Involvement of Mucosal Flora and Enterochromaffin Cell of Cecum and Descending Colon in Diarrhea-Predominant Irritable Bowel Syndrome

Jingze Yang
Shandong University Qilu Hospital
Peng Wang
Shandong University Qilu Hospital
Tong Liu
Shandong University Qilu Hospital
Lin Lin
Shandong University Qilu Hospital
Lixiang Li
Shandong University Qilu Hospital
Guanjun Kou
Shandong University Qilu Hospital
Ruchen Zhou
Shandong University Qilu Hospital
Pan Li
Shandong University Qilu Hospital
yanqing li (liyanqing@sdu.edu.cn)
Shandong University Qilu Hospital https://orcid.org/0000-0001-9325-4808

Research article

Keywords: gut microbiota, enterochromaffin cell, diarrhea-predominant irritable bowel syndrome, cecum and descending colon

Posted Date: July 8th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-103000/v2

Abstract

Background

The comparison between microbiota of cecal and colonic mucosa in irritable bowel syndrome (IBS) was rarely studied. In addition, enterochromaffin (EC) cell had interaction with IBS. The aim of this study was to investigate the relationship among gut microbiota, EC cell and diarrhea-predominant IBS (IBS-D) symptoms in cecum and descending colon.

Results

Total of 22 IBS-D patients and 22 health controls (HCs) were enrolled. The relative abundance of *Ruminococcus_torques_group* (4.91% vs. 2.20%, *P* = 0.04763) of cecum increased in IBS-D, while *Raoultella* (1.58% vs. 1.76%, *P* = 0.03117) and *Fusobacterium* (0.12% vs. 1.66%, *P* = 0.01892) were less abundant. In descending colon, the relative abundances of *Ruminococcus_torques_group* (5.94% vs. 2.29%, *P* = 0.04183) and *Dorea* (2.68% vs. 1.14%, *P* = 0.04962) of IBS-D increased but *Fusobacterium* (1.52% vs. 1.89%, *P* = 0.0345) decreased. EC cells number in cecum of IBS-D was higher than that in HCs and TPH1 level of IBS-D was higher than that of HCs in cecum and descending colon. Correlation analysis showed that *Ruminococcus_torques_group* were positively associated with HAM-A (r= 0.66, *P* = 0.004), HAM-D (r= 0.61, *P* = 0.009), EC cell number (r= 0.49, *P* = 0.047), IBS-SSS (r= 0.65, *P* = 0.004), Degree of Abdominal Pain (r = 0.63, *P* = 0.007), Frequency of Abdominal Pain (r = 0.63, *P* = 0.007), Frequency of Defecation (r = 0.60, *P* = 0.011). The abundance of *Dorea* were positively associated with EC cell number (r = 0.57, *P* = 0.018), IBS-SSS (r = 0.52, *P* = 0.034), HAM-A (r = 0.72, *P* = 0.001), HAM-D (r = 0.59, *P* = 0.012), Frequency of Abdominal Pain (r = 0.67, *P* = 0.003).

Conclusions

EC cells number increased in IBS-D patients and the expression of TPH1 was higher than HCs. In addition, our results suggested *Ruminococcus_torques_group* and *Dorea* may be targets for treatment of IBS-D but still need further studies.

Introduction

Irritable bowel syndrome (IBS) is one of functional gastrointestinal disorders and it affects about 3%-5% of adult population and severely impacts the quality of life[1]. It is characterized by diverse symptoms like abdominal pain or distension, constipation or diarrhea. According to the bowel habit, IBS is divided into diarrhea predominant (IBS-D), constipation predominant, mixed with both diarrhea and constipation and unsubtyped IBS. However, current treatment approaches of IBS are only modestly effective because of the undefined pathogenesis which is considered to be multifactorial [2].

IBS was reported to be associated with microbiota dysbiosis [3-7] and probiotic therapies were beneficial for IBS patients in the previous studies [8-10]. As for gut microbiota assessment, most studies selected

feces [5; 11; 12] as samples because of its convenience and accessibility. Nevertheless, the mucosal samples [13-15] maybe are better choices for microbiota research, which can reflect the organism's microbial community status. A systematic review summarized previous studies and suggested the lack of consistency among studies, mucosal microflora assessment in particular [16].

Microbiota plays a part in regulating serotonin (5-hydroxytryptamine, 5-HT) levels [17]. As an important neurotransmitter, 5-HT is connected with altered motility and visceral discomfort and takes part in the pathopoiesis of IBS [18]. The vast majority of 5-HT is synthesized by enterochromaffin (EC) cell, one of the most abundant enteroendocrine cells in the entire gastrointestinal tract, and stored in large dense core vesicles with acidic proteins such as chromogranin A (CgA). EC cell expresses various receptors which detect mechanical or chemical stimulations including pro-inflammatory mediators, bacterial metabolites, chemical irritants and nutrients. A study revealed that intestinal EC cells were larger in germ-free rats than specific-pathogen-free rats, which suggested that the microbiota may influence the growth or function of EC cells [19].

Intestinal flora has heterogeneous microbial communities in different segments of gut in animal and the cecum, an ideal habitat for a variety of microorganisms, is the most abundant part of intestinal flora [20]. In clinical studies, by comparing the contents of human cecum with feces, Marteau found that there was a significant difference in the composition of bacteria [21]. In cecum, microbiota produce higher concentrations of short-chain fatty acids (SCFAs) and metabolites, which promotes the production of 5-HT by increasing the expression of the rate-limiting enzyme tryptophan hydroxylase 1 (TPH1) [17]. However, the microbiota in cecal mucosa of people was rarely studied. With the stability of flora among individuals, colonic mucosa is a good choice for the microbiota researches [22]. Accordingly, we aimed to investigate the difference between cecum and descending colon about mucosal-associated microbiota and EC cell in IBS-D patients.

Methods

Participants and Questionnaires

IBS-D patients and healthy controls (HCs) were recruited from Gastroenterology Outpatient Department at Qilu Hospital, Shandong University. IBS-D patients were diagnosed by a gastroenterologist according to ROME IV criteria. The HCs were free of gastrointestinal symptoms and to attend routine health check-up. Exclusion criteria included history of any organic syndromes (coeliac disease, tumors, inflammatory bowel disease, gastrointestinal infections and psychiatric disorders), history of major abdominal surgery or using probiotics, non-steroidal anti-inflammatory drugs, anti-inflammatory drugs, Proton-Pump Inhibitors, prokinetics, antianxiety drugs, antidepressants in the past 4 weeks, accompanying with pregnancy, lactation, bladder irritation, chronic pelvic pain syndrome, dysmenorrhea, endometriosis, other painful gynecological diseases, clotting disorder and obvious mental disorders. In addition, the individuals with long-term alcohol consumption were also ruled out. All participants provided written

informed consent and the study was approved by the ethics committee of Qilu Hospital of Shandong University (ethic's number: 2019036).

The information of subjects was collected by completing questionnaires. The common information of HCs and IBS-D patients included sex, age, body mass index (BMI), Hamilton anxiety scale (HAM-A) [23] [23] [22] [24] [23] [23] [23] [23], Hamilton depression scale (HAM-D) [24]. In addition, the questionnaires of IBS-D patients included clinical symptoms and IBS Severity Scores (IBS-SSS) [25].

Subjects underwent colonoscopy and 3 biopsies from cecum and 3 biopsies from descending colon respectively were obtained using endoscopic biopsy forceps (MIN-BF-23; MICRO-TECH, Nanjing, China) during colonoscopy withdrawal. One of biopsy samples from cecum and descending colon respectively were fixed in formalin and the others were stored in liquid nitrogen immediately.

Immunofluorescence

The mucosa was fixed in formalin for 72h and then was embedded in paraffin. The paraffin block was cut into 4 μm sections using a cryostat and was mounted onto glass slides. The sections were incubated in rabbit anti-CgA antibody (1:100; Abcam, Cambridge, UK) in a humidified box at 4°C overnight and after that in Alexa Fluor 488-conjugated anti-rabbit IgG (1:400; Abcam) in a black humidified box at room temperature for 1 hour and in fluoroshield mounting medium with DAPI (Abcam) for 5 minutes. Finally, the sections were observed by fluorescence microscope (BX53, OLYMPUS, Tokyo, Japan). EC cells were identified in the crypt epithelium and showed strong nuclear and cytoplasmic staining. The number of EC cells was quantified with methods described by the previous studies [26-28]. Total of 5 nonoverlapping high-power fields (final magnification 200×) were chosen and 2 persons who were blinded to clinical data analyzed the number of EC cells.

Quantitative Real Time Polymerase Chain Reaction

The total RNA was extracted using AllPrep DNA/RNA/Protein Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. Quantitative Real Time Polymerase Chain Reaction (QRT-PCR) reactions were made by the SYBR® Green Realtime PCR Master Mix (QPK-201, TOYOBO) in a 20μl reaction volume containing the following reagents: 1μl of cDNA preparation; SYBR® Green Realtime PCR Master Mix (TOYOBO); 7.4μl PCR grade water, and 0.8μM of forward and reverse primers. QRT-PCR reactions were performed on an Applied Biosystems Stepone real-time PCR System (Thermo, Waltham, MA, USA) in triplicate using the following conditions for pre-denaturation, denaturation, annealing and extension (40 cycles): 95˚C for 1 min, 95˚C for 15 s and 58˚C for 15 s, followed by 72˚C for 45s. The specific primers sequences were as follows (5′ - 3′): TPH1: forward CTGGTTATGCTCTTGGTGTCTTTC, reverse TGCAAAGGAGAAGATGAGAGAATTTAC′ β-actin: forward ATGATGATATCGCCGCGCTC, reverse CCACCATCACGCCCTGG.

Western blot

Total of 20μg extracted protein as experimental samples were separated by 10% SDS–PAGE and transferred to polyvinylidenedifluoride membranes (Bio-Rad, Hercules, CA, USA). Then, the membranes were incubated into 5% skim milk for 2 h at room temperature. After that, the membranes were exposed to primary antibody overnight at 4˚C and were incubated for 1 h at room temperature using secondary antibody. Finally, the enhanced chemiluminescence kit (Merck Millipore, Darmstadt, Germany) was used to reveal the reaction products. The antibodies used in this study were as follows: rabbit anti-TPH1 (1:500; Abcam), anti-β-actin antibody (1:1000; Zhongshan Gold Bridge, Beijing, China), horseradish peroxidase-conjugated goat anti- rabbit IgG (1:1000; Zhongshan Gold Bridge), and goat anti-mouse IgG (1:1000; Zhongshan Gold Bridge). The results were quantified by using Image J software (National Institutes of Health, Bethesda, MD, USA).

Mucosal microbiota assessment

The microbial DNA was extracted from the biopsy samples from IBS-D patients and HCs, and then 16S rRNA sequencing was used on an Illumina MiSeq platform (San Diego, CA). as previously described[29]. The microbial composition and biodiversity were analyzed. In addition, Venn diagrams were made to express the difference of OTUs and genera between the groups. Moreover, Correlation analysis was conducted to show the relation between variables (EC cells number, HAM-A, HAM-D, IBS-SSS, Degree of Abdominal Pain, Frequency of Abdominal Pain, Frequency of Defecation) and microbial abundance.

Statistical analysis

Data were analyzed by SPSS 22.0 and R 3.1.1. The data were expressed as means ± SD for continuous variables and percentages for categorical variables. Differences between two groups were evaluated by independent - samples t-test or non-parametric test according to the data in normal distribution or not. Categorical variable data was analyzed by the chi-square test. The correlation between variables and bacterial relative abundance were assessed by using Spearman correlation analysis. *P* value less than 0.05 was regarded as significant.

Results

Clinical features of IBS-D patients and HCs

Total of 22 HCs and 22 IBS-D patients were recruited in this study and the detailed information of participants was present in Table 1. There were no differences in the aspect of sex, age and BMI ($P > 0.05$) between two groups. However, the HAM-A scores, HAD-M scores and frequency of defecation were higher in IBS-D patients than in HCs ($P < 0.0001$).

Table1. Clinical and demographic features of all study subjects

BMI, Body Mass Index; HAM-A, Hamilton anxiety scale; HAM-D, Hamilton depression scale.

Microbiota diversity in IBS-D patients and HCs

	IBS-D patients	HCs	*P*
Age, mean ± SD, years	(46.7±10.8)	(44.4±9.9)	0.469
Sex, Male/female, n (%)	9/13(41/59)	15/7(68/32)	0.564
BMI	22.6±2.3	24.1±2.5	0.066
HAM-A	6.64±3.71	1.36±1.14	< 0.0001
HAM-D	4.00±3.15	1.27±0.98	< 0.0001
Frequency of Defecation	4.59±2.06	1.18±0.39	< 0.0001
Degree of Abdominal Pain	50.00±14.80	-	-
Frequency of Abdominal Pain Duration of Disease	59.09±21.80	-	-
IBS-SSS	4.64±2.72	-	-
	194.55±51.71	-	-

Microbiota diversity was analyzed by conventional classical ecological descriptive approaches including alpha-diversity (richness as measured by number of OTUs and assessed by chao index) and beta-diversity metrics (principal co-ordinates analysis and bray-curtis distance using bacterial genus relative abundance). The results showed no difference in microbiota richness and microbiota variability between IBS-D patients and HCs. (Figure S1A and B).

Venn diagram analysis in IBS-D patients and HCs

For HCs, 770 OTUs and 333 genera were identified in descending colon and 776 OTUs and 331 genera in cecum. A total of 741 OTUs and 322 genera were shared in mucosa of descending colon and cecum. Meanwhile, IBS-D samples showed that 756 OTUs and 327 genera were identified in descending colon and 741 OTUs and 326 genera in cecum. A total of 705 OTUs and 315 genera were shared by descending colon and cecum (Figure 1).

Differences of mucosal-associated microbiota between IBS-D patients and HCs

The community composition analysis showed that the common gut microbiota were Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria and Fusobacteria. However, there was no statistical difference between IBS-D patients and HCs on the level of phylum (Figure S2). Among bacterial preponderant genera, the relative abundances of *Ruminococcus_torques_group* and *Dorea* were increased in descending colon of IBS-D patients compared with that of HCs (5.94% vs. 2.29%, $P = 0.04183$; 2.68% vs. 1.14%, $P = 0.04962$), while *Fusobacterium* was decreased in IBS-D patients (1.52% vs. 1.89%, $P = 0.0345$). Compared with microbiota of cecum in HCs, IBS-D patients showed the increased relative abundance of *Ruminococcus_torques_group* (4.91% vs. 2.20%, $P = 0.04763$) and the decreased relative abundance of *Fusobacterium* and *Raoultella* (1.58% vs. 1.76%, $P = 0.03117$; 0.12% vs. 1.66%, $P = 0.01892$) (Figure 2).

As for the comparison about mucosal associated microbiota between descending colon and cecum, there were no differences both on the level of phylum and genus (Figure S2, Figure S3).

EC cells number and TPH1 expression

The numbers of EC cells in HCs were 5.10±3.03 in cecum and 7.32±4.40 in descending colon. In contrast, in IBS-D patients, the numbers of EC cells were 8.37±5.78 in cecum and 9.27±5.96 in descending colon. The number of EC cells was significantly increased in cecum of IBS-D patients than that of HCs (P = 0.029, Figure 3). Transcriptional level of TPH1 was upregulated in descending colon (P = 0.0427, Figure 4) and cecum (P = 0.0218, Figure 4) in IBS-D patients. In addition, western blot analysis revealed that the expression of TPH1 in IBS-D patients was also significantly increased in descending colon (P = 0.0326, Figure 4) and cecum (P = 0.0424, Figure 4)

Spearman correlation between the relative abundance of microbiota and clinical parameters

We further evaluated the correlation between relative abundance of microbiota and the number of EC cells, IBS-SSS, Degree of Abdominal Pain, Frequency of Abdominal Pain, Frequency of Defecation, HAM-A and HAM-D. *Ruminococcus_torques_group*, which were increased in IBS-D patients, were positively associated with HAM-A (r= 0.66, P = 0.004), HAM-D (r= 0.61, P = 0.009), EC cell number (r= 0.49, P = 0.047), IBS-SSS (r= 0.65, P = 0.004), Degree of Abdominal Pain (r = 0.63, P = 0.007), Frequency of Abdominal Pain (r = 0.63, P = 0.007), Frequency of Defecation (r = 0.60, P = 0.011). *Dorea*, which were increased in IBS-D patients, were positively associated with EC cell number (r = 0.57, P = 0.018), IBS-SSS (r = 0.52, P = 0.034), HAM-A (r = 0.72, P = 0.001), HAM-D (r = 0.59, P = 0.012), Frequency of Abdominal Pain (r = 0.67, P = 0.003). (Figure 5).

Discussion

IBS-D has a great influence on health-related quality of life but the etiology remains poorly understood. In this study, we investigated EC cells and gut microbiota in IBS-D patients and firstly showed that there was a link among gut microbiota, EC cells and IBS-D symptoms. Not only gut microbiota changed in IBS-D patients, but also EC cells and TPH1 increased in IBS-D patients. What's more, *Ruminococcus_torques_group* and *Dorea* have a positive relationship with EC cells number and IBS symptom scores. Beyond that, comparison between IBS-D patients and HCs, this study found the microbiome variation in cecum and descending colon.

Correlation analysis revealed the positive relation between *Ruminococcus_torques_group* and the number of EC cells, which indicated EC cell can be affected by gut microbiota. Mandić showed the increased expression of leucine-rich repeat-containing G-protein-coupled receptor 5, an EC stem cell marker, in mice monoassociated with C. ramosum than germ-free mice, and suggested gut microbiota can influence the development of colonic epithelial progenitor cells towards EC cell [19]. EC cell was triangular-shaped and microvilli of the apex can extend into lumen to transmit luminal stimuli. Metabolites produced by microbiota like SCFAs can act on host colonic EC cell and regulate the peripheral 5-HT mainly by

affecting the expression of TPH1 [17]. In our study, we found that the expression of TPH1 and the relative abundance of *Ruminococcus_torques_group* both increased in IBS-D patients, which suggested the SCFA-producing *Ruminococcus_torques_group* might improve the expression of TPH1 and the synthesis of 5-HT.

Intestinal 5-HT plays an important role in intestinal motility and visceral sensation. Therefore, the increase of 5-HT in gut may be related to the generation of IBS symptoms. Treatment with TPH inhibitor or 5-HT_3 receptor antagonists were effective at relieving the symptoms of IBS [30]. As a chief source of 5-HT, the number of EC cells were increased in intestinal mucosa of IBS, postinfectious IBS in particular [26; 27; 31], which was in accordance with our results. However, current treatments are mainly targeted on reducing the availability of 5-HT and have no influence on EC cells number. It was reported that physiological stress in early life promoted differentiation of intestinal epithelial stem cells into enteroendocrine cells and induced EC cell proliferation in adult. In addition, infection and inflammation can also influence the EC cell number [32]which suggested the new therapeutic strategies for IBS.

The result of mucosal microbiota assessment showed no difference in microbiota diversity between IBS-D patients and HCs. There were studies in line with our results but also studies reporting the opposite[33], which needs further researches. However, we found that IBS-D patients showed more difference between descending colon and cecum bacterial composition both in OTUs and genera level, which suggested the change of gut microbiota may influence IBS-D patients. It may work through metabolites derived from microbiota including SCFAs, secondary bile acids, tryptophan metabolites, which transmit the signals by interacting with enteroendocrine cells, such as EC cells. SCFAs are produced by some bacterial fermentation of undigested carbohydrate. Meanwhile, the gases like carbon dioxide, hydrogen, and methane are also created, which may be connected with abdominal pain and bloating in IBS patients. Beyond that, protein residues can be fermented by bacteria to produce a variety of metabolites, including ammonia, organic acids, heterocyclic amides, phenols and indoles, which affect intestinal epithelial metabolism and thus damage intestinal health. In addition to participating in the fermentation of various ingredients in food, the intestinal microbiota can also regulate the levels of neuroactive molecules, such as nitric oxide, substance P and endocannabinoids, thereby affecting intestinal motility and visceral sensation. Gut microbiota was also related to mood disorders. Our results revealed HAM-A and HAM-D of IBS-D patients were higher than that of HCs. Besides that, we found the relative abundance of some bacteria were associated with HAM-A and HAM-D. Inflammation resulted from exotoxins secreted by certain bacteria can be transmitted to the brain, leading to mental illnesses. Sudo showed the stress responsiveness of adult can be influenced by absence of normal intestinal flora in early life and the changes can be partly reversed by early colonization of conventional gut microbiota [34]. The prophylactic effects of microbiota on anxiety and depression scores were also demonstrated and fecal microbiota transplantation can affect anxiety-like and depression-like behavior [35; 36]. Flora may be the future directions of therapy on the treatment of mood disorders.

In our study, we found *Ruminococcus_torques_group* and *Dorea* increased in IBS-D patients and correlation analysis showed positive relation among relative abundance of *Ruminococcus_torques_group*

and *Dorea* with IBS-SSS, which indicated that *Ruminococcus_torques_group* and *Dorea* played important roles in the IBS-D patients. By resolving intestinal mucin, *Ruminococcus_torques_group* can damage mucosal barrier and facilitate the IBS. Lyra revealed *Ruminococcus_torques decr*eased in the group of probiotics treatment compared with placebos[37], which suggested the pathogenicity of *Ruminococcus_torques* in IBS-D. Erja assessed the fecal flora of IBS patients and showed the abundance of *Ruminococcus_torques_group* was positively correlated with the severity of bowel symptoms[38]. *Dorea* can promote the production of intestinal gases and contribute to abdominal discomfort. Beyond that, we found the decreased abundance of *Fusobacterium* and *Raoultella* in IBS-D patients. It was reported that *Fusobacterium* may be related to the colonic adenoma [39] and inflammatory bowel disease [40] and *Raoultella* may be associated with urinary tract infection and biliary tract disease [41]. However, the connection with IBS needs further investigation.

Intestinal flora in the cecum has been found to play an important role in maintaining homeostasis in animal experiments. As the main fermentation site, cecum serves as a "bag" structure between the colon and small intestine in mouse. Portela-gomes found EC cells were mainly located in cecum and gradually decreased from the proximal colon to the distal colon in rats. In this study, comparison between cecum and descending colon mucosa, there was no significant difference in the number of EC cells, TPH1 expression, mucosal microbiota. Given that the human cecum is structurally quite different from that of rodents, its function may also be significantly different.

In conclusion, this study showed the correlation among the number of EC cells, mucosal-associated microbiota and IBS-D symptoms. *Ruminococcus_torques_group*, *Dorea* and EC cells may be considered as targets in the treatment of IBS. As for the bacterial variation in IBS-D patients, there was difference in the cecum and descending colon, which needs to pay attention in the future study.

Abbreviations

IBS: Irritable Bowel Syndrome; IBS-D: Diarrhea-Predominant IBS; 5-HT: 5-Hydroxytryptamine; EC: Enterochromaffin; CgA: Chromogranin A; TPH1: Tryptophan Hydroxylase 1; SCFAs: Short Chain Fatty Acids; HCs: Health Controls; HAM-A: Hamilton Anxiety Scale; HAM-D: Hamilton Depression Scale; IBS-SSS: IBS Severity Scores;

BMI: Body Mass Index; QRT-PCR: Quantitative Real Time Polymerase Chain Reaction; OTUs: Operational Taxonomic Units

Declarations

Ethics approval and consent to participate

The study was approved by the ethics committee of Qilu Hospital of Shandong University (ethic's number: 2019036). All participants provided written informed consent before enrollment.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by the National Natural Science Foundation of China (81873550), Key Research and Development Program of Shandong Province (2018CXGC1209), National Key R&D Program of China (2017YFC0109700), Natural Science Foundation of Shandong Province (ZR2016HB62) for laboratory supplies and research materials, and this study was also supported by the Taishan Scholars Program of Shandong Province and National Clinical Research Center for Digestive Diseases supporting technology project (2015BAI13B07) for supporting graduate student publication fees.

The funder of ZR2016HB62, P W designed the study and reviewed the manuscript.

The funder of 81873550, 2018CXGC1209, 2017YFC0109700 and 2015BAI13B07. YQ L reviewed the manuscript and gave some advices.

Authors' contributions

JZ Y designed the study, conducted the experiment, analyzed the result and wrote the article. P W designed the study and reviewed the manuscript. T L reviewed the manuscript and gave some advices. L L collected the subjects and designed the experiment. LX L designed the experiment and reviewed the manuscript. GJ K collected the subjects and reviewed the manuscript. RC Z conducted the experiment and reviewed the manuscript. P L collectd the subjects and reviewed the manuscript. YQ L reviewed the manuscript and gave some advices.

All authors have read and approved the manuscript.

Acknowledgements

Not applicable.

Author details

[1] Department of Gastroenterology, Qilu Hospital, Cheloo College of Medicine, Shandong University，Jinan, Shandong, China

[2] Laboratory of Translational Gastroenterology, Qilu Hospital, Cheloo College of Medicine, Shandong University, Jinan, Shandong, China

[3] Robot engineering laboratory for precise diagnosis and therapy of GI tumor，Qilu Hospital, Cheloo College of Medicine, Shandong University, Jinan, Shandong, China

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Figures

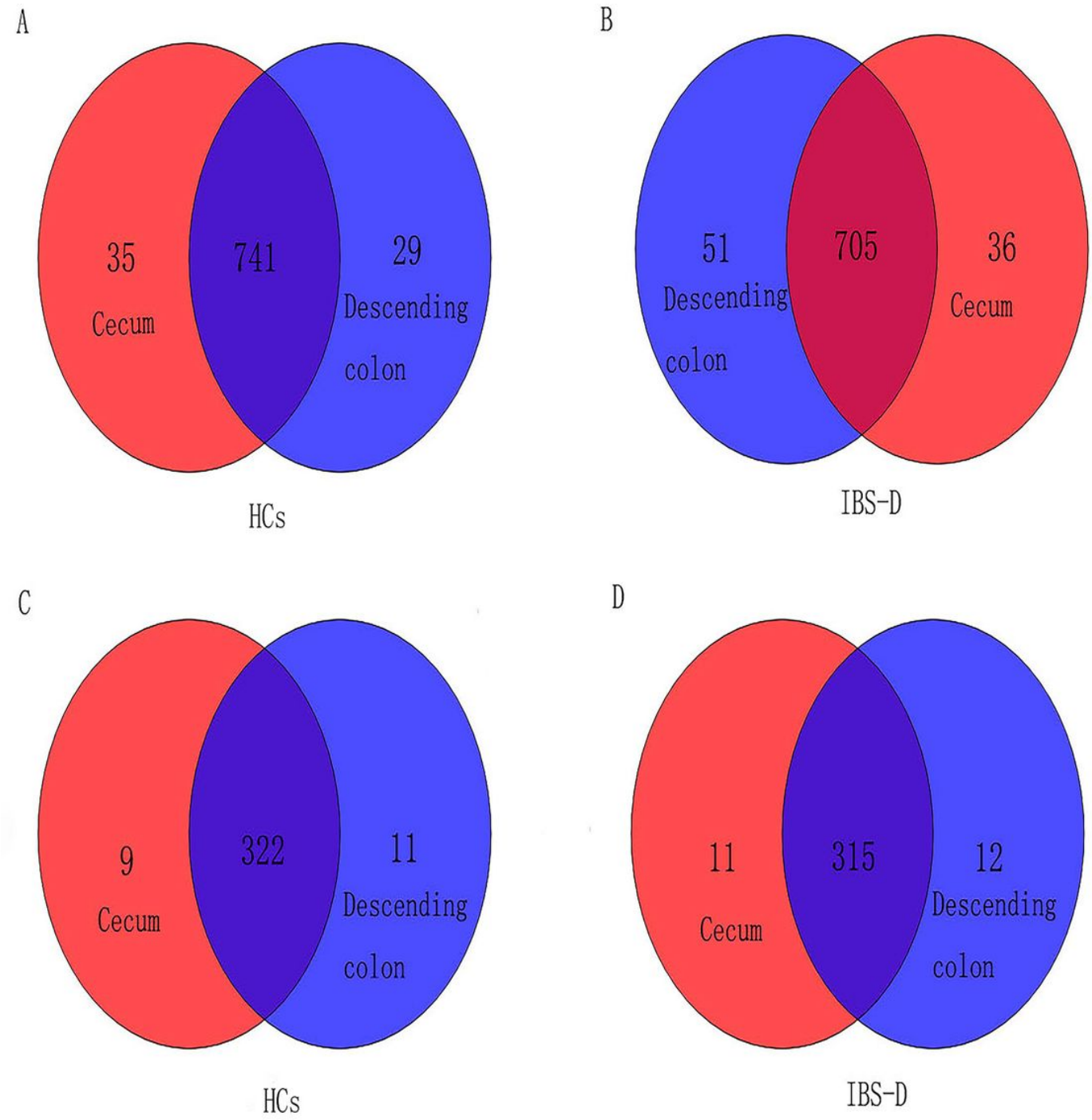

Figure 1

Shared OTUs and genera between the descending colon and cecum in the HCs and IBS-D patients were shown by Venn diagrams. a. The numbers of shared and separated OTUs from the descending colon and cecum in HCs. b. The numbers of shared and separated OTUs from the descending colon and cecum in IBS-D patients. c. The numbers of shared and separated genera from descending colon and cecum in HCs. d. The numbers of shared and separated genera from descending colon and cecum in IBS-D patients. (blue color referring to descending colon, red color referring to cecum).

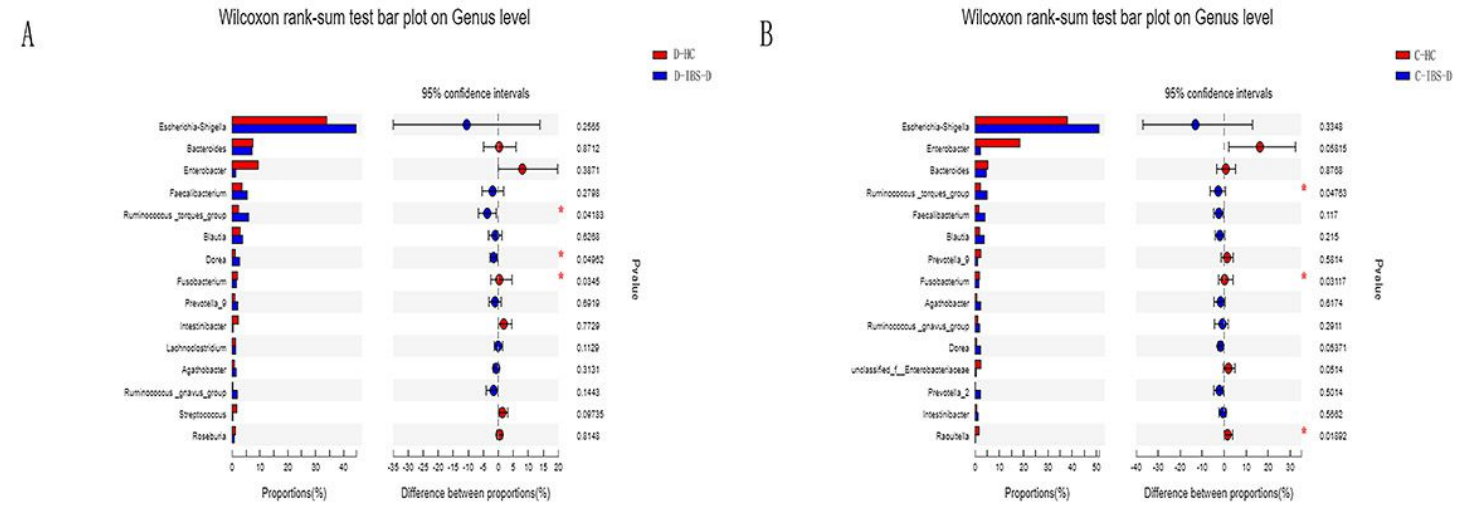

Figure 2

Relative abundances of dominant genera in descending colon and cecum in the HCs and IBS-D patients. a. Relative abundances of genera in descending colon in the HCs and IBS-D patients. b. Relative abundances of genera in cecum in the HCs and IBS-D patients (D-HC refer to the descending colon in HCs, D-IBS-D refer to the descending colon in IBS-D patients, C-HC refer to the cecum in HCs, C-IBS-D refer to the cecum in IBS-D patients) * P value < 0.05.

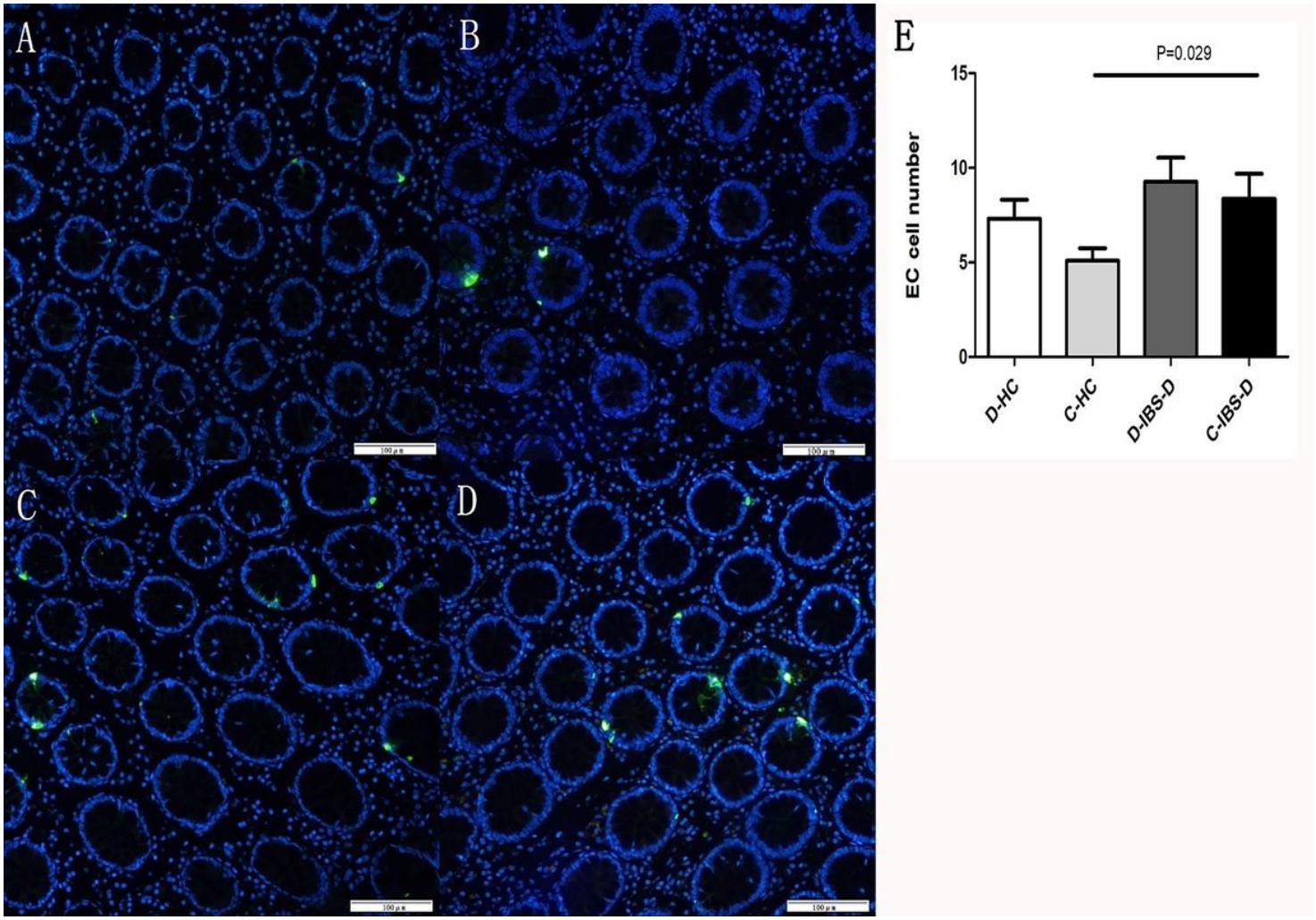

Figure 3

Immunofluorescence analysis of EC cell in the IBS-D patients and HCs a. Representative image of EC cell (green) in the descending colon of the HCs, 200×. b. Representative image of EC cell (green) in the cecum of the HCs, 200×. c. Representative image of EC cell (green) in the descending colon of the IBS-D patients, 200×. d. Representative image of EC cell (green) in the cecum of the IBS-D patients, 200×. e. The EC cells number in descending colon and cecum in the HCs and IBS-D patients and the number of EC cell of the cecum in IBS-D patients was more than in HCs. The data were displayed as mean±SD; (D-HC refer to the descending colon in HCs, D-IBS-D refer to the descending colon in IBS-D patients, C-HC refer to the cecum in HCs, C-IBS-D refer to the cecum in IBS-D patients)

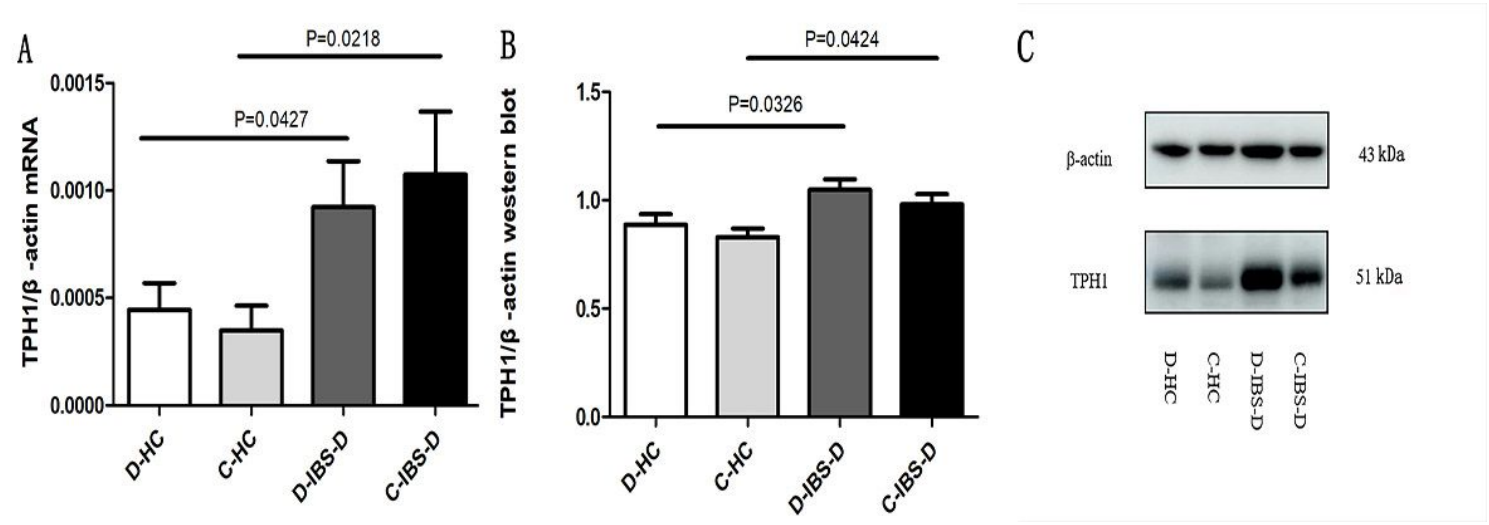

Figure 4

The expression of TPH1 in the IBS-D patients and HCs a. Quantitative Real Time Polymerase Chain Reaction analysis of Tph1/β-actin in the subjects. b. Western blot analysis of Tph1/β-actin in the subjects. c. Western blot analysis of Tph1 and β-actin in the subjects. Stripped blots were re-probed with β-actin. Data were normalized with housekeeping protein (β-actin). The full-length blots or gels are presented in Supplementary Fig. S4. (D-HC refer to the descending colon in HCs, D-IBS-D refer to the descending colon in IBS-D patients, C-HC refer to the cecum in HCs, C-IBS-D refer to the cecum in IBS-D patients)

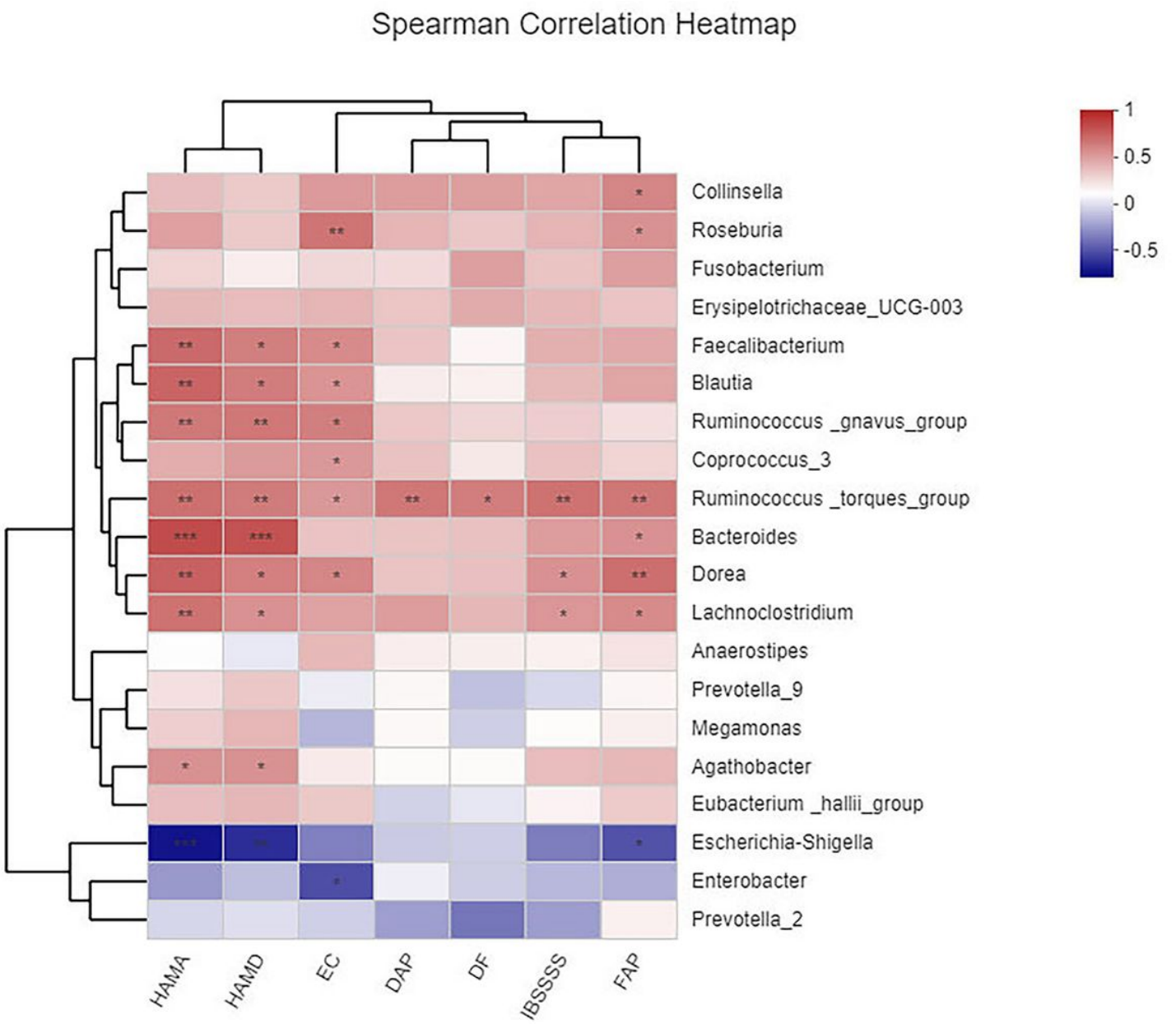

Figure 5

Relationships among the relative abundance of microbial communities and clinical parameters based on the Spearman correlation analysis (HAM-A: Hamilton Anxiety Score; HAM-D: Hamilton Depression Score; EC: Enterochromaffin Cell Number; IBSSSS: IBS Symptom Severity System; DAP: Degree of Abdominal Pain; DF: Defecation Frequency; FAP: Frequency of Abdominal Pain); ***P < 0.001; **P < 0.01; *P < 0.05.

Supplementary Files

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