 10 IBS patients and 10 healthy donors. The alpha diversity indicated no differed significance between stool specimens of IBS patients and control donors. There was no statistical significance between samples in Shannon’s diversity index (\( P = 0.910 \)), Simpson’s diversity index (\( P = 0.734 \)), Chao richness index (\( P = 0.521 \)), and Ace richness index (\( P = 0.473 \)), showing there’s no difference in the diversity and richness of bacterial species between the two groups. The coverage values were greater than 0.999, suggesting that the sequencing depth was sufficient to cover most of the microorganisms.

We used the Venn chart to reveal the microbial community composition in different groups, and found 98 species in IBS group and 94 in healthy group in genus level. Among these, 93 species appeared simultaneously in both two groups (Fig. 1A). Although there was no difference in the diversity and richness of bacterial species between the two groups, there was a significant difference in microbial community abundance between the two groups according to the ANOSIM/Adonis (Fig. 1B) and PLS-DA (Fig. 1C) analysis with unweighted unifrac in genus level. Community barplot analysis showed the microbial community composition at the genus level (Fig. 1D). We found *Lachnospiraceae UCG-004* and *Prevotella 9* were most
abundant across both groups, with a proportion of 20.96% and 12.97% of *Lachnospirceae UCG-004* in IBS and healthy control groups, and *Prevotella* 9 of 10.62% and 20.05% in IBS and healthy control groups, separately. It was found that the *Megamonas* had a high proportion of 12.74% in the microbial community of IBS patients, while it was hardly found in the specimens of healthy control groups. Test also showed different composition ratios among other bacterial species between the two groups, indicating the differences in community abundance between the two groups.

**Significant differences of genus level in microbial communities**

To determine the classified bacterial taxa with significant abundance differences between the samples of IBS patients and healthy donors, we performed biomarker analysis using the LEfSe method. Forty-two bacterial clades presented statistically significant differences (Fig. 2A, B). Among them, 22 were significantly enriched in IBS samples, while another 20 clades showed abundance advantage in normal control samples. Wilcoxon rank-test was used to examine the significant differences between groups, and there were two bacterial genus showed statistically different proportions between different groups in the top 15 species with the most abundant expression at the genus level (Fig 2C). And a total of 15 genus showed significant differences in the abundance between the two groups (Table 1).

**IBS model construction: IBS flora transplantation**

Ten stool specimens from the IBS-D patients were equivalently premixed and implanted into 7 germ free mice to construct IBS animal model; and another 6 sterile mice were transferred with the mixture of 10 stool specimens from the healthy donors as the normal controls. We also established a blank control group consisting of 6 germ free mice gavaged by aseptic saline. One week after the intestinal flora transplantation, one mouse in the IBS group died from severe intestinal infection, and another 3 mice in the IBS group had recurrent anus redness and swelling, indicating that the feces of IBS patients had more pathogenic bacteria, which might lead to severe infection. Mice were given perianal stimulation over the following 5 weeks and the feces were graded. Although the fecal particle number had no statistical significance during the 5 weeks, the mice in the IBS group showed large fluctuations (Fig. 3A). In the 3rd day after
transplantation, the IBS mice defecated shapeless stool comparing to the normal group \((P = 0.026, \text{Fig 3B})\). In the 1\textsuperscript{st} week, 4\textsuperscript{th} week and 5\textsuperscript{th} week, the stool of mice in the blank control group showed significant thinner than that in the normal group \((P = 0.026, 0.009, \text{and 0.041, respectively, Fig 3B})\), indicating the normal intestinal flora is necessary for maintaining normal intestinal function and normal stool trait.

There’s no significant difference of body weight growth between the IBS group and the normal group (Fig. 3C). However, the IBS group mice had a higher ratio of inflammatory cells/epithelial cells number comparing with the mice in the healthy control group (Fig. 3D, E), suggesting that the gut flora in the IBS patients can also produce chronic inflammatory reaction in the germ-free mice.

**Diversity of the microbial community among different test groups**

There is no significant difference between the stool specimens of mice in the IBS group and normal group by the alpha diversity. There’s no statistical significance between samples in Shannon’s diversity index \((P = 0.379)\), Simpson’s diversity index \((P = 0.471)\), Chao richness index \((P = 0.230)\), and Ace richness index \((P = 0.173)\), showing there’s no difference in the diversity and richness of bacterial species between the two groups. The coverage values were 0.897 and 0.927 in the IBS group and normal group, respectively. The blank group had low diversity and richness in the bacterial which come from the contamination in post-treatment of samples.

We also used the Venn chart to reveal the microbial community composition in different groups (Fig. 4A). There was a significant difference in microbial community abundance between the two groups according to the ANOSIM/Adonis (Fig. 4B) and PCoA (Fig. 4C) analysis with unweighted unifrac in genus level. Community barplot analysis showed the microbial community composition at the genus level (Fig. 1D). In the IBS group, the first 5 highest abundant expressed bacterial were *Fusobacterium* (24.57%), *Akkermansia* (21.41%), *Bacteroides* (24.45%), *Parabacteroides* (5.60%), and *Lachnoclostridium* (3.41%); while in the normal group, the first 4 highest abundant expressed bacterial were *Akkermansia* (41.12%), *Bacteroides* (20.07%), *Parabacteroides* (10.10%), and *Lachnoclostridium* (9.00%). The
"Fusobacterium" is abundant in the IBS group while it was hardly found in the normal group in the animal model experiment. Different composition ratios among other species between the two groups was also present, indicating the difference in community abundance between the two groups. The blank group on the other hand, showed a totally different flora expression.

**Significant difference in microbial communities between stool specimens of IBS group and normal group**

Twenty-eight bacterial clades presented statistically significant differences according to the LEfSe (Fig. 5A, B). Among them, 19 were significantly enriched in IBS samples, while another 9 clades showed decreased proportion. There were 6 genuses, including *Fusobacterium*, *Parabacteroides*, *Flavonifractor*, *Phascolarctobacterium*, *unclassified Lachnospiraceae*, and *Parasutterella*, that showed significantly different abundances between the stool specimens of IBS group and normal group, and all of them were in the top 15 most abundant expressed species (Fig 5C, Table 1). Among them, *Parasutterella* was significantly differently expressed in both screening and validation experiments, so it was considered associated with the development and progression of IBS.

**Relationship between microflora and chronic intestinal inflammation**

We used Spearman test and heatmap to show the relationship between the bacterium and intestinal chronic inflammation (Fig. 6), and found that *Phascolarctobactrium*, *Parasutterella* were significantly positively related with the subcutaneous tissue inflammatory cells/epithelial cell number ratio in all the segments of the intestine, and *Subdoligranulum*, *Anaerotruncus*, and *Fusobacterium* were positively related with the chronic intestinal inflammation in part of the intestine. On the other hand, *unclassified Lachnospiraceae*, *Parabacteroides*, *Eisenbergiella*, *Akkermansia*, *Tyzzerella*, *Flavonifractor*, and *Escherichia-Shigella* were negatively related with chronic intestinal inflammation in part of the intestine, showing these bacteria might play as probiotics in IBS patients.

**Two species of Parasutterella were related with IBS**

To investigate the relationship between two species of *Parasutterella*, *Parasutterella excrementihominis* and *Parasutterella secunda*, and IBS, Realtime Fluorescent Quantitative
PCR was performed. We found these two of Parasutterella species were both related with IBS ($P < 0.001$, Supplemental Fig. 1). The average concentration copies of Parasutterella excrementihominis were $1.13E7 \pm 1.47E7$ copies/g ($6.61 \pm 0.78 \log_{10}(\text{copies/g})$) in normal group and $9.78E8 \pm 1.66E9$ copies/g ($8.22 \pm 1.06 \log_{10}(\text{copies/g})$) in IBS group; while the average concentration copies of Parasutterella secunda were $5.49E6 \pm 1.04E7$ copies/g ($6.10 \pm 0.82 \log_{10}(\text{copies/g})$) in normal group and $1.35E8 \pm 2.30E8$ copies/g ($7.60 \pm 0.80 \log_{10}(\text{copies/g})$) in IBS group.

**Discussion**

IBS is a common functional disorder of the gastrointestinal tract. Recently, increasing evidence showed an important role of gut microbiota in the pathophysiology of IBS. Our team has written a systematic review of altered molecular signature of intestinal microbiota in IBS patients compared with healthy controls, and found that down-regulation of bacterial colonization including Lactobacillus (SMD = -0.85, $P < 0.001$, $I^2 = 28\%$), Bifidobacterium (SMD = -1.17, $P < 0.001$, $I^2 = 79.3\%$) and Faecalibacterium prausnitzii (SMD = -1.05, $P < 0.001$, $I^2 = 0.0\%$) was observed in IBS patients, particularly in IBS-D patients\(^\text{17}\). In this work, we found Bifidobacterium and Faecalibacterium down regulated in the IBS-D patients, but had no statistic difference ($P = 0.096$ and 0.381, respectively). Currently, different studies showed different specific microflora changes in IBS patients. The reason may because of the different living environment and diet structure leading to the different composition of flora of the subjects. In addition, different detection methods and technical methods may also lead to detection errors.

In the experiment, we found that the abnormal flora in IBS patients was not consistent with that in IBS mice after transplanting the intestinal flora. The reasons may be as follows. First of all, the different host intestinal conditions between human and mice make the microflora structure change after transplantation. Secondly, during the process of treatment and transplantation, some bacteria with weak activity and colonization may be lost. Last but not least, the early
detection time of intestinal flora in mice, the detection number of bacterial species was limited at that time and could not completely cover the detection results of intestinal flora in IBS patients. Nevertheless, sterile mice transplanted with fecal flora of IBS patients showed IBS related symptoms and chronic intestinal inflammation, suggesting that the occurrence and development of IBS may not be caused by the excess or absence of one bacterium. Different pathogens may cause similar symptoms. And in our experiments, we found *Parasutterella* significantly increased both in IBS patients and IBS mice, considering it one of the bacterium related to the occurrence and development of IBS.

*Parasutterella* is a genus of Gram-negative, coccobacilli, strictly anaerobic, non-s pore forming bacteria from the *Proteobacteria* phylum, *Betaproteobacteria* class and the *Sutterellaceae* family\(^\text{18, 19}\). In 2009, *Parasutterella excrementihominis*, one of the species of *Parasutterella*, was cultured and characterized\(^\text{18}\); and in 2011, *Parasutterella secunda*, another species of *Parasutterella*, was discovered\(^\text{19}\). *Parasutterella* is still a relatively new genus with limited literature. In a study concerning about the correlation between the intestinal flora and diet-induced obesity, *Parasutterella* was decreased in mice with diet-induced obesity and increased in controls and reversal of the high fat diet with the normal chow\(^\text{20}\). Additional studies suggested that the increased abundance of *Parasutterella* was associated with dysbiosis, or a decrease of intestinal flora diversity\(^\text{21, 22}\). Dysbacteriosis might be associated with many intestinal or metabolic disease, such as inflammatory bowel disease and obesity\(^\text{23}\). Recent studies have found that increase expression of *Parasutterella* in submucous ileum is associated with Crohn’s disease\(^\text{21}\) and hypertriglyceridemia-related acute necrotizing pancreatitis\(^\text{22}\). In this study, we found that *Parasutterella*, as well as the two species of *Parasutterella*, *Parasutterella excrementihominis* and *Parasutterella secunda*, were increased in the intestinal microbiota of the IBS patients and might associated with the chronic intestinal inflammation. More studies are needed to explore the role *Parasutterella* played in gut pathophysiological changes.

In conclusion, we found the 15 genuses significantly differentially expressed in the gut flora of IBS patients, and 6 genuses showed significant different abundances between the stool
specimens of mice of IBS group and normal group. Among them, *Parasutterella* was significantly differentially expressed in both screening and validation experiments, and also related with chronic intestinal inflammation, so it was considered associated with the development and progression of IBS.

**References**


[21] Chiodini RJ, Dowd SE, Chamberlin WM, Galandiuk S, Davis B, Glassing A. Microbial

This article is protected by copyright. All rights reserved.


Fig. 1. Diversity of flora community in IBS patients and normal donors. Venn chart (A) showed the flora community composition in different groups. ANOSIM/Adonis (B) and PLS-DA (C) showed a significant difference in flora community abundance between the two groups. Community barplot analysis (D) showed the flora community composition at the genus level.
Fig. 2. LEfSe method showed the different abundance of genus level in flora communities. Cladogram (A) of flora communities showed the difference from phylum to species level. LDA score (B) identified the size differentiation between IBS and normal groups with a threshold value of 2. Wilcoxon rank-sum test bar plot (C) showed the top 15 species with the most abundant expression at the genus level. *0.01 < P <= 0.05, **0.001 < P <= 0.01.
Fig. 3. The physiological and pathological manifestations of the IBS model. Fecal particle number had no statistical significance during the 5 weeks (A), while fecal characteristic showed diverse during the 5 weeks (B) after flora transplantation. *P < 0.05. There’s no significant difference of body weight growth between the IBS group and the normal group (C). We observed the morphology of the intestinal segments of mice by HE stains (D, magnification, 100×three pics above), and found a higher ratio of inflammatory cells/epithelial cells number in IBS group (E).
Fig. 4. Diversity of the flora community among different groups in animal experiment. Venn chart (A) showed the flora community composition in different groups. ANOSIM/Adonis (B) and PCoA (C) showed a significant difference in flora community abundance between the IBS and normal groups. Community barplot analysis (D) showed the flora community composition at the genus level.
Fig. 5. LEfSe method showed the different abundance of genus level in flora communities in animal experiment. Cladogram (A) of flora communities showed the difference from phylum to species level. LDA score (B) identified the size differentiation between IBS and normal groups with a threshold value of 2. Wilcoxon rank-sum test bar plot (C) showed the top 15 species with the most abundant expression at the genus level. *0.01 < P <= 0.05, **0.001 < P <= 0.01, ***P < 0.001.
Fig. 6. Relationship between microflora and chronic intestinal inflammation. Spearman test and heatmap to show the relationship between the bacterium and intestinal chronic inflammation.
Table 1. The differential expressed bacteria between IBS and normal group

<table>
<thead>
<tr>
<th>Genus name</th>
<th>P value</th>
<th>Lower CI</th>
<th>Upper CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>In patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Megamonas</td>
<td>0.006</td>
<td>-28.08</td>
<td>2.600</td>
</tr>
<tr>
<td>Mitsuokella</td>
<td>0.046</td>
<td>-2.065</td>
<td>7.160</td>
</tr>
<tr>
<td>Megasphaera</td>
<td>0.003</td>
<td>0.008</td>
<td>3.676</td>
</tr>
<tr>
<td>Prevotella_2</td>
<td>0.035</td>
<td>-4.498</td>
<td>1.200</td>
</tr>
<tr>
<td>Lachnospiraceae_UCG-004</td>
<td>&lt;0.001</td>
<td>0.717</td>
<td>1.570</td>
</tr>
<tr>
<td>Lachnospiraceae_NK4A136_group</td>
<td>0.031</td>
<td>-0.045</td>
<td>1.017</td>
</tr>
<tr>
<td>Tyzzerella_3</td>
<td>0.039</td>
<td>0.063</td>
<td>0.923</td>
</tr>
<tr>
<td>Dorea</td>
<td>0.049</td>
<td>-0.392</td>
<td>0.140</td>
</tr>
<tr>
<td>Tyzzerella</td>
<td>0.006</td>
<td>-0.060</td>
<td>0.857</td>
</tr>
<tr>
<td>Ruminococcaceae_UCG-013</td>
<td>0.016</td>
<td>0.065</td>
<td>0.307</td>
</tr>
<tr>
<td>Holdemanella</td>
<td>0.035</td>
<td>-0.632</td>
<td>0.186</td>
</tr>
<tr>
<td>Parasutterella</td>
<td>0.002</td>
<td>-0.367</td>
<td>0.066</td>
</tr>
<tr>
<td>Paraprevotella</td>
<td>0.030</td>
<td>-0.206</td>
<td>0.005</td>
</tr>
<tr>
<td>Coprococcus_1</td>
<td>0.007</td>
<td>0.017</td>
<td>0.107</td>
</tr>
<tr>
<td>Odoribacter</td>
<td>0.015</td>
<td>-0.103</td>
<td>0.001</td>
</tr>
<tr>
<td>In mice</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fusobacterium</td>
<td>0.003</td>
<td>-36.950</td>
<td>-12.190</td>
</tr>
<tr>
<td>Parabacteroides</td>
<td>0.013</td>
<td>1.222</td>
<td>7.786</td>
</tr>
<tr>
<td>Flavonifractor</td>
<td>0.033</td>
<td>0.232</td>
<td>3.660</td>
</tr>
<tr>
<td>Phascolarctobacterium</td>
<td>0.003</td>
<td>-2.916</td>
<td>-0.734</td>
</tr>
<tr>
<td>unclassified_f_Lachnospiraceae</td>
<td>0.028</td>
<td>-0.839</td>
<td>4.245</td>
</tr>
<tr>
<td>Parasutterella</td>
<td>0.009</td>
<td>-2.571</td>
<td>-0.349</td>
</tr>
</tbody>
</table>