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# Integrated Gut Microbiome–Metabolome Profiles of Diarrhea-Predominant Irritable Bowel Syndrome: A Study from a Chinese Cohort

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**Background: Irritable bowel syndrome (IBS) is a prevalent gastrointestinal disorder, yet its underlying mechanism remains incompletely understood. This study aimed to elucidate gut microbiome dysbiosis and metabolic perturbations among Chinese patients with diarrhea-predominant IBS (IBS-D).**

**Methods: Fecal samples were collected from 55 IBS-D patients (according to Rome IV criteria) and 29 healthy controls. Gut microbiome-metabolome signatures were obtained through 16S ribonucleic acid (rRNA) amplicon sequencing and untargeted metabolomics. Integrated bioinformatics analysis was conducted to investigate microbiome-metabolome characteristics in IBS-D patients.**

**Results: Significant differences in microbiome profiles were observed between IBS-D patients and healthy volunteers. Utilizing machine learning algorithms, our investigation revealed a notable increase in gut microbes, including *Sutterella*, *Lachnospira*, *Bacteroides*, and *Fusobacterium*, in the IBS-D patients ($p < 0.05$). Conversely, *Bifidobacterium*, *Blautia*, and *Romboutsia* exhibited a decrease in IBS-D patients ($p < 0.05$). Furthermore, functional analysis indicated potential alterations in gut lipopolysaccharide (LPS) biosynthesis and disruptions in energy metabolism functions among IBS-D patients. In terms of metabolome profiles, significant upregulation was observed in metabolites such as 5′-S-methyl-5′-thioadenosine, S-adenosyl-methionine, creatine, adenine, and gamma-aminobutyric acid (GABA) in individuals with IBS-D ($p < 0.05$), suggesting a potentially pivotal role of these metabolites in the microbiota-gut-brain axis. Additionally, our study identified several significant associations between metabolites and microbes, further enhancing our understanding of the intricate interplay within the IBS-D microbiome.**

**Conclusions: Our research highlights a microbiome-metabolome pattern in individuals with IBS-D, indicating that gut microbiome and fecal metabolites can serve as valuable indicators to distinguish between IBS-D patients and healthy individuals.**

**Keywords:** biomarkers; dysbiosis; gut microbiome; irritable bowel syndrome; metabolome

## Introduction

Irritable bowel syndrome (IBS) is a prevalent functional gastrointestinal disorder characterized by symptoms such as abdominal pain, bloating, and alterations in bowel habits, affecting approximately 1 in 10 individuals globally [1]. Depending on the predominant symptoms, IBS can be categorized into four subtypes: diarrhea-predominant IBS (IBS-D), constipation, mixed bowel habits, and unclassified [2]. Despite its widespread prevalence, the etiology of IBS remains poorly understood. Genetics, dietary habits, and gut microbiota are the risk factors for IBS [1].

In recent years, heightened attention has focused on the role of gut microbiota and its metabolites in the pathophysiology of IBS. Dysbiosis within the gut microbiota disrupts intestinal function, compromises mucosal barrier integrity, and induces inflammatory responses, resulting in IBS symptoms. Comparative analyses between the gut microbiota of IBS patients and healthy controls have revealed reduced microbial diversity in IBS patients, coupled with significant fluctuations in the abundance of specific bacterial species [3–5]. Notably, intestinal dysbiosis contributes to alterations in fecal metabolites, as many metabolites are derived from microbial activity. Various metabolites, including short-chain fatty acids (SCFAs), encompassing propionate, acetic acid, and butyrate, enhance intestinal peristalsis, reinforce mucosal barrier function, exert anti-inflammatory properties, and play a pivotal role in preserving intestinal homeostasis [6–8]. Additionally, specific microbial metabolites, including sulfide [9] and bile salt metabolites [10,11], may adversely impact intestinal motility and inflammation in IBS.

While previous studies have reported various microbiota and metabolite signatures of IBS [12], a consensus on

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**Table 1. Characteristics of the study cohort.**

| Characteristics | IBS-D patients | Healthy controls | *p*-value | $\chi^2$ | *t* | Z-score |
|---|---|---|---|---|---|---|
| Subjects (n) | 55 | 29 | / | / | / | / |
| Sex (male/female) | 29/26 | 15/14 | 0.929 | 0.008 | / | / |
| Age (y) [mean ± SD (min, max)] | 54.34 ± 9.04 (35, 72) | 53.21 ± 9.68 (42, 74) | 0.584 | / | 0.618 | / |
| Height (m) [Median ($P_{25}$, $P_{75}$)] | 1.64 (1.59, 1.70) | 1.67 (1.61, 1.70) | 0.696 | / | / | 0.395 |
| Weight (kg) [mean ± SD (min, max)] | 62.10 ± 9.80 (44.0, 80.0) | 62.50 ± 10.99 (43.8, 93.0) | 0.869 | / | 0.165 | / |
| BMI (kg/m$^2$) [mean ± SD (min, max)] | 22.92 ± 2.76 (17.04, 29.38) | 22.43 ± 2.66 (17.97, 27.17) | 0.444 | / | 0.770 | / |

IBS-D, diarrhea-predominant IBS; BMI, Body Mass Index; IBS, irritable bowel syndrome; SD, Standard Deviation.

the precise alterations in gut microbiome-metabolome composition in IBS remains elusive. The composition of gut microbiota and its associated metabolites may be influenced by various factors that vary among racial groups, including genetics, heredity, diet, and IBS subtypes [13]. To date, most research on dysbiosis in IBS has originated from Western countries. Consequently, there exists a significant research gap in understanding microbiome-metabolome dysbiosis, especially among Chinese patients with IBS, notably those with IBS-D.

In this study, we aimed to identify the fecal microbiome-metabolome profiles of IBS-D within a Chinese cohort through microflora sequencing and metabolic mass spectrometry. This integrative analysis enabled us to identify bacterial and metabolic biomarkers that potentially contribute to IBS pathogenesis, offering a stronger foundation for potential therapeutic interventions.

## Materials and Methods

### *Cohort Description and Study Design*

The patients were enrolled from Huashan Hospital Affiliated to Fudan University between May 2021 and October 2022. A total of 55 IBS-D patients who met the Rome IV diagnostic criteria were enrolled in the outpatient department [2]. All IBS-D patients underwent fecal occult blood tests and colonoscopies to exclude organic intestinal diseases. Healthy volunteers were recruited from the physical examination center of Huashan Hospital. Inclusion criteria included healthy adults, regardless of gender, who had received a physical examination in the past 6 months confirming the absence of organic diseases. Exclusion criteria included any history of gastrointestinal tumors; serious systemic illnesses such as advanced cardiac or renal conditions; severe mental disorders; pregnancy or lactation; use of antibiotics, probiotics, synbiotics, laxatives, or other antidiarrheal agents within the last month; unique eating or drinking habits (e.g., vegetarianism, alcoholism, or diets related to specific religious or social traditions). To minimize the impact of geographical and dietary factors, only participants who had resided in Shanghai, China, for more than six months were included. For two weeks before enrollment, participants were required to adhere to a balanced and nutritious diet, including 300–500 grams of grains daily, vegetables, and fruits, with approximately 300 grams of proteins from meat, dairy, and eggs daily. Participants were also advised to avoid overeating, spicy foods, alcohol, and carbonated drinks. Written informed consent was obtained from all participants. The study was approved by the Ethics Committee of the Huashan Hospital, with approval code KY2019-471. All the fresh fecal samples were collected using a fecal sample collection kit (#R1180, KMDH Gene Tech, Guangzhou, China) and stored in liquid nitrogen for subsequent analysis.

To analyze the clinical characteristics (including sex, age, height, weight, and Body Mass Index (BMI)) of the IBS-D patients and healthy controls, data were tabulated in Table 1. For normally distributed data, the Student's *t*-test was used for continuous variables, and the chi-square test was used for categorical variables. The Mann-Whitney U test was utilized for non-normally distributed data. All statistical analyses were performed using SPSS 18.0 software (IBM, Armonk, NY, USA). A $p$-value $< 0.05$ was considered statistically significant. All clinical experiments were conducted following the ethical standards of the Declaration of Helsinki.

### *DNA Extraction, Amplification, and 16S rRNA Sequencing*

Genomic DNA was extracted from the collected specimens using the Cetyltrimethylammonium bromide/Sodium dodecyl sulfate (CTAB/SDS) method [10,11]. The extracted DNA was diluted in sterile water and used as the polymerase chain reaction (PCR) amplification template. Oligonucleotide sequences targeting the V3-4 region of the 16S ribonucleic acid (rRNA) for PCR reaction were: 5′-CCTAYGGGRBGCASCAG (forward primer) and 5′-GGACTACNNGGGTATCTAAT (reverse primer). Following PCR amplification, the sequencing libraries were prepared using the NEBNext® Ultra™ II DNA Library Prep Kit (E7645, Illumina, San Diego, CA, USA). The prepared libraries were subsequently sequenced on an Illumina NovaSeq 6000 system (Illumina, San Diego, CA, USA), producing paired-end reads with a length of 250 base pairs each.

### *Microbiota Analysis*

Raw 16S RNA sequencing data for each sample were processed using QIIME2 software (Version 2.0, Novogene, Shanghai, China). Functional analyses, linear discriminant analysis effect size (LEfSe), and alpha and beta diversity assessments were performed using the R 'microeco' package (https://github.com/ChiLiubio/microeco) [14,15]. The differences in the abundance of taxa between the patient and control groups were calculated using the 'edgeR' package (https://bioconductor.org/packages/release/bioc/html/edgeR.html) [16]. Biomarker identification was conducted using the 'RandomForest' package (https://cran.r-project.org/web/packages/randomForest/), with analysis divided between a training cohort (n = 62) and a validation cohort (n = 22). Enterotype classification was performed using Dirichlet multinomial mixtures (DMM) [17] and partitioning around medoids (PAM)-based clustering, including the calculation of Bray-Curtis distances. Between-class analysis (BCA) was used to validate clustering outcomes and identify the factors influencing enterotypes.

### *UHPLC-MS/MS Analysis*

Analytical profiling was performed using a Vanquish UHPLC system (Version 2.0, Thermo Fisher, Waltham, MA, USA) paired with an Orbitrap Q Exactive™ HF mass spectrometer (#IQLAAEGAAPFALGMAZR, Thermo Fisher Scientific, Beijing, China) for ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). The processing of UHPLC-MS/MS-generated raw data files was executed by Compound Discoverer 3.1 (Thermo Fisher Scientific, Beijing, China), which facilitated the alignment of peaks, the selection of peaks, and the quantification of each metabolite. Following peak normalization, molecular formula prediction, and peak matching, a comprehensive metabolite identification yielded 989 metabolites in positive ion mode and 602 metabolites in negative ion mode across 84 samples.

### *Metabolomic Data Analysis*

Metabolomic data, comprising metabolites detected in negative and positive electrospray ionization modes, underwent a two-step normalization process involving log transformation followed by Pareto scaling. The 'ropls' package (https://bioconductor.org/packages/release/bioc/html/ropls.html) was utilized to perform partial least squares discriminant analysis (PLS-DA). Differential metabolite analysis was performed using the 'Rvolcano' package (https://bioconductor.org/packages/release/bioc/html/EnhancedVolcano.html) [18], with metabolites meeting the criteria of $p < 0.05$ and $|\log2FoldChange| > 1$ considered significant. Quantitative Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of metabolites was conducted using MetaboAnalyst (https://www.metaboanalyst.ca/) [19]. MetOrigin (https://metorigin.met-bioinformatics.cn/home/) was utilized for metabolite traceability analysis and metabolite functional enrichment analysis [20]. Integrative analysis of the microbiome and metabolome was accomplished through Spearman's correlation test using SPSS 18.0 software (IBM, Armonk, CA, USA).

## Results

### *Information of Cohort: IBS-D Patients and Healthy Controls*

A total of 29 healthy controls and 55 IBS-D patients were enrolled in the study. Stool samples from all 84 participants underwent untargeted mass spectrometry and 16S rRNA sequencing. Baseline characteristics are presented in Table 1. There were no significant differences in age, gender, or BMI between the IBS-D patients and the healthy controls ($p > 0.05$).

### *IBS-D Patients Harbor Distinct Microbial Patterns*

We initially assessed the microbial community composition and distribution at the amplicon sequence variants (ASV) level between healthy controls and IBS-D patients. Chao1, Pielou's evenness, and Shannon indices indicated no significant difference in $\alpha$-diversity between IBS patients and healthy controls ($p > 0.05$, Fig. 1A). However, the $\beta$-diversity analysis, based on the Bray-Curtis distance, revealed a significant difference in the composition and abundance of gut microbiota between the two groups ($p < 0.01$, Fig. 1B). Additionally, $\beta$-diversity analysis demonstrated that the gut microbiome of IBS-D patients exhibited higher interindividual variation compared to healthy adults ($p < 0.01$, Fig. 1C).

Subsequently, we examined microbial distributions at the phylum level. One of the most notable differences observed between IBS-D patients and healthy individuals was the over-representation of *Bacteroidota* ($p < 0.01$). Meanwhile, *Firmicutes* and *Actinobacteriota* were more abundant in healthy individuals, whereas *Fusobacteriota* was enriched in the IBS-D group ($p < 0.05$, Fig. 2A). At the genus level, the relative abundance of *Bacteroides*, *Prevotella*, *Faecalibacterium*, and *Fusobacterium* was higher, while the abundance of *Blautia*, *Bifidobacterium*, and *Romboutsia* was lower in IBS-D patients ($p < 0.01$, Fig. 2B), indicating differences in community abundance between the two groups.

Next, we employed the LEfSe algorithm to identify bacterial biomarkers within the two groups, identifying a total of 20 biomarkers (|Linear Discriminant Analysis (LDA) Score| >4). We observed that the families *Bifidobacteriaceae* and *Peptostreptococcaceae*, as well as the genera *Bifidobacterium*, *Blautia*, and *Romboutsia*, were increased in the control group ($p < 0.05$). Conversely, the families *Bacteroidaceae* and *Prevotellaceae*, along with the genera *Bacteroides* and *Prevotella*, were enriched in the IBS-D group ($p < 0.05$, Fig. 2C,D), consistent with the results of the differential analysis performed by 'edgeR' ($p < 0.05$, Fig. 2E).

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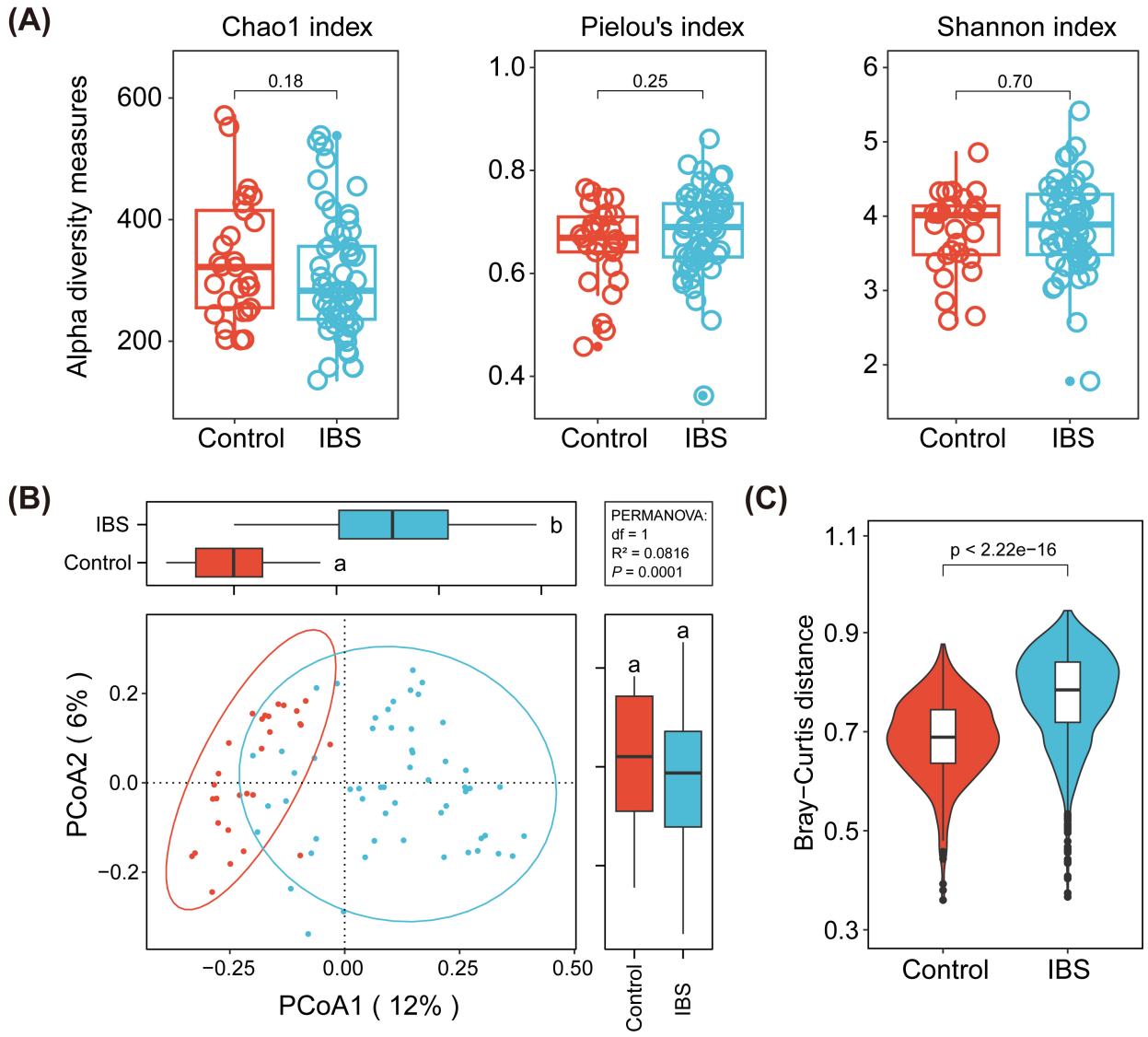


**Fig. 1. Characteristics of the gut microbial community in IBS-D patients.** (A) Comparison of $\alpha$-diversity using the Chao1 index, Pielou's evenness index, and Shannon index between the IBS-D (n = 55) and control groups (n = 29). (B) Principal Coordinates Analysis (PCoA) illustrating the comparison of gut microbial composition between IBS-D patients (n = 55) and healthy controls (n = 29) using Bray-Curtis distances. A 95% confidence interval represents each group. The boxplot illustrates the distribution of PCoA1 and PCoA2 scores within each group. Groups with different letters in the boxplot were significantly different based on a one-way analysis of variance (ANOVA) with two-sided Tukey's post hoc test ($p < 0.05$). (C) Assessment of microbial composition dissimilarity using Bray-Curtis distances between irritable bowel syndrome (IBS) patients (n = 55) and healthy controls (n = 29).

It has been reported that human microbial communities could be typically characterized by different enterotypes [21]. Thus, we conducted enterotype analysis using DMM and PAM methods at the genus level [22], identifying two distinct optimal enterotype clusters in our cohort (**Supplementary Fig. 1A,B**). This analysis revealed that enterotype 2 (driven by *Bacteroides*) was more enriched in the IBS group ($p < 0.05$, **Supplementary Fig. 1C,D**), while enterotype 1 (driven by *Blautia*) was predominated in the healthy control group ($p < 0.05$). These findings further indicate that IBS patients exhibit unique microbial communities.

### *Identifying Signature Microbiota for IBS-D Diagnosis*

To further identify potential microbial biomarkers for IBS-D diagnosis, we developed a machine-learning classifier using the random forest algorithm. Through this approach, we identified the top 30 taxa at the genus level that exhibited the most significant differences between IBS patients and healthy individuals ($p < 0.05$, Fig. 3A). Among these 30 bacterial species, 15 were enriched, while 15 were deficient in the IBS group ($p < 0.05$, **Supplementary Fig. 2**). These findings demonstrated that IBS patients were characterized by high enrichment of genera such as *Sut-*

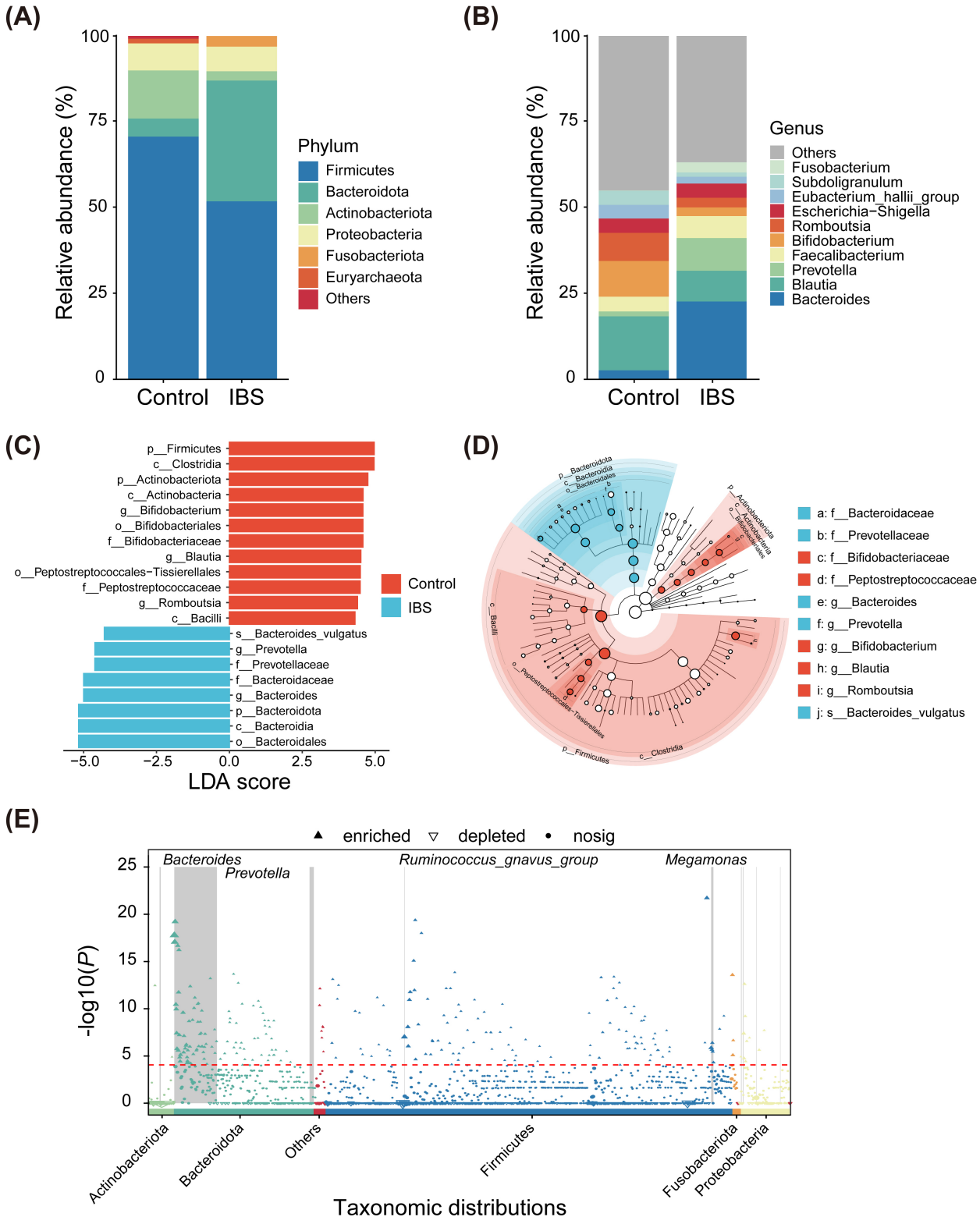


**Fig. 2. Discrepancies in gut microbiome composition in IBS-D patients.** (A) Composition of the gut microbiome at the phylum level between IBS-D patients and healthy controls. (B) Composition of the gut microbiome at the genus level between IBS-D patients and healthy controls. (C) Bar plot illustrating the differences between IBS-D patients and healthy controls identified through linear discriminant analysis effect size (LEfSe) analysis (two-sided Kruskal-Wallis test between classes, $p < 0.05$, |LDA| >4). (D) Taxonomic cladogram generated from LEfSe analysis of 16S ribonucleic acid (rRNA) gene sequences. Only LDA scores >4 are shown. (E) Manhattan plot showing the genera with significant differences between IBS-D patients and healthy controls. LDA, Linear Discriminant Analysis.

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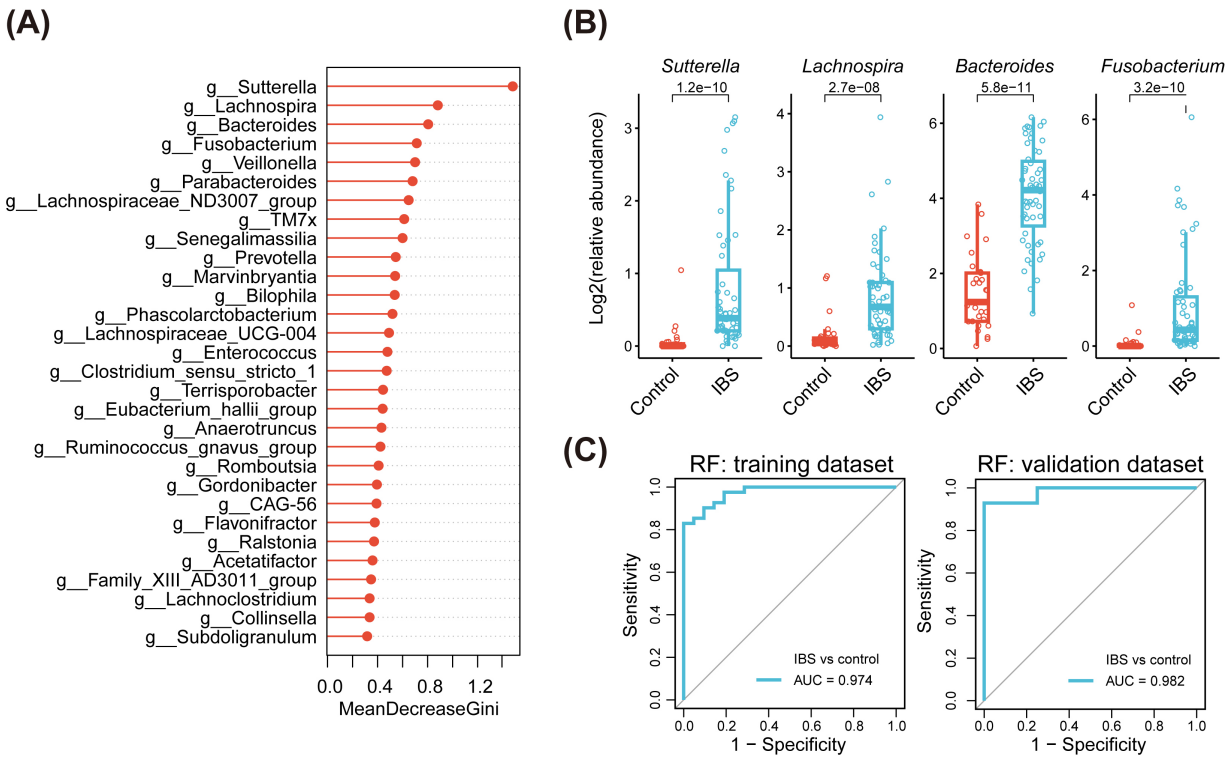


**Fig. 3. Identification of IBS-D diagnostic markers using random forest model.** (A) Selection of the optimal marker in the random forest model using the training dataset (n = 62) based on ranked MeanDecreaseGini values. (B) Box plot showing the top 4 markers of the IBS-D gut flora in the random forest model. The Wilcoxon rank-sum test (two-sided) was used. (C) Receiver operating characteristic (ROC) curves for the random forest model based on the 30 features in the training dataset (n = 62) and validation dataset (n = 22). The 95% CIs are presented in blue-shaded areas. AUCs, areas under the curve; RF, Radio Frequency; CIs, Confidence Intervals.

*terella*, *Lachnospira*, *Bacteroides*, and *Fusobacterium* ($p < 0.05$, Fig. 3B). Utilizing these 30 markers, we effectively classified IBS-D and healthy individuals, achieving areas under the curve (AUCs) value of 0.974. In the validation cohort, these biomarkers differentiated IBS-D patients from healthy individuals with AUC value of 0.982 (Fig. 3C). These findings indicate that gut microbial signatures could serve as a distinctive fingerprint, predicting whether a fecal sample originates from IBS-D patient with high sensitivity and specificity.

### *Identification of Unique Metabolic Signatures for IBS Diagnosis and Treatment*

The interaction between the host and microbiome is primarily driven by metabolic processes within the microbial community. In addition to assessing microbial composition, we analyzed microbial metabolic activity using microbiome-metabolome data. Functional profiles predicted by Tax4Fun revealed that the IBS-D group exhibited downregulation in most energy metabolism pathways while showing enhancement in pathways associated with lipopolysaccharide (LPS) and glycan biosynthesis ($p < 0.05$, Fig. 4A), suggesting a significant role of microbiome-derived antigens or metabolites in IBS-associated gut dysbiosis.

To further explore the metabolic alterations of gut flora during IBS, we performed untargeted metabolomics analysis in IBS-D patients and control groups. The fecal metabolic signatures of IBS patients were significantly distinct from healthy controls, as illustrated by Principal Component Analysis (PCA) and PLS-DA (Fig. 4B and **Supplementary Fig. 3A**). Through differential analysis, we identified 48 significantly upregulated metabolites and 38 downregulated metabolites in the IBS-D group compared to controls ($p < 0.05$, Fig. 4C). Among these 86 significantly differential metabolites, MetOrigin metabolite traceability analysis identified 8 differential metabolites originating from microbiota, 27 from microbiota-host co-metabolism, and 1 from the host (**Supplementary Table 1** and **Supplementary Fig. 3B,C**). Notably, various metabolites exhibiting significant alterations in the IBS-D group, including 5′-S-methyl-5′-thioadenosine, S-adenosyl-methionine, and gamma-aminobutyric acid (GABA), are associated with the gut microbiota (**Supplementary Table 1** and Fig. 4C), suggesting the involvement of the gut microbiota in the pathophysiology of IBS.

To further analyze these altered metabolites, we conducted quantitative KEGG enrichment analysis using MetaboAnalyst. Compared to the control group, cysteine and methionine metabolism pathways, glycerophospho-

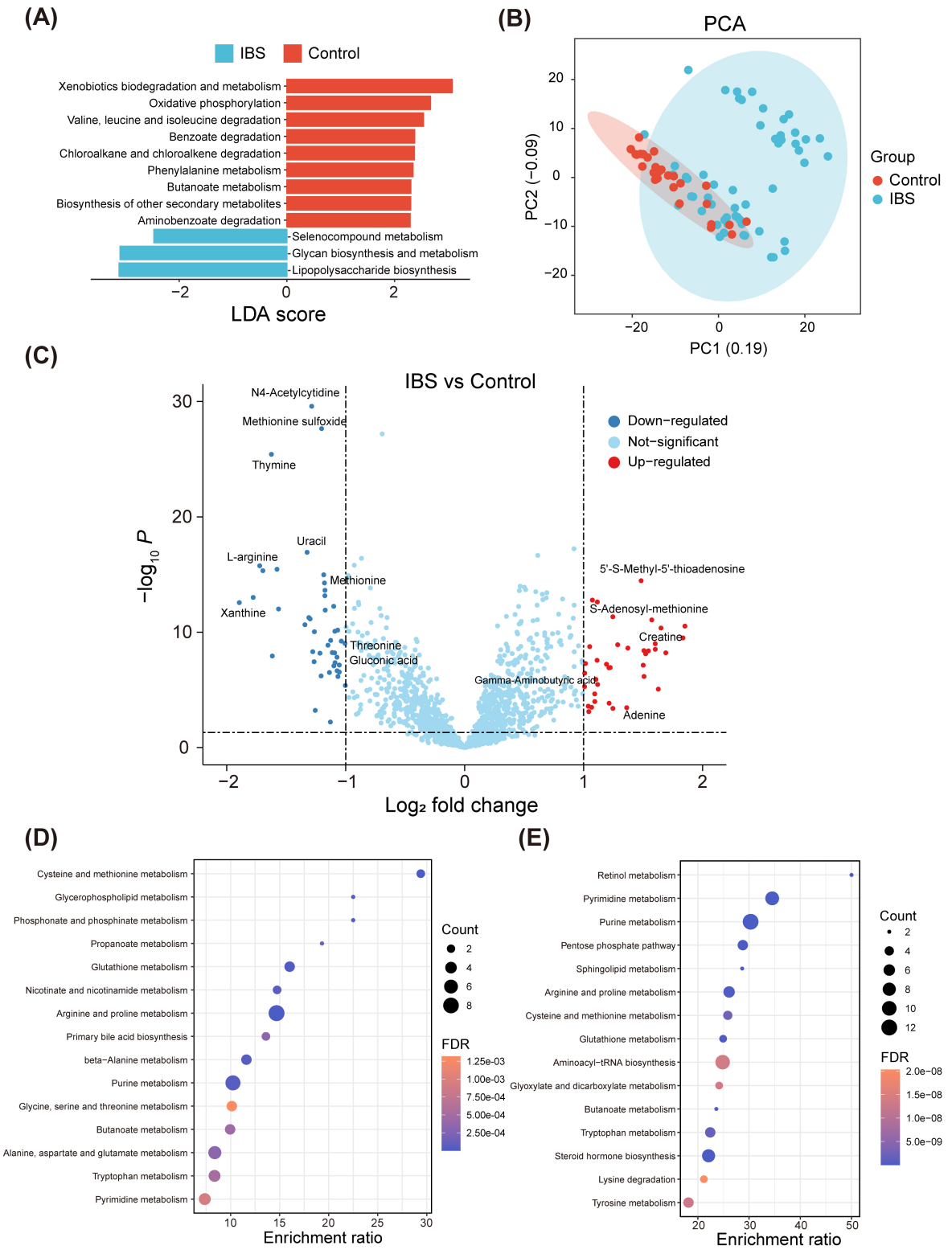


**Fig. 4. Gut metabolomic alterations in IBS-D patients.** (A) Relative abundance of metabolism pathways (LDA >2) in the gut predicted by Tax4Fun and analyzed by LEfSe. (B) Principal Component Analysis (PCA) plot comparing the IBS-D group (n = 55) and the control group (n = 29). (C) Volcano plot illustrating differentially expressed metabolites between the two groups. Each dot represents an individual metabolite, colored red when a metabolite is significantly upregulated and blue when a gene is significantly downregulated in the IBS-D group. (D) Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment pathways based on upregulated metabolites in the IBS-D group compared to the control group. (E) KEGG enrichment pathways based on downregulated metabolites in the IBS-D group compared to the control group. FDR, false discovery rate.

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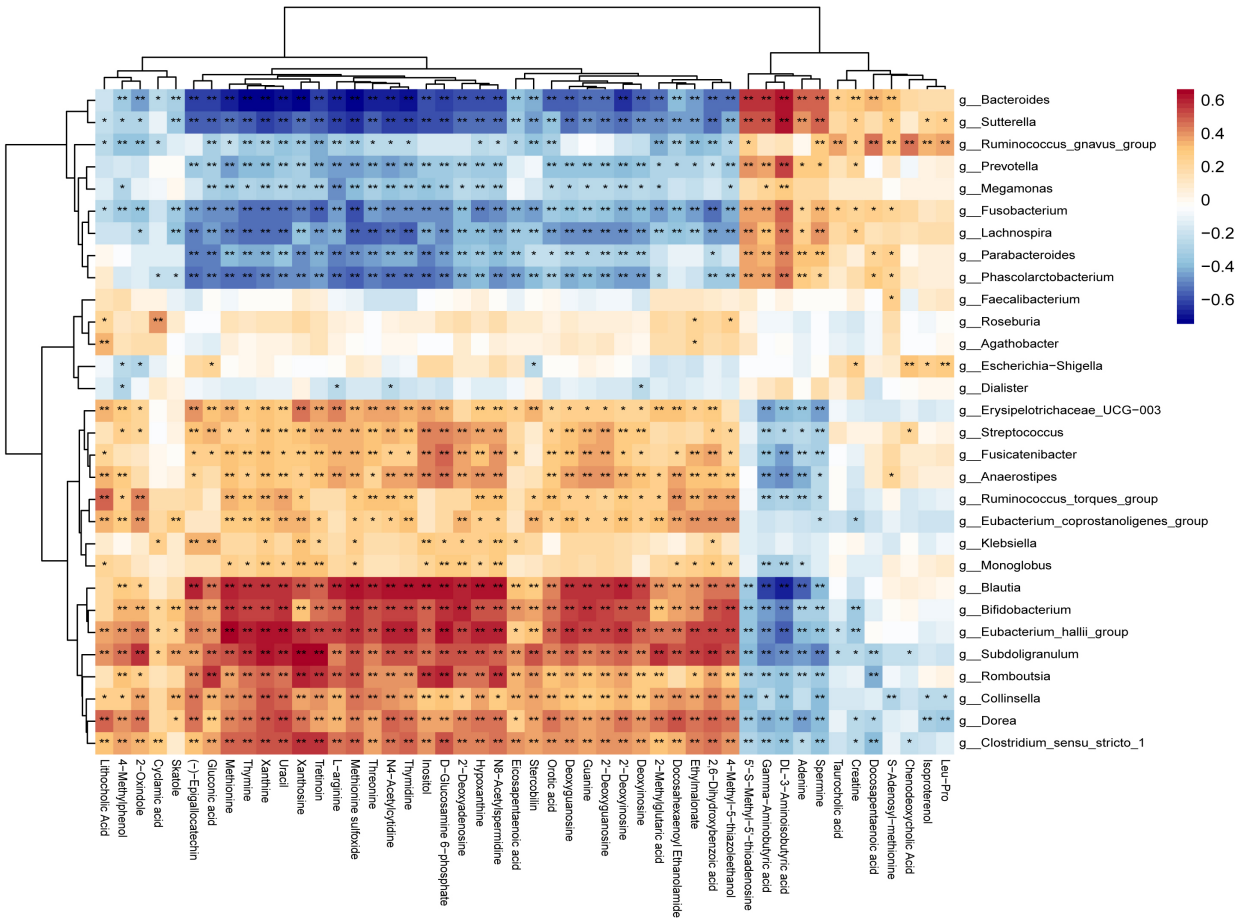


**Fig. 5. Correlation between gut microbiome and fecal metabolites in IBS-D patients.** Heatmap illustrating the correlation analysis of the gut microbiome and fecal metabolites. Spearman's rank correlation was conducted for the 30 most abundant genera and 48 differential metabolites from the IBS group versus the control group. *$p < 0.05$, **$p < 0.01$.

lipid metabolism, and glutathione metabolism were significantly enriched in the IBS-D group ($p < 0.05$, Fig. 4D). Conversely, retinol, pyrimidine, and purine metabolism pathways were strongly enriched in the control group ($p < 0.05$, Fig. 4E and **Supplementary Fig. 3D**). In summary, our findings indicate a notable shift in gut metabolites among IBS-D patients, encompassing alterations in amino acid, purine, and carbohydrate metabolisms.

### *Correlation Analysis between Microbiomes and Metabolites*

To explore the potential associations between modified intestinal genera and metabolites, we conducted correlation analysis involving differential genera and metabolites. Our results revealed several significant metabolite-microbe associations. For example, metabolites such as gamma-aminobutyric acid, 5′-S-methyl-5′-thioadenosine, and 3-aminoisobutyric acid, which exhibited a significant increase in the IBS-D group, demonstrated strong positive correlations ($p < 0.01$) with genera such as *Bacteroides*, *Sutterella*, and *Prevotella*. Conversely, reduced metabolites associated with amino acid and purine metabolism, such as methionine, thymine, and L-arginine, exhibited positive correlations ($p < 0.01$) with *Blautia*, *Bifidobacterium*, and *Eubacterium_hallii_group* ($p < 0.05$, Fig. 5).

## Discussion

Previous studies have highlighted the critical role of gut microbiota in IBS. However, the microbiota and metabolic profiles in IBS patients remain inconclusive [23]. In this study, we employed a dual-omics approach to elucidate the pathogenesis of IBS, focusing on host-microbial interactions and the functional aspects of gut microbiota. While no significant differences in community structures were observed between IBS patients and healthy controls, the utilization of LEfSe analysis and machine learning techniques effectively allowed us to distinguish IBS from healthy controls.

Based on the LEfSe results, *Bacteroides* and *Prevotella* were strongly enriched in the IBS group, while *Bifidobacterium*, *Blautia*, and *Romboutsia* emerged as the most differentially abundant bacterial taxa in healthy controls. Further analysis using random forest identified a heightened

enrichment of genera such as *Sutterella*, *Lachnospira*, *Bacteroides*, and *Fusobacterium* in the IBS group. A systematic review has indicated that the genus *Bacteroides* (phylum *Bacteroidetes*) could potentially be a harmful microbiota in individuals with IBS, while the genus *Bifidobacterium* demonstrated a significant decrease in IBS patients regardless of IBS subtype [23]. Moreover, an increased abundance of *Prevotella* has been associated with a high risk of diarrhea-predominant IBS [24], and elevated levels of *Lachnospira* also significantly increased in IBS patients [25]. Additionally, while *Fusobacteria* are rare constituents of the fecal microbiota, they have been previously cultured from biopsies of inflamed gut mucosa [26]. These findings suggest the presence of reproducible microorganism signatures in IBS.

Gut microbiota and metabolome are crucial in the development of gastrointestinal disorders. Our functional analysis, utilizing 16S rRNA-seq, revealed a reduction in energy metabolism pathways and an enrichment in pathways related to LPS and glycan biosynthesis in the gut microbiome of IBS patients. Based on these findings, we hypothesize that the gut microbiome in IBS may contribute to gut inflammation and dysbiosis through antigen release.

The disrupted interaction between the gut and the brain is considered a primary pathophysiological mechanism in IBS [27]. The gut microbiota influences gut motility, inflammatory responses, and brain function by producing metabolites and neurotransmitters. Studies have shown that abnormalities in the intestinal flora can lead to disturbed intestinal motility, increased inflammatory response, and heightened symptoms of IBS [28–30]. For example, metabolites such as SCFAs can regulate the function of the intestinal nervous system by activating the G protein-coupled receptor 41 (GPR 41) in intestinal epithelial cells, thus impacting intestinal peristalsis and inflammatory responses [31]. Furthermore, specific bacterial metabolites, such as the neurotransmitter serotonin, transmit signals through the gut-brain axis, influencing mood regulation and pain perception [32]. Study has also identified connections between the gut microbiome and systemic neurohormonal activity, as well as stress reactivity, in individuals with IBS [33].

In our study, we observed a significant increase in gamma-aminobutyric acid (GABA), a primary mediator of inhibitory transmission in the mammalian nervous system, in the IBS group. However, another reported a significant decrease in GABA levels in the serum of IBS-D patients compared to controls [34]. This inconsistency may be attributed to the contribution of bacteria in GABA production and consumption [35]. Additionally, we identified metabolites, including 5′-S-methyl-5′-thioadenosine and 3-aminoisobutyric acid, which were significantly increased in the IBS group, while metabolites such as methionine, thymine, and L-arginine were decreased. Currently, there is limited research on these metabolites and their roles in the gut-brain axis. Further investigations into the regulatory effects of these metabolites derived from the intestinal flora are warranted, as the correlation analysis suggests a potential internal correlation between gut microbiota and metabolites.

Most studies on IBS, especially investigations into microbiome-metabolome signatures, originated primarily in Western countries. This study aimed to enhance our understanding of the alterations and functional roles of microbiota and metabolites in IBS patients in China. Most of our findings, including a significant increase of *Bacteroidota* and a decrease in *Firmicutes* indicating dysbiosis in gut microbiota among IBS patients, align with observations from a previous Chinese cohort study [36]. The potential biomarkers identified in this study could be evaluated as participants in the inflammation associated with IBS and subsequently considered for the development of novel clinical therapies based on microbiota-metabolic regulation for the prevention and treatment of IBS. Further elucidation of their functions in gut metabolism and potential interactions with inflammation and the neurological system is crucial for future investigations.

Moreover, since the present study was a pilot and bioinformatics-oriented investigation aimed at identifying signatures of IBS in terms of microorganisms and metabolites, future animal models and pre-clinical experiments are warranted to validate our findings at the animal and cellular levels. Additionally, further clinical data are needed to validate our findings from a clinical perspective, thereby enhancing the reliability of our conclusions.

## Conclusions

The present study investigates the correlations between microorganisms and metabolites with the pathogenesis of IBS-D, confirming that multiple microorganisms (including *Sutterella, Lachnospira*, *Bacteroides*, *Fusobacterium*, *Bifidobacterium*, *Blautia*, and *Romboutsia*) and metabolites (5′-S-methyl-5′-thioadenosine, S-adenosyl-methionine, creatine, adenine, and gamma-aminobutyric acid) are closely associated with IBS-D progression. Furthermore, we construct metabolites-microbe relationships. Our findings will shed light on the exploration of novel treatment strategies for IBS-D from the perspectives of metabolites and microbes.

## Availability of Data and Materials

All the data had been included in the manuscript, and the original raw data could be obtained from the corresponding authors with reasonable request.

## Author Contributions

ZQ and KF were co-first authors of the present study and were responsible for the conception, methodology, in-

vestigations, data collection, data curation, data presentation and manuscript drafting of this work. HZ, LL and DQ conducted part of the investigations and experiments, and they provided technical support for the present study and also helped to draft the first version of the manuscript. WJ, WG and JL mainly helped to conduct the bioinformatics-related experiments, and they were responsible for the data curation, visualization and interpretation, and also participated in drafting the first version of the manuscript. As the co-corresponding authors, JZ and JC provided guidance, conception, supervision, manuscript drafting, funding acquisition and proof of the final version of the manuscript. All authors contributed to important editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

The study was approved by the Ethics Committee of the Huashan Hospital, with approval code KY2019-471. Written informed consent was obtained from all participants. All clinical experiments were conducted following the ethical standards of the Declaration of Helsinki.

## Acknowledgment

Not applicable.

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## Conflict of Interest

The authors declare no conflict of interest.

## Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.23812/j.biol.regul.homeost.agents.20243807.444.

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