

# Sex-based differences in fecal short-chain fatty acid and gut microbiota in irritable bowel syndrome patients

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**Objective:** To explore alterations in fecal short-chain fatty acids (SCFA) and gut microbiota in patients with diarrhea-predominant irritable bowel disease (IBS-D) and their relationships with clinical manifestations.

**Methods:** We recruited 162 patients with IBS-D and 66 healthy controls (HC). Their manifestations and psychological status were evaluated using the IBS severity scoring system and the Hospital Anxiety and Depression Scale (HADS). Colorectal visceral sensitivity was evaluated using a barostat. Systemic inflammation was evaluated using plasma cytokine levels. Fecal SCFA were quantified using ultra-performance liquid chromatography-tandem mass spectrometry, and fecal microbiota communities were analyzed using 16S rRNA sequencing.

**Results:** More men presented with IBS-D than women in our patient cohort. Patients with IBS-D had more severe manifestations, higher HADS score, and a higher rate of previous infectious enteritis than HC. Notably, female patients had significantly higher HADS scores than male patients. Male patients had significantly higher levels of plasma interleukin (IL)-12, fecal propionate and colorectal visceral sensitivity than male HC, while no differences were observed between female patients and female HC. Fecal acetate, butyrate and valerate correlated with the initial visceral sensory threshold, stressors, and IL-10 and IL-12 levels. The propionate-producing *Prevotella 9* genus was significantly increased in male patients and positively correlated with fecal propionate.

**Conclusion:** Distinct sex-based differences in clinical manifestations, fecal SCFA and microbiota richness are found in Chinese patients with IBS-D, which may be used to diagnose dysbiosis in these patients.

## KEYWORDS

cytokine, diagnosis, gut microbiota, irritable bowel syndrome, volatile fatty acid

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## 1 | INTRODUCTION

Irritable bowel syndrome (IBS) is a prevalent functional gastrointestinal disorder that presents as abdominal pain with altered bowel habits. The prevalence of IBS ranges from 1.1% to 35.5% worldwide, and from 9.5% to 9.8% in Asia. Diarrhea-predominant IBS (IBS-D) is the most prevalent subtype of IBS, accounting for approximately 40% of all cases.<sup>1</sup> The pathophysiology and etiology of IBS remain unclear. Visceral hypersensitivity, gastrointestinal dysmotility, low-grade inflammation of the intestinal mucosa, and dysfunction of the brain-gut interaction have been considered to participate in the pathogenesis of IBS, all of which may be associated with intestinal immune dysfunction related to altered compositions of intestinal microbiota and their metabolites.<sup>2</sup>

Fecal short-chain fatty acids (SCFA) are the most important metabolites of the intestinal flora that play important roles in the pathogenesis of IBS.<sup>3</sup> We have conducted a systematic review and meta-analysis and found that the levels of fecal SCFA differ between IBS patients and healthy controls (HC), suggesting that fecal SCFA may be regarded as potential diagnostic biomarkers of IBS.<sup>4</sup> Nevertheless, the relationship between changes in the content of fecal SCFA and clinical manifestations of the patients with IBS remains undetermined. Furthermore, recent studies have revealed that men and women have sex-specific differences in their immune systems and gut microbiota composition.<sup>5,6</sup> Therefore, we conducted a sex-based case-control study in patients with IBS-D to explore changes in fecal SCFA and gut microbiota and their relationships with clinical manifestations of the disease.

## 2 | PARTICIPANTS AND METHODS

### 2.1 | Participants

Patients with IBS-D were consecutively enrolled from the Outpatient Department of Gastroenterology, Peking University Third Hospital (Beijing, China) from March 2015 to December 2017. The inclusion criteria of the patients were as follows: (a) patients who met the Rome III diagnostic criteria for IBS-D;<sup>7</sup> (b) aged 18–65 years; (c) negative for blood routine test, serum biochemistry, routine stool test, fecal occult blood test, and fecal parasite screening; and (d) exclusion of organic gastrointestinal diseases, such as microscopic colitis, Crohn's disease, ulcerative colitis, and cancer, on colonoscopy and biopsy. The exclusion criteria were as follows: (a) with organic gastrointestinal diseases; (b) having serious comorbidities or complication of the cardiovascular system, liver, lung, kidney, blood, endocrine, and nervous system, or autoimmune diseases; (c) current infection of the respiratory, digestive, or urinary system; (d) history of abdominal surgery other than appendectomy; (e) use of antibiotics within one month, or use of probiotics, laxatives, or antidiarrheal drugs within two weeks before their enrollment; and (f) pregnancy or lactation.

An age- and sex-matched asymptomatic HC group was recruited from the surrounding communities and WeChat groups, with the

following inclusion criteria: (a) aged 18–65 years; (b) with no gastrointestinal symptoms or abnormal results for blood routine test, serum biochemistry, routine stool test, fecal occult blood test, and fecal parasite screening; and (c) negative colonoscopic findings. The exclusion criteria of the HC group were the same as those applied to the IBS-D patients.

All participants provided complete information in a case report form and completed an IBS severity scoring system (IBS-SSS),<sup>8</sup> the Hospital Anxiety and Depression Scale (HADS),<sup>9</sup> and indicators of current stressors.<sup>10</sup> The maximum score of IBS-SSS is 500, which represents the sum of five individual scores for the severity of abdominal pain, frequency of abdominal pain, distension, dissatisfaction with bowel habits, and quality of life measure. The HADS score is used to evaluate the psychological condition of the participants. And current stressors were evaluated according to the following categories: economic status, work and study, family relationships, interpersonal relationships, and intimate relationships. Each category was awarded one point.

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Medical Ethics Committee of Peking University Third Hospital (no. IRB00001052-14091). Written informed consent was obtained from each participant prior to their enrollment. A flowchart of participant recruitment is shown in Figure 1.

### 2.2 | Evaluation of visceral sensitivity

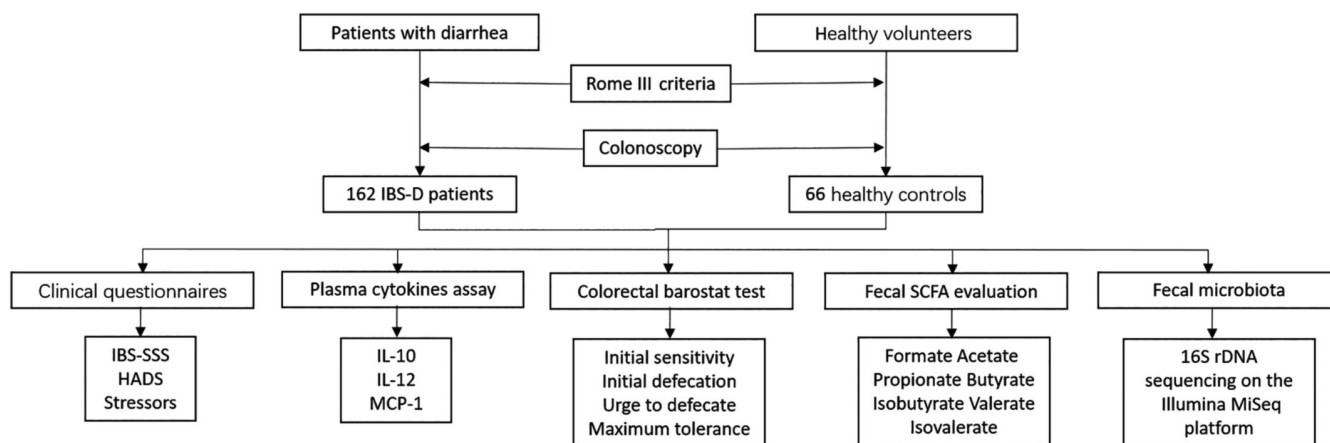
Visceral sensitivity was evaluated using a barostat (Distender II; G&J Electronics, Toronto, Canada) as described in the previous study.<sup>11</sup> When performing the study, the rectum was empty of feces on a digital rectal examination. The participants then underwent sequential conditioning isobaric distensions of 4 mmHg every 60 seconds to a maximum of 60 mmHg or patient tolerance. The pressure of the initial sensitivity (the minimum pressure at which the participants perceived rectal distension), initial defecation (the minimum pressure at which the participants felt awareness of impending defecation) and urge to defecate (the minimum pressure at which the participants felt the urge to defecate), as well as the maximum tolerance threshold (pressure at which the participants reported intolerable discomfort), were recorded.

### 2.3 | Assessment of plasma cytokines

Plasma levels of monocyte chemotactic protein-1 (MCP-1), interleukin (IL)-10, and IL-12 were measured using the enzyme-linked immunosorbent assay kits (BMS281, BMS215/2 and BMS238, respectively; eBioscience, San Diego, CA), and the ratio of IL-10 to IL-12<sup>12</sup> were calculated for the assessment of systematic inflammation.

### 2.4 | Analysis of fecal SCFA

Fresh stool samples were collected from all participants and were immediately stored at  $-80^{\circ}\text{C}$  until analysis. Fecal levels of formate,



**FIGURE 1** Flowchart of participant enrollment. Abbreviations: HADS, Hospital Anxiety and Depression Scale; IBS-D, diarrhea-predominant irritable bowel disease; IBS-SSS, irritable bowel disease severity scoring system; IL, interleukin; MCP-1, monocyte chemotactic protein-1; SCFA, short-chain fatty acids

acetate, propionate, butyrate, isobutyrate, valerate and isovalerate were quantified using a C-labeled chemical derivatization method and a slightly modified ultra-performance liquid chromatography-tandem mass spectrometry method.<sup>13,14</sup> The raw mass spectrometry data were processed and analyzed using the Xcalibur version 3.0 (Thermo Fisher Scientific, Carlsbad, CA).

## 2.5 | DNA extraction and sequencing of fecal microbiota

DNA was extracted from fecal samples with the E.Z.N.A. Soil DNA Kit following the manufacturer's instructions (Omega Bio-Tek, Norcross, GA). The hypervariable regions (V3 to V4) of the bacterial 16S ribosomal RNA gene were amplified with two primers, 338F (5'-ACTC CTACGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). Polymerase chain reaction was performed as follows: denaturation at 95°C for 3 minutes; 27 cycles at 95°C for 30 seconds, at 55°C for 30 seconds and at 72°C for 45 seconds; and a final extension at 72°C for 10 minutes. Purified amplicons were pooled in equimolar amounts. Sequencing was performed using the Illumina MiSeq PE300 sequencing platform (Illumina, San Diego, CA) according to the manufacturer's instructions.

## 2.6 | Bioinformatic analysis

Raw sequencing data were processed by using the quantitative insights into microbial ecology (QIIME) platform. Bioinformatic analysis was performed using the Majorbio Cloud Platform (Majorbio, Shanghai, China). Operational taxonomic units (OTU) were assigned to phylum, class, order, family and genus taxa, and their relative abundance was calculated.<sup>15,16</sup> Alpha diversity based on the normalized OTU profile was analyzed with the abundance-based coverage

estimator, the Chao1 estimator and the Shannon and Simpson diversity indices.<sup>13</sup>

## 2.7 | Statistical analysis

SPSS Statistics version 22 (IBM, Armonk, NY, USA) was used for statistical analysis. Continuous variables were expressed as mean  $\pm$  standard deviation or median and interquartile range (IQR), while categorical variables were expressed as numbers and percentages or frequencies. The normal distribution of the continuous variables was determined by using the Kolmogorov-Smirnov test. Differences in normally distributed continuous variables were compared by using the Student's *t*-test, whereas those in two independent non-abnormally distributed variables were compared by using the Mann-Whitney *U*-test. Any statistically significant difference between the mean or median value of three or more independent groups was evaluated by using a one-way ANOVA or the Kruskal-Wallis test, respectively. RStudio version 3.4.3 (R Foundation, Boston, MA) was used for the correlation analysis, with Pearson's correlation for normally distributed continuous variables, otherwise Spearman's correlation was performed. A *P* value of less than 0.05 was considered statistically significant.

A Kruskal-Wallis test was used for the comparison of abundance of genera in the microbial communities among different groups (samples), and a post hoc test (Scheffe's method) was performed to evaluate the significance of the observed differences. A non-parametric factorial Kruskal-Wallis sum-rank test was first used to identify significant difference from phylum to genus among the groups. The linear discriminant analysis (LDA) effect size (LEfSe) algorithm was used to estimate the effect of the core bacterial abundance that could be used for discrimination.<sup>17</sup> LDA >3 and *P* < 0.05 were used to identify significantly enriched microbial communities.

### 3 | RESULTS

#### 3.1 | Female IBS-D patients had higher HADS score and male patients exhibited higher visceral hypersensitivity

A total of 162 patients with IBS-D and 66 age- and sex-matched HC were included in the study (Table S1). There were no significant differences in height, weight, or body mass index (BMI) between the IBS-D and HC groups. However, IBS-SSS and its five constituent parts, HAD score, and stressor score were significantly higher in the IBS-D patients than in the HC group. A well-documented medical history was obtained from 69 patients with IBS-D and 44 HC. Among them, IBS-D patients were more likely to have a previous history of infectious enteritis than HC (42.0% [29/69] vs 6.8% [3/44],  $P < 0.001$ ). Moreover, men were more likely to have a history of infectious enteritis (66.7% [22/33]) than women (30.3% [10/33]). Patients with IBS-D presented with significant visceral hypersensitivity and initial sensitivity (8.00 mmHg vs 11.00 mmHg,  $P = 0.025$ ), initial defecation (14.03 mmHg vs 19.00 mmHg,  $P < 0.001$ ), urge to defecation (24.00 mmHg vs 28.00 mmHg,  $P = 0.008$ ) and maximum tolerance (32.00 mmHg vs 38.00 mmHg,  $P = 0.028$ ) thresholds that were significantly lower than those of the HC group.

In our cohort, IBS-D was more commonly seen in men ( $n = 114$ ) than in women ( $n = 48$ ). To explore the potential pathophysiological mechanisms of the obvious sex-specific differences in IBS-D morbidities, a subgroup analysis was conducted based on patient's sex. Female IBS-D patients had a lower BMI than female HC ( $P = 0.028$ ), whereas no difference was observed between male IBS-D patients and male HC ( $P = 0.224$ ). Notably, HADS score was significantly higher in female IBS-D patients than in male patients (18.00 vs 15.00,

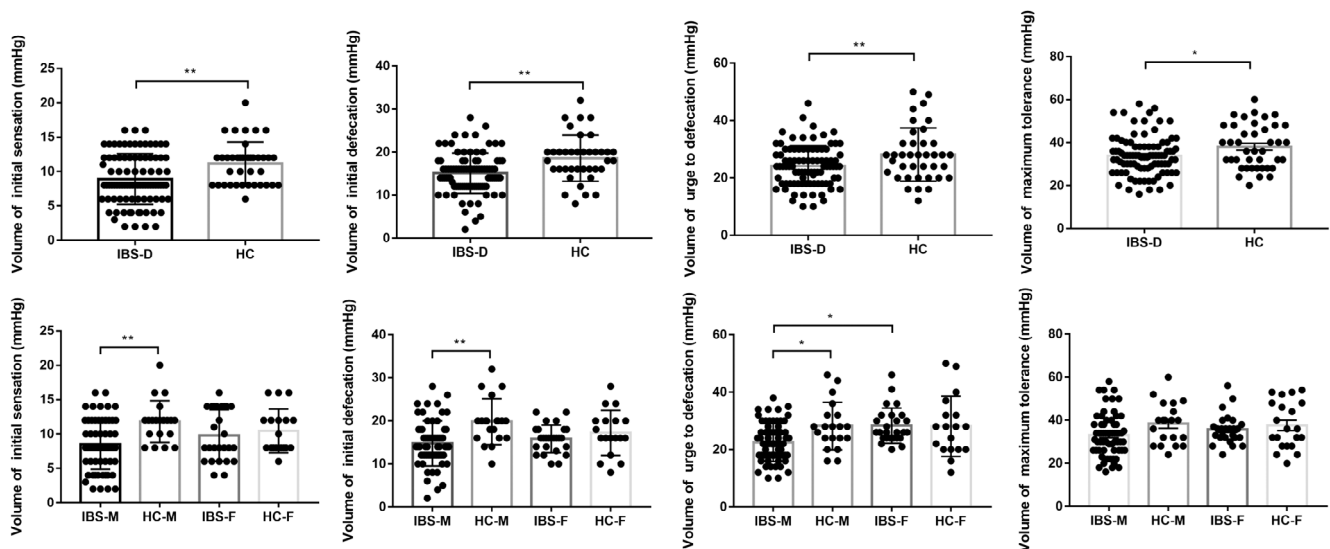
$P < 0.05$ ). Male patients with IBS-D exhibited significantly lower tolerance to urge to defecation than the other subgroups ( $P < 0.05$ ); however, there were no significant differences in visceral hypersensitivity between female patients with IBS-D and female HC (Figure 2).

#### 3.2 | Male patients with IBS-D exhibited particular systematic inflammation

Plasma IL-12 level was significantly higher in the IBS-D group than in the HC group ( $P = 0.037$ ), the difference of which might be due to a high proportion of male patients as they had higher IL-12 level than other groups ( $P < 0.05$ ; Figure 3). This result indicates that male patients with IBS-D may have more severe systematic inflammation.

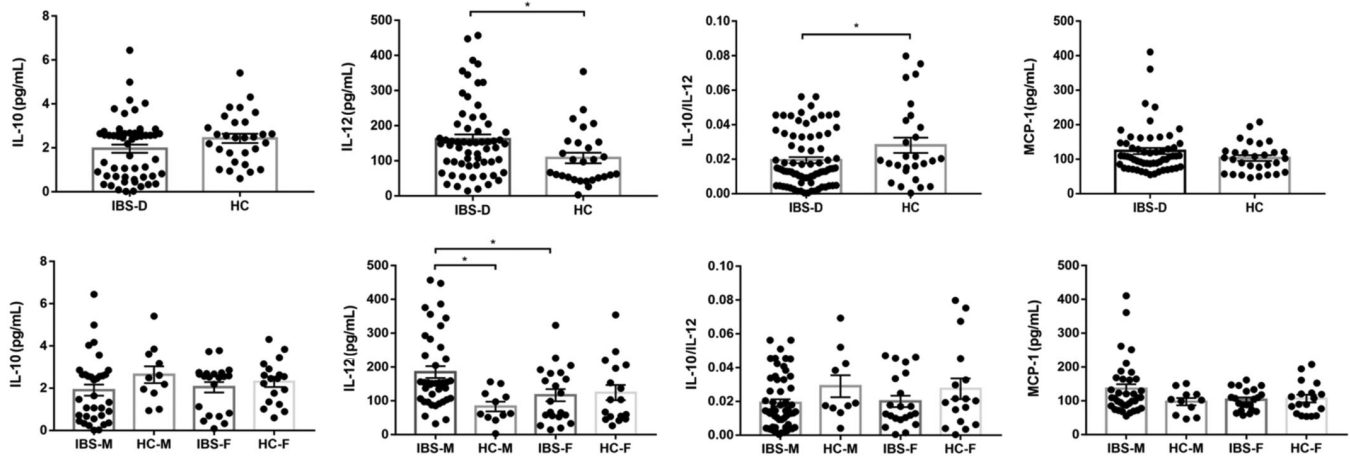
#### 3.3 | Male IBS-D patients had higher levels of fecal propionate and varied fecal SCFA correlated with pathophysiological parameters

The proportion of acetate was significantly lower in the IBS-D group compared with the HC group (43.20% vs 46.59%,  $P < 0.001$ ). While the proportion and concentration of propionate were significantly higher in the IBS-D group (25.17% vs 20.79%,  $P = 0.002$ ; and 47.71 mg/g vs 36.23 mg/g,  $P = 0.009$ ; Table S2.1). According to the subgroup analysis, changes in both the proportion and concentration of propionate were more significant in male IBS-D patients than in female patients ( $P < 0.01$  and  $< 0.001$ , respectively; Table S2.2, Figure 4). Figure 5 shows the positive correlation of fecal acetate levels with the initial visceral sensitivity threshold ( $r = 0.50$ ,

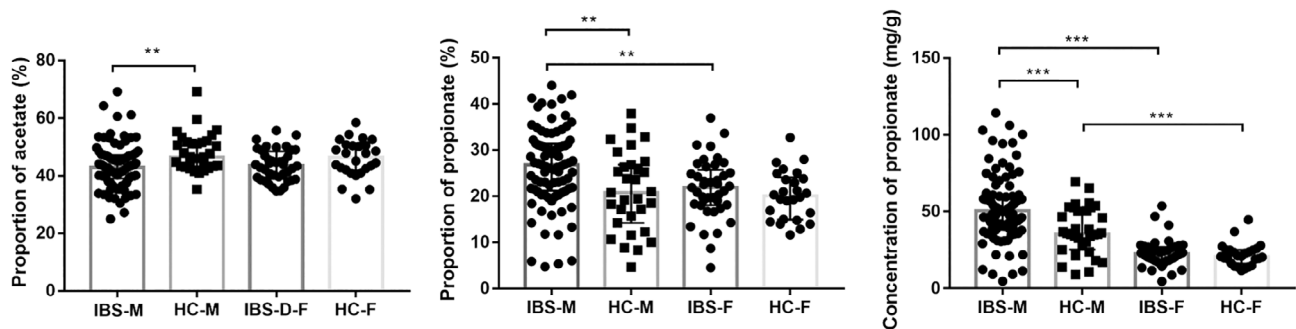


**FIGURE 2** Comparison of colorectal visceral sensitivity between patients with diarrhea-predominant irritable bowel disease (IBS-D) and healthy control (HC) by using the Mann-Whitney  $U$ -test, as well as the comparison among subgroups of male IBS-D patients (IBS-M;  $n = 71$ ), female IBS-D patients (IBS-F;  $n = 27$ ), female HC (HC-F;  $n = 17$ ) and male HC (HC-M;  $n = 22$ ) by using the Kruskal-Wallis test.

\* $P < 0.05$ ; \*\* $P < 0.01$



**FIGURE 3** Comparison of plasma levels of interleukin (IL)-10, IL-12, and monocyte chemotactic protein (MCP)-1, and the ratio of IL-10 to IL-12 (IL-10/IL-12) between patients with diarrhea-predominant irritable bowel disease (IBS-D) and healthy control (HC) by using the Mann-Whitney *U*-test, as well as the comparison among subgroups of male IBS-D patients (IBS-M; *n* = 36), female IBS-D patients (IBS-F; *n* = 21), female HC (HC-F; *n* = 18) and male HC (HC-M; *n* = 11) by using the Kruskal-Wallis test. \**P* < 0.05



**FIGURE 4** Comparison of proportions of fecal acetate and propionate and concentration of propionate among the male diarrhea-predominant irritable bowel disease (IBS-D) patients (IBS-M; *n* = 87), female IBS-D patients (IBS-F; *n* = 41), male healthy control (HC-M; *n* = 19) and female healthy control (HC-F; *n* = 26) by using the Kruskal-Wallis test. \*\**P* < 0.01; \*\*\**P* < 0.001

*P* < 0.001) and their negative correlation with stressors ( $r = -0.35$ , *P* < 0.001). Fecal butyrate was positively correlated with plasma IL-10 level ( $r = 0.33$ , *P* < 0.001) and negatively correlated with plasma IL-12 level ( $r = -0.30$ , *P* = 0.002). Fecal valerate was positively correlated with stressors ( $r = 0.40$ , *P* < 0.001).

We also found that participants with a history of infectious enteritis had a higher fecal propionate concentration (50.75 mg/g [36.01–61.48 mg/g] vs 39.06 mg/g [30.82–53.53 mg/g], *P* = 0.027; Figure S1) and proportion (26.89% [22.13%–30.85%] vs 21.53% [17.82%–29.27%], *P* = 0.018) than those without.

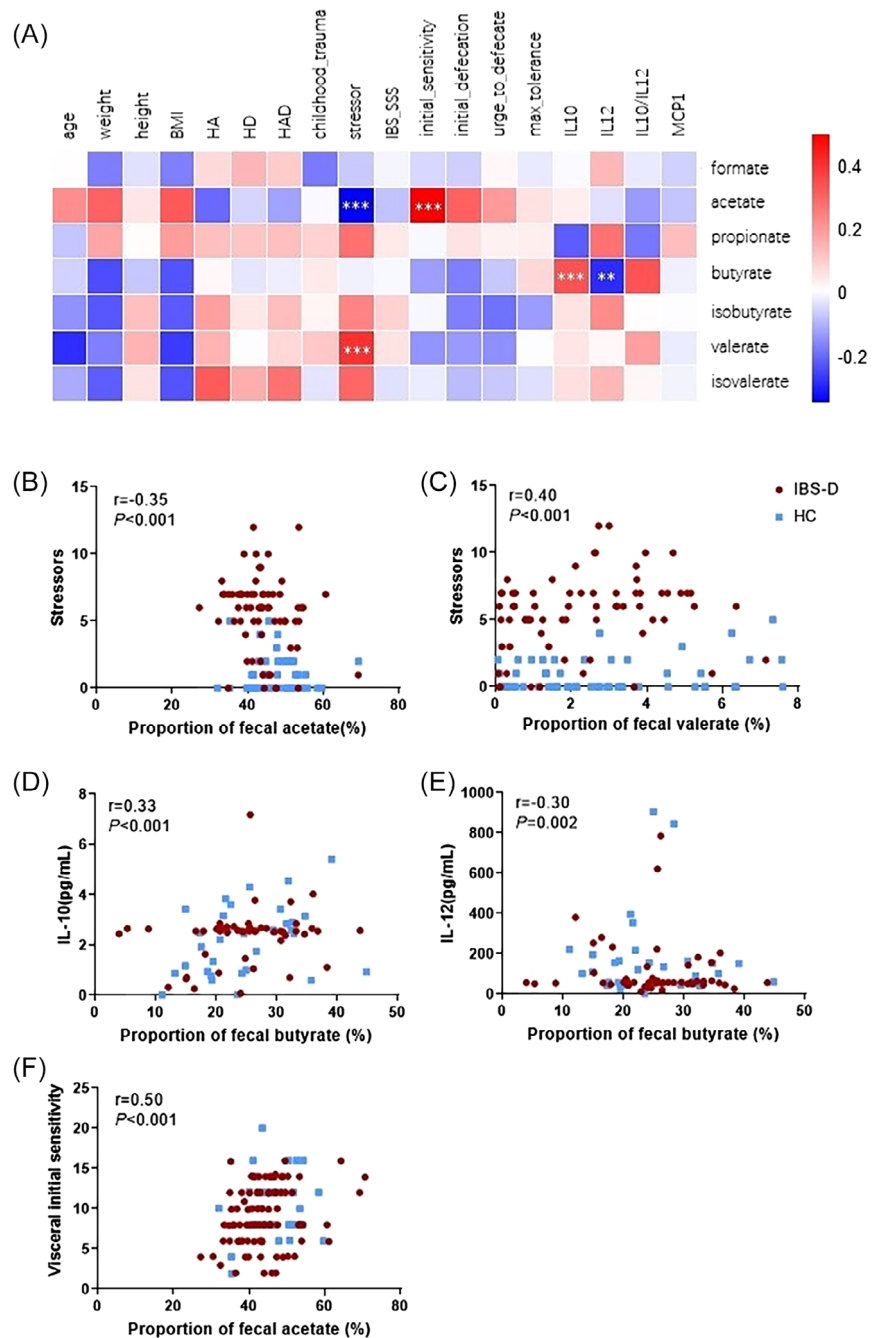
### 3.4 | Male patients with IBS-D presented significant gut dysbiosis

Altogether 98 fecal samples were collected from 69 patients with IBS-D (55 men and 14 women) and 29 HC (22 men and seven women) for

the analysis of microbiota richness. No significant differences in the fecal microbiota richness were found between male IBS-D patients and male HC, or between female patients and female HC as well (Table S3). There were 1240 OTU in male patients with IBS-D, 665 OTU in male HC, 1285 OTU in female patients with IBS-D and 668 OTU in female HC. In total, 456 OTU were found in all the four groups.

The differences in gut microbiota between each group were further determined at the genus level. A significant increase in *Prevotella 9* and *Escherichia-Shigella* were found in the male IBS-D group when compared with the male HC (*P* = 0.025 and 0.026; Table S4). The abundance of *Bacteroides* had a decreasing trend in the male IBS-D group compared with male HC (*P* = 0.079). In the LEfSe analysis, a LDA cut-off score of 3 was used to estimate the discriminatory impact of each community on phylogenetic distribution. A total of eight taxa were indicated as key members with Prevotellaceae, genus of *Prevotella 9*, *Prevotella 2*, *Alloprevotella*, *Escherichia-Shigella*, *norank f*

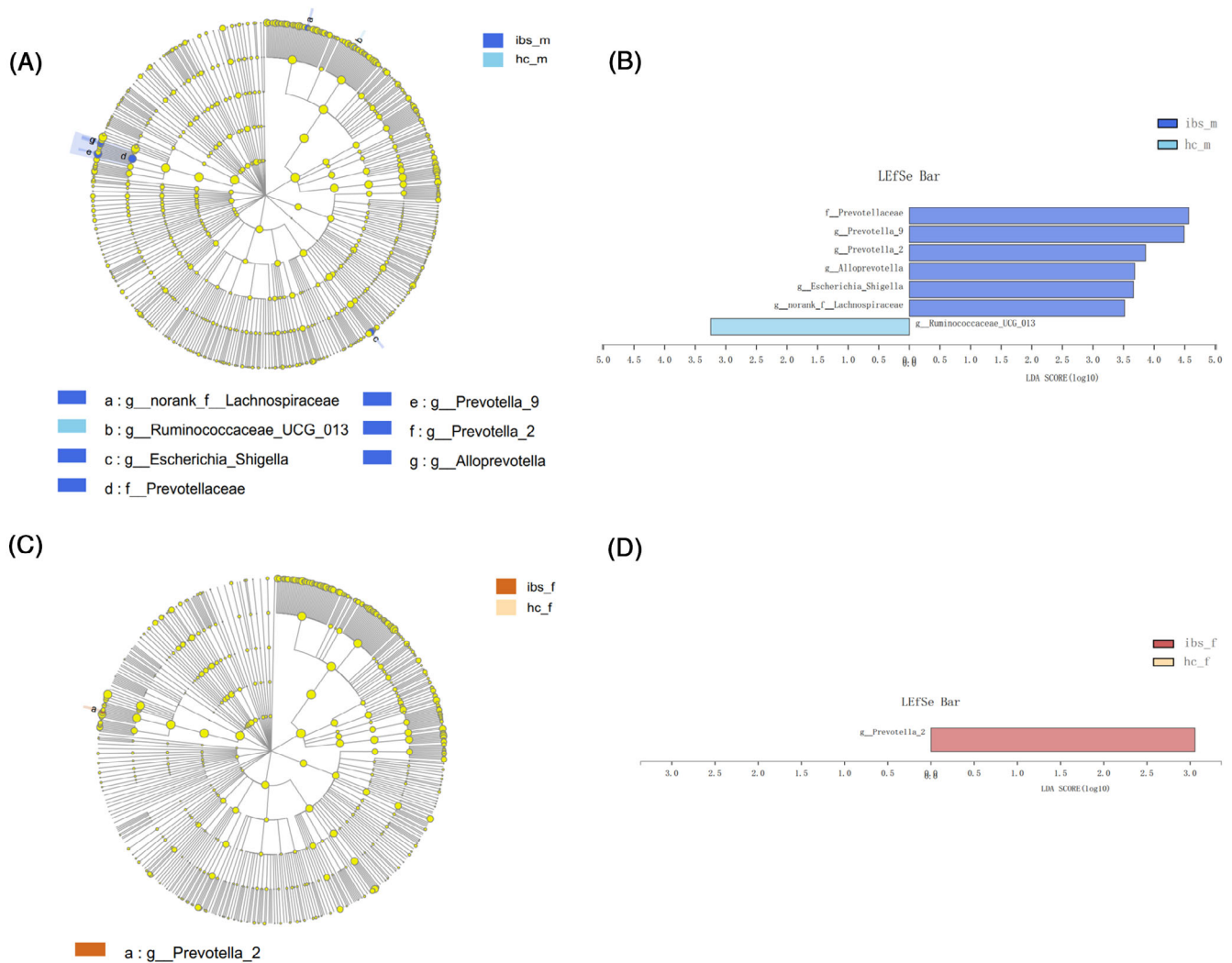
**FIGURE 5** A, Correlation analysis of fecal short-chain fatty acid and clinical manifestation of the patients. Correlation analyses of B, acetate and C, valerate with stressors; butyrate with D, interleukin (IL)-10 and E, IL-12; and E, acetate with visceral initial sensitivity. Abbreviations: BMI, body mass index; HA, Hospital Anxiety; HADS, Hospital Anxiety and Depression Scale; HD, Hospital Depression



*Lachnospiraceae*, *Ruminococcaceae* UCG-013 in male groups (Figure 6A, B) and genus of *Prevotella* 2 in female groups (Figure 6C,D).

A Spearman's correlation analysis was conducted to explore the correlation between phenotypes and microbes based on participant's sex. At the genus level in male groups, changes in *Prevotella* 9 were significantly positively related to fecal propionate levels ( $r = 0.40$ ,  $P < 0.01$ ). *Faecalibacterium* ( $r = 0.34$ ,  $P < 0.01$ ), *Ruminococcaceae* UCG-013 ( $r = 0.43$ ,  $P < 0.001$ ), *Ruminococcus* 1 ( $r = 0.50$ ,  $P < 0.001$ ), *Ruminococcus* 2 ( $r = 0.35$ ,  $P < 0.01$ ) and the *Eubacterium rectale* group ( $r = 0.50$ ,  $P < 0.001$ ) were positively correlated with the levels of fecal

butyrate. Additionally, changes of *Bacteroides* were negatively associated with the levels of serum IL-12 ( $r = -0.35$ ,  $P < 0.01$ ) and fecal propionate ( $r = -0.40$ ,  $P < 0.01$ ). The change in *Faecalibacterium* ( $r = -0.40$ ,  $P < 0.01$ ) and *Fusicatenibacter* ( $r = -0.35$ ,  $P < 0.01$ ) had a weak correlation with the level of serum IL-12. *Ruminococcaceae* UCG-013 ( $r = 0.33$ ,  $P < 0.05$ ) was significantly positively associated with serum IL-10 level. *Escherichia-Shigella* ( $r = 0.45$ ,  $P < 0.001$ ) was significantly positively correlated with Hospital Depression (HD) scores (Figure 7A). While in the female groups, although there were some correlations between certain genera and phenotypes



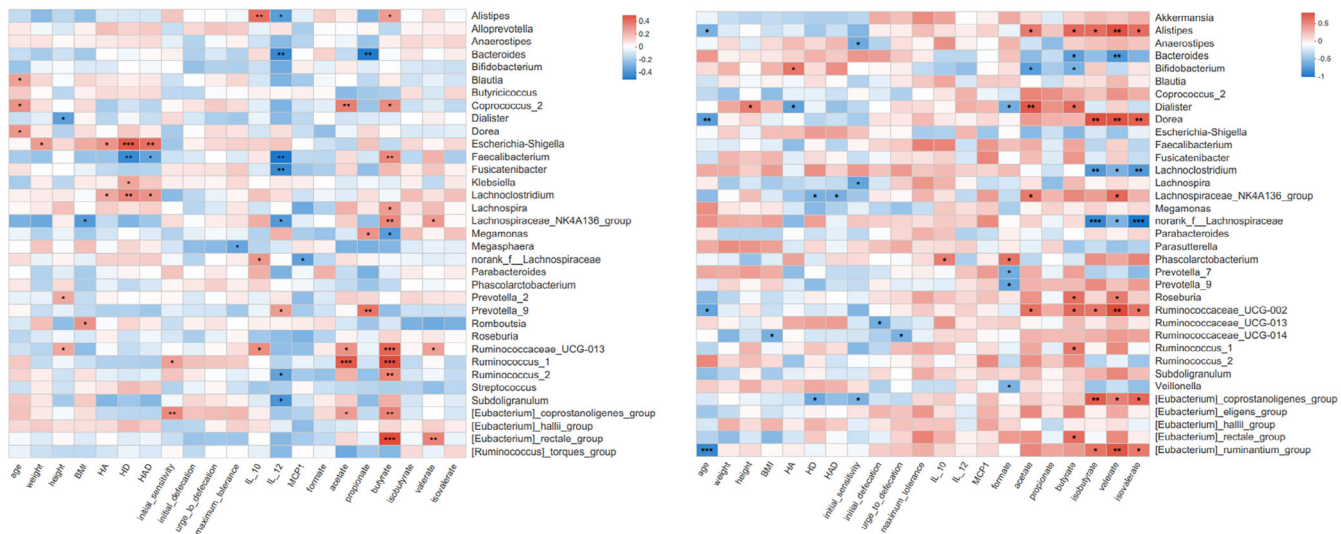
**FIGURE 6** Gut microbiota composition. A, Linear discriminant analysis effect size (LEfSe) analysis and B, histogram of LEfSe analysis from phylum to genus between male patients with diarrhea-predominant irritable bowel syndrome (IBS-D) and male healthy control (HC). C, LEfSe analysis and D, histogram of LEfSe analysis from phylum to genus between female IBS patients and female HC. Abbreviation: LDA, linear discriminant analysis [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

(Figure 7B), no significant correlation between clinical phenotypes and the most representative taxon (which was differentially abundant), *Prevotella 2* was observed.

## 4 | DISCUSSION

To the best of our knowledge, this is the first study to report changes in the fecal SCFA in Chinese patients with IBS-D. There were more male than female in our consecutively enrolled IBS-D cohort, the distribution of which was similar to that in a multicenter population-based study conducted in Southeast China that observed relatively more men with IBS-D (58.2%) although the difference was not significant.<sup>18</sup> A representative national survey of Japanese adolescents with IBS also found that the prevalence of IBS-D was higher among boys,

whereas that of constipation-predominant IBS (IBS-C) was higher among girls.<sup>19</sup> However, a female predominance has been reported among patients with IBS-D in the USA and other western countries.<sup>20-22</sup> To explore the possible reason for the difference in sex distribution we conducted a further subgroup analysis of patients with IBS-D. According to our data, male patients had a higher incidence of previous infectious enteritis than did female patients. The same tendency was observed for colorectal visceral sensitivity. Plasma IL-12 levels were significantly higher in male patients with IBS-D than in male HC while this difference was not significant in women, suggesting that male patients might present with higher level of inflammation. An increased risk of exposure to pathogens or a poor diet could cause long-term low-grade inflammation of the intestine and high colorectal visceral sensitivity.<sup>23-25</sup> Our results indicate that the pathogenesis of male Chinese patients with IBS-D may be



**FIGURE 7** Correlation analysis between phenotypes and microbes at the genus level in A, male groups and B, female groups. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$

associated with post-infectious low-grade intestinal inflammation. However, there were no such changes in the systematic inflammation grade or visceral sensitivity in female patients, whereas they had significantly higher HADS scores than male patients, suggesting that gastrointestinal syndromes in women are more likely related to anxiety. More data and further explorations on the microbiota and brain-gut axis function are needed to determine the pathophysiological mechanism in female patients with IBS-D.

SCFA are primary metabolites of the intestinal microbiota. The proportions and concentrations of SCFA may differ among individuals due to factors such as fermentation substrates, bacterial structures, dietary habits, intestinal movement and genetic polymorphisms.<sup>26</sup> In our study the ratio of acetate, propionate and butyrate in the HC group was approximately 5:2:2, and the concentrations in the intestinal lumen ranged from 30  $\mu\text{g/g}$  to 90  $\mu\text{g/g}$ , which are consistent with previous reports.<sup>27-30</sup>

Studies have reported various beneficial and detrimental effects of SCFA on host metabolisms.<sup>31,32</sup> One study reported that acetate, propionate and butyrate inhibited the release of tumor necrosis factor- $\alpha$  and alleviated colonic inflammation.<sup>33</sup> However, another group revealed that acetate and propionate significantly decreased mucosal paracellular permeability and transepithelial net fluid flux while increased mucosal bicarbonate secretion.<sup>34</sup> In our study we observed a significantly decreased proportion of fecal acetate and increased proportion and concentration of propionate in the IBS-D group. This finding was consistent with the results of our previous meta-analysis.<sup>4</sup> One possible explanation may be the abnormal absorption of SCFA in the intestinal mucosal epithelium and signal transduction system of IBS patients, resulted in decreased SCFA entry into the peripheral blood and the inability of G-protein-coupled receptors to exert normal physiological functions. The correlation analysis performed during our study showed that acetate and butyrate were somewhat beneficial for patients

with IBS-D, as acetate level was moderately correlated with lower visceral sensitivity, and butyrate level had a weak correlation with higher plasma IL-10 and lower IL-12 levels. Patients with previous infectious enteritis had higher propionate levels. Altered luminal chemo-sensing and aberrant signaling in response to SCFA may contribute to the symptoms observed in patients with a suppressed barrier function.<sup>6</sup> Previous studies suggested that the favorability of a certain SCFA for the host might be determined by its concentration and proportion.<sup>6,35</sup> Sex-based differences in fecal SCFA were also found in our study. Male patients with IBS-D had higher fecal propionate level than female patients.

Further gut microbiome analysis showed a significant increase in *Prevotella 9* in the male IBS-D group at the genus level. Moreover, a positive association was observed between *Prevotella 9* and fecal propionate level. This finding is consistent with the previous study that the growth of *Prevotella* had a concomitant increase in propionate production in vitro.<sup>36</sup> *Prevotella* strains have been reported to be associated with chronic inflammatory conditions, which could explain the reason why our male IBS-D patients had higher levels of systematic inflammation than other groups to some degree.<sup>37,38</sup> *Escherichia-Shigella*, as bacterial pathogen that is frequently associated with diarrheal disease,<sup>39</sup> was found with increasing abundance in male IBS-D patients and was positively correlated with HD scores. A previous intestinal infection of *Escherichia-Shigella* may play a role in the pathogenesis of IBS-D. The LEfSe analysis of male patients with IBS-D and male HC found that *Ruminococcaceae UCG-013* played a key role in the distinction between HC and patients with IBS-D. Butyrate-producing *Ruminococcaceae*<sup>40,41</sup> were reported to be beneficial bacterium that could maintain a balanced composition of gut microbiota, and reduce the inflammatory response in the host.<sup>42</sup> This finding was consistent with ours that fecal butyrate level had a weak correlation with higher plasma IL-10 and lower IL-12 levels.



Although there was no significant differences in the abundance of *Faecalibacterium*, *Ruminococcus 1*, *Ruminococcus 2*, (*Eubacterium*) *rectale* groups between male patients and male HC, these genera were positively correlated with fecal butyrate level, which are known as butyrate-producing bacteria.<sup>40,41</sup> The change of *Faecalibacterium* and *Fusicatenibacter* were negatively associated with plasma IL-12 level. One report noted that *Faecalibacterium* decreased inflammatory cytokines and increased anti-inflammatory cytokines.<sup>43</sup>

However, no significant differences in gut microbiota abundance were found in the female groups. This result was consistent with the SCFA comparison in female groups. The LEfSe analysis indicated that only *Prevotella 2* was the most representative taxa in female patients with IBS-D. Sex-based differences in fecal SCFA and clinical manifestations were related to gut microbiota, which could also be affected by a previous intestinal infection.<sup>44</sup> A murine model study revealed that male mice were more vulnerable to the anxiogenic effects of high-fat diet and exhibited decreased locomotion activity in response to stress when compared with female mice.<sup>45</sup> Data from one study suggested that the sex-specific gut microbiota composition contributed to sex differences in the immune system.<sup>46</sup>

One of the important pathogenic mechanism of IBS-D is dysbiosis. SCFA, as the main gut flora metabolisms, can reflect the intestinal condition effectively. In the present study, through concurrent fecal SCFA measurement and fecal microbiota 16S rRNA sequencing we found that the sex-based changes in fecal SCFA might be a potential indicator of dysbiosis in patients with IBS-D, which could assist physicians in making individualized diagnosis.

There were some limitations to our study. First, the aim of the present study did not extend to the influence of diet on the SCFA metabolism in patients with IBS-D. A dietary questionnaire would be required to ensure more rigorous and well-founded results. Second, large, well-designed, multicenter cohort studies of fecal SCFA and gut microbiota are required to strengthen the data and evidence.

In conclusion, sex-based differences on clinical manifestations of the patients, a previous history of infectious enteritis, systematic inflammation, colorectal visceral sensitivity, fecal SCFA and gut microbiota in IBS-D suggest that the pathogenesis of IBS-D might differ between Chinese male and female patients. Sex-based changes in fecal SCFA can be used as a potential indicator of dysbiosis in IBS-D, and assist physicians to design individualized treatment strategies.

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#### CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

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