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Fecal microbiota transplantation improves bile acid malabsorption in patients with inflammatory bowel disease: results of microbiota and metabolites from two cohort studies

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Abstract

Background Bile acid malabsorption (BAM) or bile acid diarrhea (BAD) complicates more than 30% of Crohn's disease (CD), yet no non-invasive biomarker reliably identifies patients who will benefit from fecal microbiota transplantation (FMT). We investigated whether serum 7α-hydroxy-4-cholesten-3-one (C4), a hepatic bile-acid synthesis precursor, can predict BAM and FMT response in inflammatory bowel disease (IBD).

Methods We included 106 pairs of IBD patients treated with FMT from two longitudinal cohorts of prospective trials and 24 matched healthy individuals to identify a multi-omics analysis of microbiota-metabolism and evaluate real-world effectiveness of FMT. Fecal and serum samples before and after FMT along with medical information were collected and detected through 16S rRNA amplicon sequencing and untargeted liquid chromatography mass spectrometry. Mice models were used to preliminarily verify the exacerbation of colitis through administration of primary BAs and treated by FMT.

Results Patients in BAM group tended to achieve sustained higher and stable clinical response (66.67% vs. 49.41%) and remission (52.38% vs. 40.00%) than non-BAM group at 3 months after FMT, along with a significantly decrease of C4 ($P < 0.001$), improvement of obvious abdominal pain and diarrhea, which was especially obvious in CD patients with ileal resection and ileal /ileocolonic type. Random forest classifiers predicted BAM in IBD patients with 18 or top 4 differential OTUs, showing an area under the curve of 0.92 and 0.83, respectively. Furthermore, results from primary bile acid-induced colitis mice models reinforced these findings.

Conclusions Serum C4 and a minimal gut microbiota may identify IBD patients with BAM who are most likely to achieve durable remission after FMT. These translatable biomarkers can guide precision use of microbiota-directed therapy.

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Trial registration ClinicalTrials.gov: NCT01790061 and NCT01793831.

Keywords Inflammatory bowel disease, Bile acid malabsorption, Washed microbiota transplantation, Pain, Transendoscopic enteral tubing

Background

Bile acids (BAs) are hepatocyte-derived steroidal molecules essential for lipid emulsification and metabolic signaling. After hepatic conjugation, BAs enter the duodenum, where 95% undergo efficient ileal reabsorption via the enterohepatic circulation[1]. The residual BA flow into the colon, undergoing microbial biotransformation including deconjugation, dehydrogenation, and dihydroxylation to generate secondary BAs such as deoxycholic acid (DCA) and lithocholic acid (LCA). This microbial processing is critical for BA pool homeostasis and receptor-mediated signaling[2, 3]. In inflammatory bowel disease (IBD), which encompasses Crohn's disease (CD) and ulcerative colitis (UC), ileal even ileocolonic inflammation or resection, and backwash ileitis disrupt BA reabsorption, leading to excessive colonic BA influx[4, 5]. This results in bile acid malabsorption (BAM) and bile acid diarrhea (BAD), the condition affecting about 30% of CD patients with ileal involvement and contributing to steatorrhea and epithelial injury[5]. BAD represents a prevalent etiology of chronic watery diarrhea, with a prevalence rate of 1% within the general population[6, 7], a recent study also reported that BAD is associated with an increased incidence of gastrointestinal cancers [8]. While bile acid sequestrants (e.g., cholestyramine and colesevelam) ameliorate diarrhea [2, 9, 10], their efficacy is hampered by poor compliance, dose titration challenges, and aggravation of fat-soluble vitamin deficiencies [11]. Critically, these agents fail to correct the underlying microbial dysregulation driving BA dysmetabolism.

Current BAM diagnostics rely on cumbersome methods like ^{75}SeHCAT retention or 48-h fecal BA quantification, only accessible in less than 10 European countries [12]. Patients with BAM often exhibit increased synthesis of BA precursors [13]. Serum 7α-hydroxy-4-cholesten-3-one (C4), a BA synthesis precursor, emerges as a promising biomarker, with levels showing different standards based on interindividual variability for predicting BAD[2, 14–17]. Studies have shown a strong correlation between C4 levels and BAD diagnosed with ^{75}SeHCAT[18]. Moreover, when compared to SeHCAT, C4 has been found to have similar test characteristics[11, 19].

Despite decades of BA studies, diverse biological roles for BAs have been identified just recently due to developments in understanding the human microbiota[20]. Fecal microbiota transplantation (FMT), as the reconstruction technique of gut microbiota, represents a paradigm shift by restoring microbial BA-metabolizing functions. Despite mechanistic links between dysbiosis and BAM in IBD, clinical evidence for the efficacy of FMT remains sparse. Prior studies lack multi-omics integration and longitudinal cohort validation. Thus, in this study we aimed to evaluate the efficacy of FMT in IBD patients with BAM or BAD identified by serum C4, and explore the value of the random forest model in predicting the occurrence of BAM in IBD patients based on baseline microbiota.

Methods

Study design and clinical assessment

This study recruited healthy Chinese volunteers as a control group (healthy controls matched with patients, age, and sex) and donors of FMT and was derived and part of two registered trials (ClinicalTrials.gov: NCT01790061 and NCT01793831) which conducted at the Second Affiliated Hospital of Nanjing Medical University. We selected the study cohort (37 UC and 69 CD) based on the availability of paired clinical samples and associated data before and after FMT treatment in patients with IBD who underwent microbiota transplantation between 2013 and 2020. Demographics and clinical characteristics were thoroughly recorded before FMT. The daily score of abdominal pain, frequency of defecation, and clinical efficacy of treatments were recorded and evaluated at baseline, within 1 week, and 1 and 3 months after the initial FMT. In UC patients, clinical response was defined as a decrease of partial Mayo score ≥ 3 and $\geq 30\%$ from baseline, in addition to a decrease in the rectal bleeding sub-score of ≥ 1 or final rectal bleeding sub-score of ≤ 1[21]. Clinical remission was defined as a partial Mayo Score ≤ 1. In CD patients, clinical response was defined as the decrease of Harvey Bradshaw index (HBI) score > 3 and clinical remission was defined as HBI score ≤ 4. Loss to follow-up was excluded.

Patients and healthy

This study recruited 24 healthy Chinese volunteers as healthy controls. Healthy Chinese volunteers were those who have regular bowel habits every day with the Bristol Stool Scale ranging from 3 to 4; newly appeared gastrointestinal symptoms (e.g., abdominal pain, diarrhea, nausea, vomiting) and newly appeared illness or general symptoms (various organ systems) for at least 6 months were excluded. The inclusion criteria for patients with

IBD are the following: patients who were diagnosed as IBD based on a combination of typical clinical symptoms, endoscopy, and histological criteria for at least 6 months; patients with mild, moderate, and severe active UC (Mayo score from 3 to12) or CD (Harvey Bradshaw index ≥ 5); patients exhibited a poor response (defined by recognized international guidelines or expert consensus) and patients who failed to achieve satisfactory efficacy from conventional medications such as 5-aminosalicylic acid, steroids, cyclosporine, azathioprine, anti-TNF antibody, and traditional Chinese medicine; patients who were available of complete and paired clinical samples and associated clinical records before and after FMT treatment in patients with IBD who underwent microbiota transplantation. Excluded criteria are the following: patients missed blood samples; had a follow-up period < 3 months; under the age of 10 years; suffered from other intestinal diseases, e.g., Clostridioides difficile infection, or from other severe diseases, such as malignant neoplasm, serious liver or kidney diseases, or cardiopulmonary failure, refused to complete the follow-up, and underwent FMT.

Donors, FMT procedure and quality control

Adolescents (preferably aged 6–24 years old) who passed questionnaire screening, face-to-face screening, and laboratory screening step-by-step are potential donors in the clinical practice according to our previous study. Before April 2014, fecal microbiota was prepared by manual methods. Beginning in April 2014, the method for preparation of washed microbiota is improved and based on the automatic microbiota purification system (GenFMTer, FMT Medical, Nanjing, China) followed with centrifugation plus suspension for three times in a Good Manufacture Practice (GMP) level laboratory room, which is a development on methodology for FMT[22–24].

Mouse models

All experimental protocols were approved by the animal ethics committee of Nanjing Medical University. The male C57BL/6 mice weighted 20–22 g at 6–8 weeks from Animal Center of Nanjing Medical University were used (each group $n = 8$). Antibiotics pretreatment included the following: Ampicillin: 0.5 g/L, vancomycin: 0.25 g/L, metronidazole: 0.5 g/L, neomycin: 0.5 g/L and gentamicin: 0.5 g/L along with 1 packet of artificial sweetener in per 250 ml of drinking water as previously described[25], beginning 1 week prior to 2.5% DSS (Dextran Sulfate Sodium, MW: 36,000–50,000 Da, MP Biomedicals, USA) administration. The supplementation was CA (400 mg/kg) and CDCA (100 mg/kg) at the dose of 0.2 mL per mouse[26]. (1) Acute modeling group (Fig. 6a): All mice treated with antibiotics for 1 week before 2.5% DSS administration. In injury phase, Ctrl and CA/CDCA groups drunk normal water and were gavaged with PBS. CA or CDCA once daily for 1 week. DSS and CA/CDCA + DSS groups drunk 2.5% DSS water and were gavaged with PBS, CA, or CDCA once daily for 1 week. In recovery phase, all mice had water for 2 days. (2) Treatment group (Fig. S7a): All mice treated with antibiotics for 1 week before 2.5% DSS administration. In injury phase, Ctrl group drunk normal water and were gavaged with PBS. CA/CDCA + DSS groups drunk 2.5% DSS water and were gavaged with PBS, CA, or CDCA once daily for 1 week. FMT treatment group drunk 2.5% DSS water and were gavaged with PBS, CA, or CDCA together with fecal microbiota for 1 week. In recovery phase, all mice had water for 2 days. (3) Chronic modeling group (Fig. S9a): Ctrl, CA, and CDCA groups treated with normal water for the whole process and were gavaged with PBS, CA, or CDCA for 1 month. Anti + Ctrl/CA/CDCA groups treated with antibiotics for 1-week before returning to normal water and were gavaged with PBS, CA, or CDCA for 1 month. Serum, liver, spleen, and colon were taken to evaluate the inflammation and for Elisa (IL-1b, IL-10, and TNF-a) and qPCR (Muc2, Occludin, ZO-1, IL-1b, IL-10, and TNF-a) examination.

Serum metabolomics

Serum samples at baseline before FMT and 1 week after FMT and IBD patients were collected and stored at − 80℃ until untargeted metabolomics analysis based on liquid chromatography mass spectrometry (LC–MS/MS, Waters 2D UPLC system: Waters, Milton, MA; Q-Exactive mass spectrometer: Thermo Fisher Scientific, Wilmington, MA, USA, at a resolution of 70,000) [27]. High-resolution mass spectrometry (HRMS) was combined with the high-quality secondary spectrum information database (XCMS, mzCloud, Metlin, KEGG, HMDB, LIPIDMAPS) to match and identify the molecular characteristic peaks. Quality control (QC) samples were used (combined with BGILibrary) and a Coefficient of Variance (CV) less than 30% in QC samples were retained. Differential metabolites screening thresholds (OPLS-DA model): $|\log2FC| \geq 1$ & OPLS-DA_VIP ≥ 1 & P-value ≤ 0.05, combined with the RSD of QC samples was less than 15%. Due to the lack of SeHCAT retention in China, the gold standard for quantifying BAM [28] and uncertain value of C4 in different studies, we used the highest upper limit (relative abundance based on LC–MS/MS) of C4 in the serum of healthy controls to define the pathological increase of C4 in IBD patients. Higher than the highest upper limit of healthy controls was defined as BAM, otherwise defined as non-BAM.

Microbiome profiling and bioinformatics

Stool samples at baseline before FMT and 1 week after FMT from healthy donors and IBD patients were collected and stored at − 80℃. And then stool samples were sequenced for the V3–V4 region of 16S rRNA genes. Microbial DNA extraction and sequencing were carried out using an Illumina MiSeq platform (Illumina, Inc., San Diego, CA, USA). The mothur (version 1.33.3, http://www.mothur.org/), UPARSE 7.1 (http://drive5.com/uparse), and R (version 4.0.2, https://www.r-project.org/) software applications were used for processing the raw 16S rRNA gene sequences, Operational taxonomic units (OTUs) clustering, and analysis [29]. Specifically, UPARSE pipeline was employed to cluster OTUs having a sequence similarity of ≥ 97%. Taxonomic differences were identified through linear discriminant analysis of effect size (LEfSe) and abundance levels of genera were considered in the LEfSe and Wilcoxon signed-rank test. In addition, principal coordinates analysis (PCoA) based on the distance matrix of Bray–Curtis dissimilarity was performed. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) (http://picrust.github.io/picrust/tutorials/genome_prediction.html) program based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database was used to predict the functional alteration of microbiota [30, 31]. The interaction between differential microbiota and specific KEGG pathway was conducted by the Mantel test [32]. To examine the interactions among different species and metabolites, we constructed co-occurrence patterns based on 16S rRNA and LC–MS/MS data using the Spearman correlation coefficient. The co-occurrence patterns between significant correlated taxa or microbiota were illustrated by Cytoscape version 3.10.1 (https://cytoscape.org/).

Statistical analysis

All analyses were carried out using IBM SPSS Statistics 23.0.0 (SPSS Inc., Chicago, IL, USA), R version 4.0.0 (R Development Core Team, Vienna, Austria), and Python 3.7.7. Continuous data were expressed with mean ± standard deviation (SD). Categorical data were described as numbers (percentages). We compared groups using unpaired Student's t test and one-way ANOVA for normal continuous variables, a Mann–Whitney U test for skewed continuous variables, and a chi-square test or Fisher's exact test for categorical variables. Differences were considered significant when $P < 0.05$. The Spearman correlation was used for correlation analysis. The differences between serum metabolites and fecal microbiota detected before and after FMT were analyzed by Wilcoxon matched pairs signed rank. Machine learning using the "caret" package was performed to examine the complex relationships among metabolites and thus predict patient outcomes as accurately as possible. Training and testing datasets were derived and used in the feature selection in the training dataset, and then random forest (RF) algorithms were used to develop a classification model. The sklearn package of python was used to process the samples according to ratio of the training set: testing set to be 0.8:0.2. The model parameters of the training set were adjusted by fivefold CV, and the optimal parameters were selected and evaluated by the test set. The performance of the markers was analyzed by calculating the area under the receiver-operating characteristic curve (AUC).

Results

Incidence of BAM in patients with IBD

This study included 37 pairs of patients with UC and 69 pairs of patients with CD before and after treatment into the final analysis, along with a healthy control group of 24 individuals without liver and gastrointestinal diseases. The serum level of C4 in patients with IBD considered to be pathologically elevated when exceeding the upper limit of healthy control. As shown in Fig. 1a, 21 patients with IBD were considered as BAM and their serum C4 levels were significantly higher than those of healthy controls and non-BAM group ($P < 0.0001$, respectively). A total of 20.29% (14/69) of patients with CD and 18.92% (7/37) of patients with UC were diagnosed with BAM using the upper limit of C4 in healthy controls (Fig. 1b, c). Clinical characters such as age, sex, disease activity, and age of diagnosis had no difference between BAM group and non-BAM group (Table 1).

Additionally, among the BAM group in patients with CD, the proportion of ileal type and ileocolonic type were as high as 85.71% (12/14), and 50.00% (7/14) of the patients had a history of ileal resection (Table 1), only 12.73% (7/55) of non-BAM patients had a history of ileal resection ($P = 0.005$). The C4 levels in CD patients with ileal resection were higher than that in non-resection group ($P < 0.005$, Fig. 1d).

IBD patients with BAM have altered serum metabolome and fecal microbiome

A total of 2012 metabolites classified into 193 metabolic class or super classes based on HMDB taxonomy were identified using untargeted metabolomic analysis. When comparing metabolites related to BA synthesis between BAM group and non-BAM group, C4 and chenodeoxycholic acid 3-sulfate significantly increased in BAM group before FMT, while taurochenodeoxycholate-3-sulfate, glycochenodeoxycholate-3-sulfate, and taurochenodeoxycholic acid (TCDCA) showed a significant decrease trend (Fig. 1f). Concurrently, the level of sulfated BAs

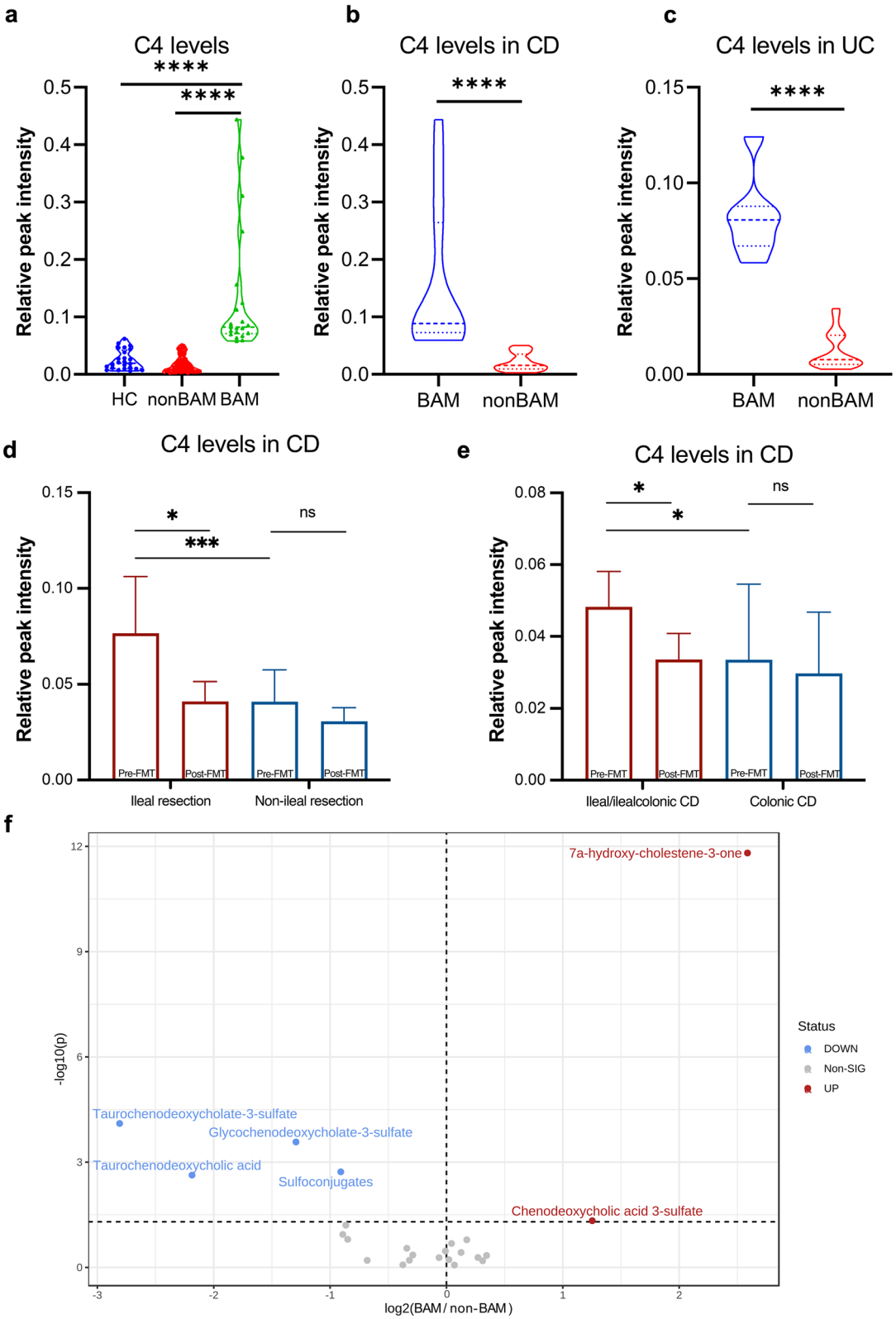

Fig. 1 The serum level of C4 in patients with IBD and HC, and metabolites related to bile acid synthesis. The serum level of C4 **a** between patients with IBD and healthy controls. **b** in CD patients with or without BAM. **c** in UC patients with or without BAM. **d** in CD patients with ileal resection/non-ileal resection. **e** in CD patients with ileal/ileocolonic lesions. **f** Differential metabolites related to bile acid synthesis between BAM group and non-BAM group. CD, Crohn's disease; UC, Ulcerative Colitis; HC, healthy control; BAM, Bile Acid Malabsorption. **** $p < 0.0001$

Table 1 Characteristics of BAM and non-BAM in patients with IBD

Characteristics	BAM (*N* = 21)		Non-BAM (*N* = 85)		*p* value
	CD (*N* = 14)	UC (*N* = 7)	CD (*N* = 55)	UC (*N* = 30)	
Age (years, *mean* ± SD)	36.8 ± 14.1	42.6 ± 13.8	34.6 ± 14.7	36.7 ± 11.9	0.36
Sex (male, *n*)	7 (50.0)	2 (28.6)	35 (63.6)	19 (63.3)	0.14
Disease activity (*n, %*), pre FMT					0.74
Remission	0	0	0	0	
Mild	4 (28.6)	0	11 (20.0)	1 (3.33)	
Moderate	10 (71.4)	3 (42.9)	42 (76.4)	10 (33.3)	
Severe	0	4 (57.1)	2 (3.6)	19 (63.3)	
Disease activity (*n, %*), 1 m post FMT					
Response	9 (64.3)	4 (57.1)	39 (70.9)	17 (56.7)	0.80
Remission	9 (64.3)	2 (28.6)	32 (58.2)	5 (16.7)	0.48
Disease activity (*n, %*), 3 m post FMT					
Response	10 (71.4)	4 (57.1)	30 (54.5)	12 (40.0)	0.22
Remission	9 (64.3)	2 (28.6)	26 (47.3)	8 (26.7)	0.33
Surgery (*n, %*)	9 (64.3)	2 (28.6)	26 (47.3)	10 (33.3)	0.47
Ileal resection	7 (50.0)	0	7 (12.7)	0	0.005
Others	2 (14.3)	2 (28.6)	19 (34.5)	10 (33.3)	
Drug history (*n, %*)					NA
No treatment	0	0	0	0	
Mesalazine	13 (92.9)	6 (85.7)	52 (94.5)	28 (93.3)	
Immunosuppressant	9 (64.3)	0	22 (40.0)	4 (13.3)	
Anti-TNFα	3 (21.4)	0	17 (30.9)	3 (10.0)	
Traditional Chinese medicine	9 (64.3)	6 (85.7)	32 (58.2)	25 (83.3)	
Steroids Dependence (*n, %*)	3 (21.4)	3 (42.9)	11 (20.0)	11 (36.7)	
Classification of UC (*n, %*)					NA
Ulcerative proctitis (E1)	NA	1 (14.3)	NA	1 (3.3)	
Distal UC (E2)	NA	1 (14.3)	NA	4 (13.3)	
Extensive UC or ulcerative pancolitis (E3)	NA	5 (71.4)	NA	25 (83.3)	
CD Montreal classification					
Age of diagnosis (***n***, ***%***)					0.53
≤ 16 years (A1)	2 (14.3)	0	5 (9.1)	3 (10)	
17–40 years (A2)	8 (57.1)	5 (71.4)	39 (70.9)	23 (76.7)	
> 40 years (A3)	4 (28.6)	2 (28.6)	11 (20.0)	4 (13.3)	
Location (*n, %*)					NA
Ileal CD (L1)	4 (28.6)	NA	10 (18.2)	NA	
Colonic CD (L2)	2 (14.3)	NA	10 (18.2)	NA	
Ileocolonic CD (L3)	8 (57.1)	NA	34 (61.8)	NA	
Upper GI (L4)	1 (7.1)	NA	5 (9.1)	NA	
Behavior (*n, %*)					
Non-stricturing, non-penetrating (B1)	4 (28.6)	NA	20 (36.4)	NA	
Stricturing (B2)	7 (50.0)	NA	28 (50.9)	NA	
Penetrating (B3)	3 (21.4)	NA	7 (12.7)	NA	

BAM, bile acid malabsorption; non-BAM, no bile acid malabsorption; UC, ulcerative colitis; CD, Crohn's disease; SD, standard deviation; NA, not applicable

catalyzed by enzymes such as sulfotransferase 2A1 (SULT2A1) in the liver, also decreased significantly in BAM group (Fig. 1f).

There were significant differences in serum metabolism between disease groups (BAM and non-BAM) and health group (Additional file 1: Fig. S1a, b) both in the negative mode and positive mode. A total of 238 serum metabolites exhibited significant differences were observed in serum metabolome between BAM and non-BAM groups (Additional file 1: Fig. S1c, d).

PLS-DA analysis in metabolites showed significant differences between disease groups and healthy group (Additional file 1: Fig. S2a), while OPLS-DA analysis showed no difference between disease groups (Additional file 1: Fig. S2b). There were differences in 12 metabolic classes, in which quinone and hydroquinone lipids, retinoids, glycerophosphoethanolamines, oxosteroids and cholestane steroids, and glycerophosphocholines significantly increased in the BAM group ($P < 0.05$, Additional file 1: Fig. S2c), which belong to the "Lipids and Lipid-like Molecules" superclass. The predominant differential metabolites (55.56%, 75/135) such as glycerophosphocholines and glycerophosphoethanolamines in BAM group belonged to Lipids and Lipid-like Molecules superclass (Additional file 2: Table S1).

The 16S rRNA sequencing data clustered and annotated 875 OTUs, the distribution of OTUs before FMT across different classifications was illustrated in Additional file 1: Fig. S3a (Venn). There were 385 OTUs shared among the BAM, non-BAM, and healthy groups, while 150, 74, and 4 OTUs were uniquely present in healthy group, non-BAM, and BAM groups, respectively. The top 15 genera were displayed in Additional file 1: Fig. S3b. Notably, the composition of *Bacteroides and Parabacteroides* were similar in BAM, non-BAM, and healthy groups. At the genus level of the dominant bacteria in the disease group, pathogens including *Escherichia* and *Enterococcus* showed the highest proportion in BAM group, followed by non-BAM group, while *Veillonella* demonstrates the opposite trend. *Streptococcus* and *Lachnoclostridium* exhibited a similar composition in BAM and non-BAM groups. Conversely, *Prevotella*, *Faecalibacterium*, *Subdoligranulum*, *Ruminococcus*, and *Megamonas* were relatively enriched in healthy individuals.

At the diversity level, both BAM and non-BAM groups exhibited lower richness and Shannon diversity compared to healthy group, but there was no significant difference between BAM and non-BAM groups (Additional file 1: Fig. S3c, d). The analysis of β-diversity revealed that both BAM group and non-BAM group exhibited significant separation from healthy group (Additional file 1: Fig. S3e).

Results from the LEfSe analysis at the genus level (Additional file 1: Fig. S3f) revealed that the characteristic microbiota of healthy group interestingly resembled those with high abundance in the healthy condition. Microbiota of non-BAM group were characterized by genera such as *Gemella*, *Parasutterella*, *Morganella*, *Tyzzerella*, *Peptostreptococcus*, and *Bifidobacterium*. Elevated levels of *Escherichia*, *Enterococcus*, *Fusobacterium*, *Akkermansia*, and *Erysipelatoclostridium* were related to the occurrence of BAM.

To further clarify the microbial differences associated with BAM in patients with IBD, we compared and identified 18 differential microbiota at the OTU level between disease groups (Additional file 1: Fig. S4). Of these, 15 OTUs were dominant in BAM group, only 3 OTUs exhibited high relative abundance in non-BAM group, such as *Bacteroides coprocola* DSM 17136 (OTU48), *Alloprevolella* sp. (OTU60), and *Barnesiella* sp. (OTU117).

Simultaneously, we further conducted Mantel analysis correlating the differential microbiota with predicted KEGG pathways. As shown in Fig. 2a in BAM group, a high correlation was observed among *Selenomonas* sp. oral taxon 136 (OTU480), *Lachnoanaerobaculum* sp. (OTU349), and *Selenomonas artemidis* (OTU434). *Stomatobaculum* sp. (OTU715) was highly correlated with *Eubacterium brachy* ATCC 33089 (OTU592) and showed moderate positive correlation with KEGG pathways of amino acid metabolism ($r = 0.465$), digestive system ($r = 0.309$), and metabolism of cofactors and vitamins ($r = 0.415$). OTU592 also demonstrated a positive correlation with the digestive system pathway ($r = 0.367$). *Stomatobaculum* sp. (OTU631) exhibited a positive correlation with cellular processes and signaling ($r = 0.317$).

In the non-BAM group (shown in Fig. 2b), only *Bacteroides* sp. (OTU315) was not enriched. Similarly, OTU480, OTU349, and OTU434 continued to exhibit high correlations. Moreover, OTU631 had a high correlation with OTU349, OTU480, and *Leptotrichia* sp. oral clone FP036 (OTU447). Out of 20 annotated KEGG pathway categories, 12 pathways showed some correlations with the changes in OTUs.

Interaction and prediction between differential microbiota and metabolites

As illustrated in Additional file 1: Fig. S5a and b, there is a close correlation between differential microbiota and metabolites in non-BAM group. Conversely, the connections between differential taxa in BAM group are not so close, with only a significant negative correlation between OTU631 and 7alpha-hydroxy-3-oxochol-4-en-24-oic acid. However, the relationship between different microbiota in non-BAM group was relatively close, with more positive correlations between the differential metabolites and microbiota. For example, OTU592 is associated with glycochenodeoxycholate-3-sulfate and taurochenodeoxycholate-3-sulfate, OTU60 is linked with glycochenodeoxycholate-3-sulfate. Gitogenin is related to *Actinomycetaceae* sp. (OTU531) and *Megasphaera micronuciformis* (OTU607) and positively related to C4, while *Scardovia wiggsiae* F0424 (OTU391) is directly associated with C4.

Furthermore, we constructed a random forest model based on 18 OTUs to predict the occurrence of BAM

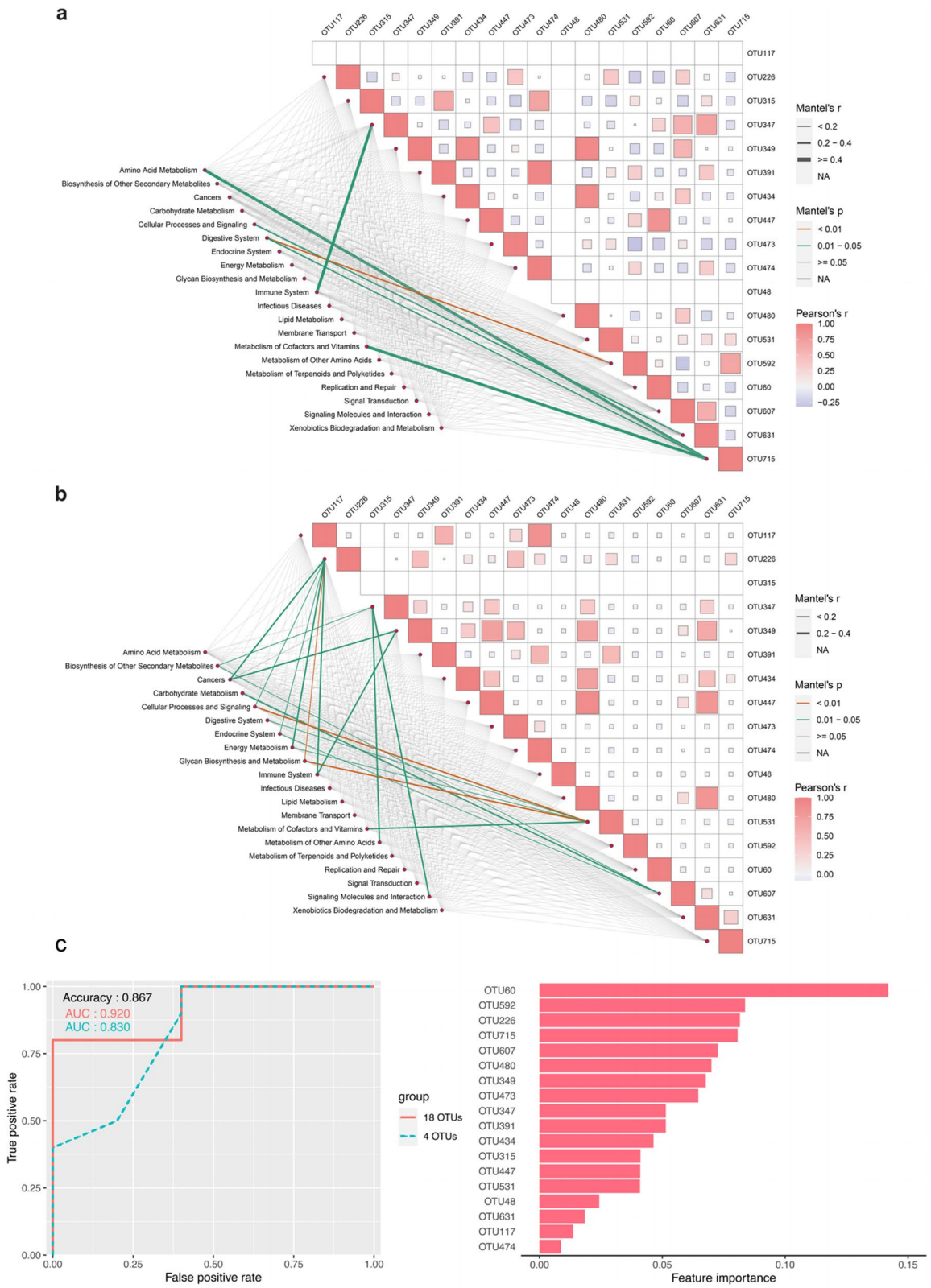

Fig. 2 Mantel analysis correlating the differential microbiota with predicted KEGG pathways and random forest classifier. **a** Mantel analysis in BAM group. **b** Mantel analysis in non-BAM group. **c** A random forest classifier constructed with 18 differential OTUs to predict the occurrence of BAM in patients with IBD. Adjusted *P* values were shown through false discovery rate control. BAM, Bile Acid Malabsorption

in patients with IBD. As shown in Fig. 2c, the AUC reached 0.92 on the test set, with an accuracy of 86.7% and an F1-score of 0.909. After selecting the top 4 OTUs (OTU60, OTU226: *Eubacterium nodatum* group, OTU592, and OTU715) based on importance ranking for another round of random forest model construction, the results indicated a slight decrease in AUC on the test set to 0.83, but the F1-score and accuracy remained unchanged.

BAM and IBD improved after FMT

Of the BAM group, 1-month post-FMT, 61.90% (13/21) and 52.38% (11/21) achieved clinical response and clinical remission, respectively, while 65.88% (56/85) and 43.53% (37/85) achieved clinical response and clinical remission in non-BAM group (Fig. 3a). 66.67% (14/21) and 52.38% (11/21) achieved clinical response and clinical remission in BAM group, respectively, while 49.41% (42/85) and 40.00% (34/85) achieved clinical response and clinical remission in non-BAM group 3-month post-FMT (Fig. 3a). Whether the patients with IBD were diagnosed as BAM or non-BAM had no effect on the clinical efficacy 1 month and 3 months post-FMT, either in clinical response ($P=0.80$, 1 month post-FMT; $P=0.22$, 3 months post-FMT) or in clinical remission ($P=0.48$, 1 month post-FMT; $P=0.33$, 3 months post-FMT). Compared with non-BAM group, BAM group had a higher proportion of improvement in abdominal pain and diarrhea after FMT. As illustrated in Fig. 3b, the number of patients in BAM group with abdominal pain decreased from 85.71% (18/21) to 42.86% (9/21) 1 month post-FMT and to 38.10% (8/21) 3 months post-FMT. While in non-BAM group, 52.94% (45/85) and 57.65% (49/85) of patients still had abdominal pain, 1 month and 3 months post FMT, respectively. In terms of diarrhea (Fig. 3c), 42.86% (9/21) and 54.12% (46/85) of patients (BAM vs. non-BAM) complained of diarrhea 1 month post FMT, while at 3 months post FMT, there were 42.86% (9/21) and 52.94% (45/85) of patients (BAM vs. non-BAM) still had diarrhea. The detailed changes of abdominal pain grades and diarrhea times are shown in Fig. 3f–i.

The C4 levels in CD patients with ileal resection decreased significantly after FMT, while the decrease of C4 in non-resection group showed no significant difference ($P=0.12$, Fig. 1d). Patients with ileal/ileocolonic lesions got more obvious decrease of C4 than colonic lesions group ($P=0.01$ vs. $P=0.53$, Fig. 1e). CD patients with ileal/ileocolonic lesions or ileal resection were attended to achieve higher and more stable rate of clinical response and remission than colonic CD or non-resection (Fig. 3d, e).

In BAM group, the symptoms of diarrhea and abdominal pain improved when the level of serum C4 decreased significantly after FMT ($P<0.001$, Fig. 4a). There was no significant change in the level of BAs except glycochenodeoxycholate-3-sulfate (Fig. 4a). In non-BAM group, the decrease trend of C4 had no significant difference and even exhibited an increase trend in some patients. However, bile acid metabolism changed significantly in non-BAM group, the levels of CA, CDCA, DCA, glycolithocholic acid, glycoursodeoxycholic acid, and chenodeoxycholic acid 3-sulfate increased significantly after FMT (Fig. 4b).

Significant improvement in lipid metabolism can be observed at both metabolic classes and single metabolite levels in BAM group (Additional file 2: Table S2). The levels of Lactones, Fatty alcohol esters, Fatty acid esters, Monoradylglycerols, Cholestane steroids, Triradylcglycerols, Lineolic acids and derivatives, Vitamin D and derivatives, Glycerophosphoethanolamines, Diradylglycerols, and Retinoids significantly decreased after FMT (Fig. 4c), which belonged to Lipids and Lipid-like Molecules superclass. A similar distribution can also be observed in metabolites which significantly decreased after FMT in BAM group, 42.75% (115/269) of which belonged to Lipids and Lipid-like Molecules superclass (Additional file 1: Table S2).

In non-BAM group, with a significant improvement in lipid metabolism 1 week post-FMT (Additional file 2: Table S3). The levels of Lactones, Fatty alcohol esters, Fatty acid esters, Monoradylglycerols, Vitamin D and derivatives, Triradylcglycerols, Endocannabinoids, Triterpenoids, Monoterpenoids, and Tetraterpenoids significantly decreased after FMT, which belonged to Lipids and Lipid-like Molecules superclass (Fig. 4d).

The 16S rRNA sequencing data clustered and annotated 934 OTUs. The top 10 genera were displayed in Fig. 5a. The dominant genera before and after treatment are similar with each other. Notably, the pathogenic genus Fusobacterium, ranked 7th, is absent in healthy group. Surprisingly, among the genera with low abundance in healthy group, such as *Escherichia* and *Veillonella*, there was a decreasing trend after FMT treatment in both BAM and non-BAM group. Similarly, among the genera with high abundance in healthy group, such as *Faecalibacterium*, *Prevotella*, and *Subdoligranulum*, there was an increasing trend after FMT treatment.

At the diversity level, the richness and Shannon diversity of both BAM non-BAM group after FMT were still lower than those of healthy group but had a trend towards healthy group. However, there is no significant difference between BAM and non-BAM group (Fig. 5b, c). PCoA analysis revealed that the distribution of samples after FMT in BAM and non-BAM groups tended to converge towards healthy group (Fig. 5d).

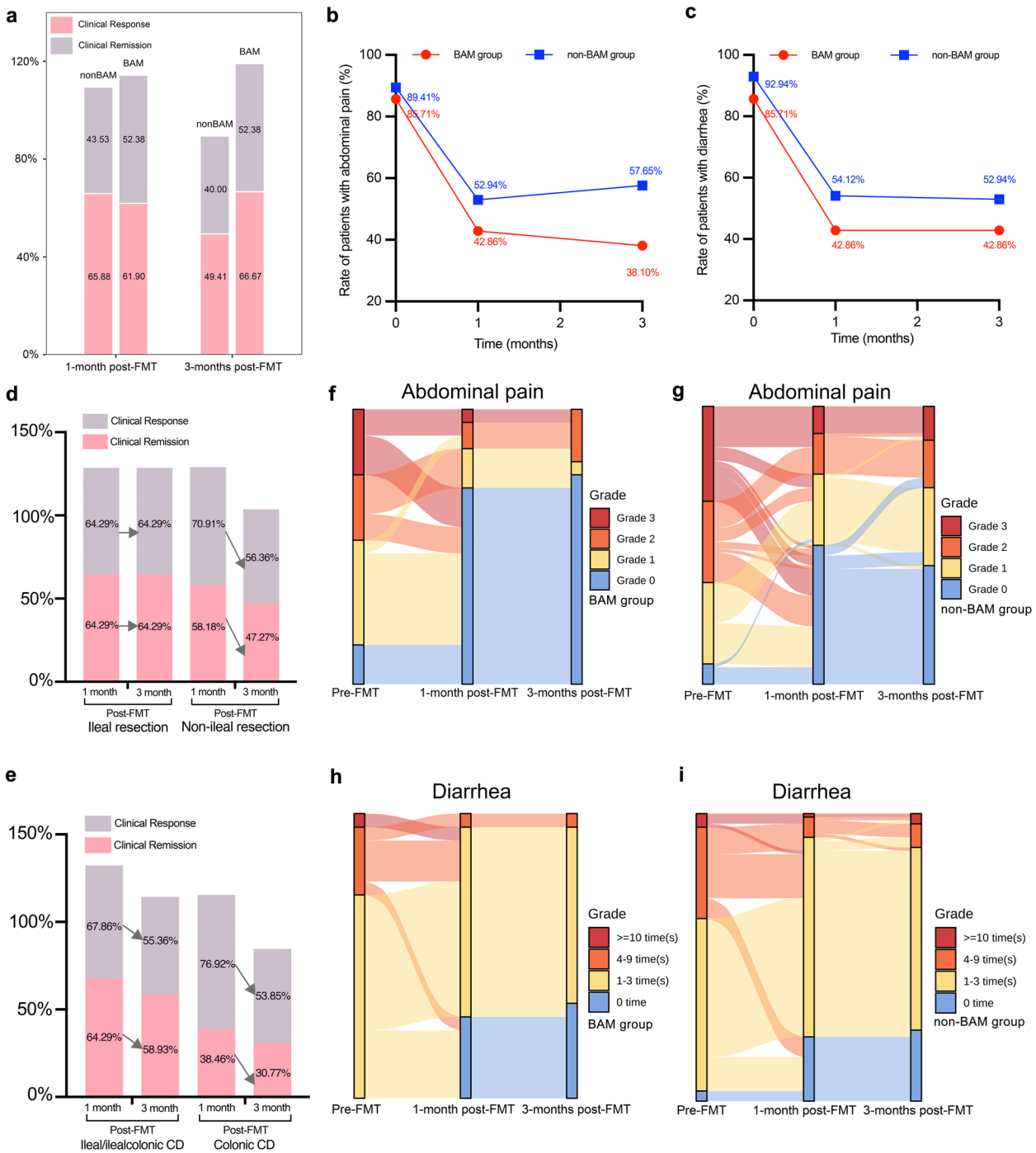

Fig. 3 Patients' clinical outcomes in BAM and non-BAM group. **a** Proportion of clinical response and remission. **b** Rate of patients with abdominal pain in BAM group before and after FMT. **c** Rate of patients with diarrhea in BAM group before and after FMT. **d** Proportion of clinical response and remission in CD patients with ileal resection/non-ileal resection. **e** Proportion of clinical response and remission in CD patients with ileal/ileocolonic lesions. **f, g** Sankey diagram of grade changes in patients with abdominal pain in BAM group and non-BAM group before and after FMT. **h, i** Sankey diagram of times changes in patients with diarrhea in BAM group and non-BAM group before and after FMT. BAM, Bile Acid Malabsorption

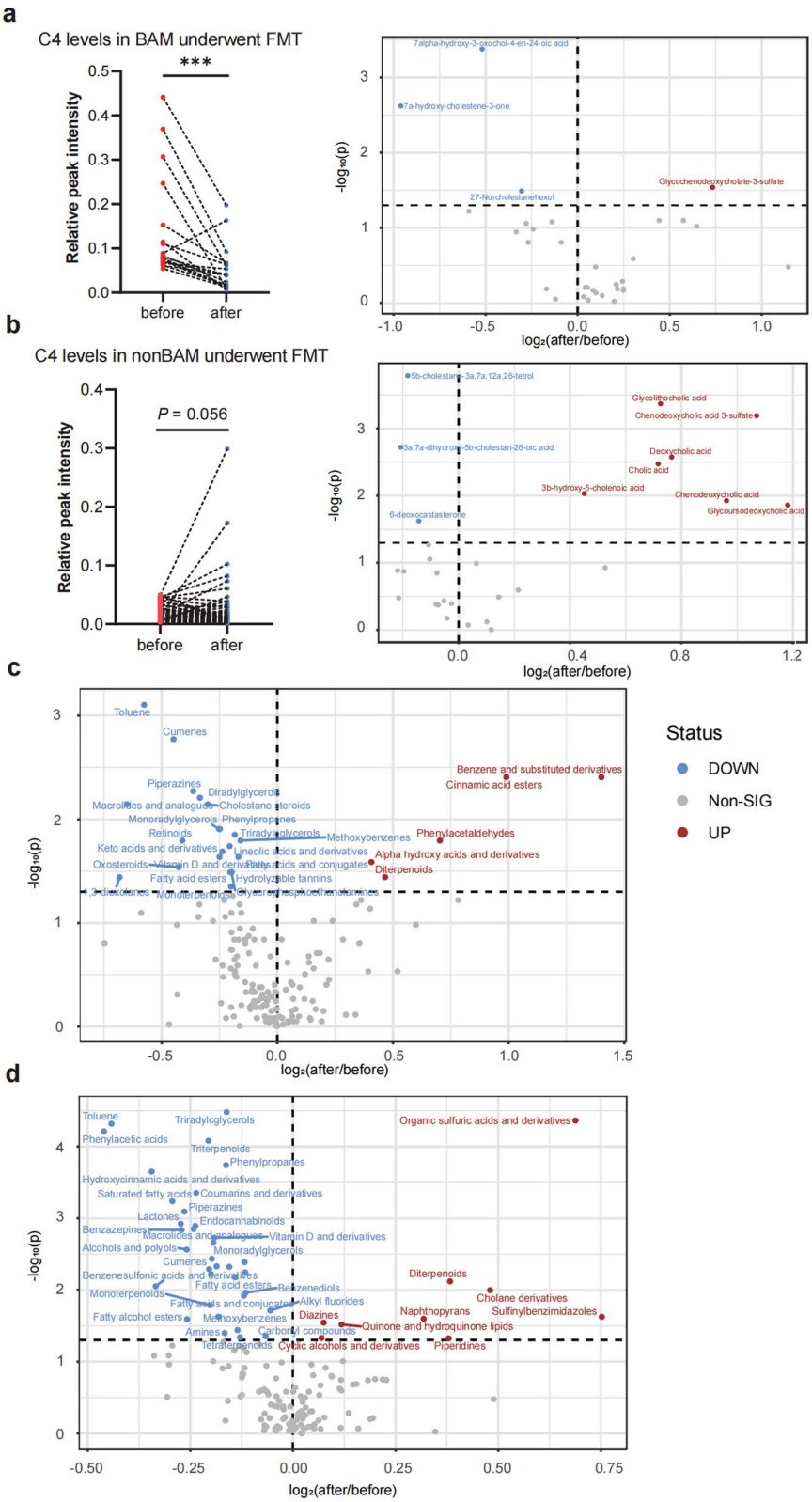

Fig. 4 The serum level of C4 and metabolites changes related to bile acid synthesis in BAM and non-BAM group. **a** C4 level in BAM after FMT and Volcano plot of bile acids in BAM patients before and after FMT. **b** C4 level in non-BAM after FMT and Volcano plot of bile acids in non-BAM patients before and after FMT. **c** Volcano plot of other differential metabolic classes in BAM before and after FMT. **d** Volcano plot of other differential metabolic classes in non-BAM before and after FMT. BAM, Bile Acid Malabsorption. *** $p < 0.001$

The abundance of differential microbiota between BAM and non-BAM groups after FMT were shown in Fig. 5e. In BAM group, most genera exhibited significantly decreased abundance after FMT, such as OTU480, OTU226, *Veillonella* sp. (OTU347), OTU349, *Shuttleworthia satelles* DSM 14600 (OTU473), and OTU531, etc. Except for a fraction of genera such as OTU48 ($P=0.003$) and OTU117 ($P=0.008$) were significantly increased, and OTU447, OTU60, and OTU592 showed an increasing trend. Notably, after FMT treatment, both the response group (here the response status included clinical response and clinical remission) and non-response group experienced a significant increase in OTU48 and OTU117, demonstrating a transition from absence to presence. Additionally, compared to the non-response group, the response group exhibited relatively lower growth of OTU48 but higher growth of OTU117. Furthermore, OTU60 and OTU715 started to show enrichment in response group compared to their baseline levels before FMT, while OTU715 still displayed a decreasing trend in non-response group. Similarly, OTU447 and OTU391 exhibited an opposite trend of enrichment between response group and non-response group.

Within non-BAM group, the differential genera displayed trends similar to which observed in the BAM group, except for OTU48 and OTU592. The abovementioned genera OTU48 and OTU592 exhibited a declining trend after FMT treatment in non-BAM group, with OTU48 notably showing an opposing trend of increase in non-response group ($P=0.012$). While the majority of microbiota exhibited no statistically differences, other OTUs that showed opposing trends between response group and non-response group within non-BAM group were *Actinomyces dentalis* (OTU474), OTU480 (up in non-response group, $P=0.022$), OTU226, OTU715, OTU60, OTU607, OTU434, and OTU447. The differential microbiota may be focal points of our future research.

FMT can improve PBA supplementation-exacerbated colitis in acute mice models

In an acute enteritis mice model (Fig. 6a), disease activity index (DAI) scores progressively increased after PBA (CA and CDCA) supplementation (Fig. 6f). From day 4, DAI scores in CA + DSS group were stably and significantly higher than that in single DSS group, and at day 5, DAI scores in CDCA + DSS group began to have the same rising trend. At the endpoint, the differences of DAI between groups were most pronounced, with CA + DSS group showing a significant increase over the DSS group ($P<0.001$), and CA group showing a significant increase over control group ($P<0.001$) (Fig. 6f). Even after DSS withdrawn, the exacerbated colitis phenotype continued. The severity of colitis was inversely correlated with colon length and positively correlated with spleen index. Mice in CA + DSS group had significantly shorter colons than those in the DSS group (Fig. 6c, g, $P=0.007$), and their spleen index were significantly higher than DSS group (Fig. 6d, h). At the endpoint, representative images of the colon from different groups showed smooth mucosa in control group while CA and CDCA groups exhibited unclear vasculature with granular and reddened surfaces in mucosa. The DSS group showed rough mucosa with small erosions and bleeding, and CA + DSS and CDCA + DSS groups displayed extensive ulceration and obvious spontaneous bleeding (Fig. 6e). Histopathological scores (including severity of inflammation, extent of injury, and crypt damage) in CA + DSS and CDCA + DSS group were significantly higher than DSS group (Fig. 6b, i). We measured inflammatory cytokine expression in the serum to explore whether CA and CDCA supplementation exacerbated colitis by modulating cytokine levels. As shown in Additional file 1: Fig. S6a and b, compared to the DSS group, CA and CDCA significantly increased the expression of pro-inflammatory cytokines such as TNF-α and IL-1β in single-DSS mice. However, the anti-inflammatory cytokine IL-10 was significantly decreased in CA + DSS and CDCA + DSS groups (Additional file 1: Fig. S6c).

Treatment with FMT from healthy donors ameliorated the exacerbated colitis phenotype induced by CA and CDCA (Fig. S7a). This was evidenced by reduced DAI scores: which progressively decreased in the treatment experiment and were significantly lower than untreated CA/CDCA + DSS group (Fig. S7f). The colon length, spleen index, and histopathological scores in

(See figure on next page.)

Fig. 5 Microbial signatures associated with short-term responsiveness to FMT in BAM, non-BAM and HC group. **a** Relative abundance of the top 10 genera after FMT. **b, c** Comparison of α-diversity (richness and Shannon index) before and after FMT. **d** Comparison of β-diversity (PCoA of Bray–Curtis distance) before and after FMT. **e** Heat map of differential microbiota between response and non-response group (here the response status included clinical response and clinical remission) in BAM and non-BAM group. Adjusted *P* values were shown through false discovery rate control. HC, healthy control; BAM, Bile Acid Malabsorption; BAM_R, patients with BAM and have response after FMT; BAM_NR, patients with BAM and have no response after FMT; nonBAM_R, patients have no BAM and have response after FMT; nonBAM_NR, patients have no BAM and have no response after FMT; NA, no significance. * $p<0.05$; ** $p<0.01$; *** $p<0.001$

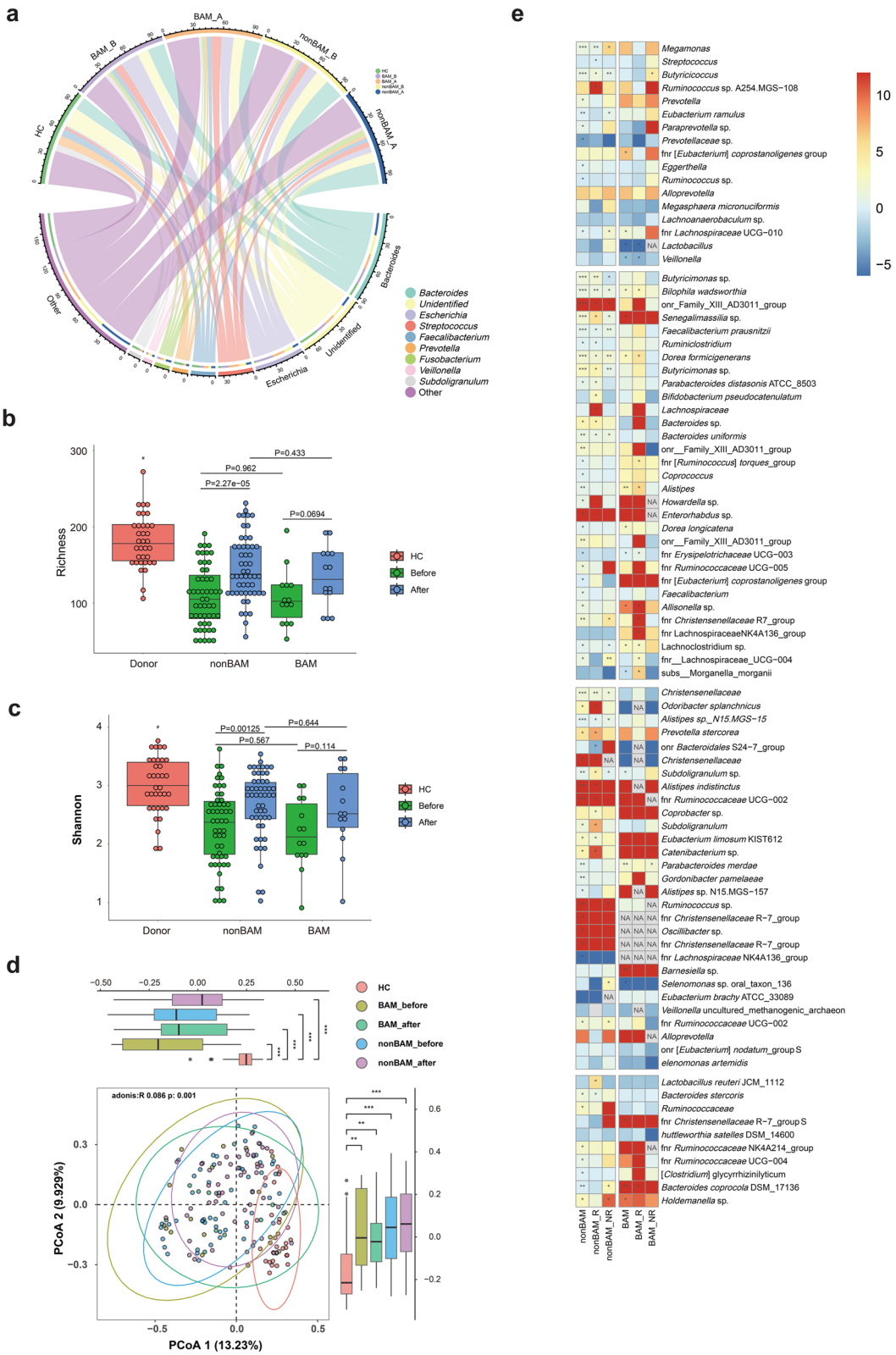

Fig. 5 (See legend on previous page.)

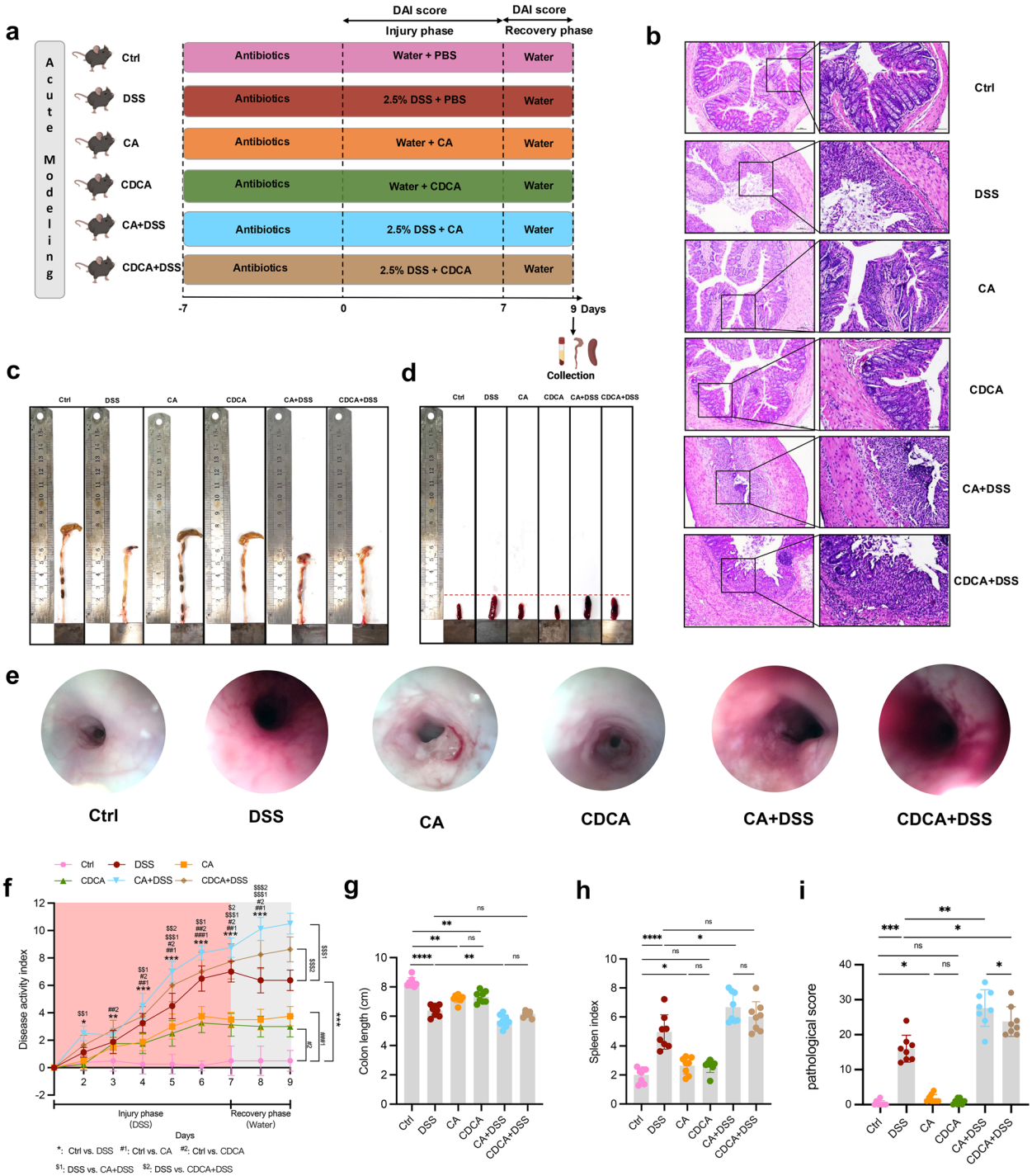

Fig. 6 Primary BA administration in acute modeling mice. **a** Flow chart. **b** Representative H&E images, **c** Representative colon images, **d** Representative spleen images, **e** Representative endoscopic images of the colon, **f** DAI score, **g** Colon length, **h** Spleen index, **i** Pathological score of acute modeling mice. ^{ns} $p > 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

FMT-treated CA/CDCA + DSS group were also significantly different from untreated CA/CDCA + DSS group (Fig. S7b–d, g–i). At the endpoint, representative images of the colon showed healed smooth mucosa in FMT-treated CA/CDCA + DSS group (Fig. S7e). Intestinal barrier integrity indicated that FMT treatment significantly increased mRNA levels of tight junction proteins in the colonic tissue compared to untreated CA/

CDCA + DSS group, including Zonula Occludens-1 (ZO-1) and Occludin (Additional file 1: Fig. S8b, c, $P < 0.001$), as well as the mucus layer protein Mucin 2 (Muc2) (Additional file 1: Fig. S8a, $P < 0.001$). Additionally, FMT reduced the mRNA expression of pro-inflammatory cytokines such as TNF-α and IL-1β in the intestinal mucosa compared to untreated CA/CDCA + DSS group (Additional file 1: Fig. S6h, i, $P < 0.001$), while significantly increasing IL-10 levels (Additional file 1: Fig. S6g, $P < 0.001$). These changes of cytokine levels in serum were consistent with those observed in the colon (Additionalfile 1: Fig. S6d, e, f). Interestingly, FMT treatment significantly reduced C4 levels in CA + DSS/CDCA + DSS groups (Additional file 1: Fig. S8d, $P < 0.001$), accompanied by a decrease in cytochrome P450 7A1 (CYP7A1), cytochrome P450 8B1 (CYP8B1), and an accumulation of cytochrome P450 27A1 (CYP27A1), both of which are involved in the synthesis and metabolism of C4 and BAs (Additional file 1: Fig. S8e-g).

PBA supplementation-exacerbated colitis in chronic mice models

We also explored the significant role of primary BAs (PBAs, CA, and CDCA) in inducing chronic intestinal injury in mice model (Fig. S9a). After 7 days' antibiotics supplementation and PBA supplementation for 1 month, DAI evaluation for mild to moderate intestinal injury was observed and more obvious than non-antibiotics group (Fig. S9g). Histopathological scores and representative images showed local accumulation of inflammatory cells and some crypt damage in antibiotics + PBA supplementation (Fig. S9b, e). The colon length and spleen index in antibiotics + PBA supplementation group were also significantly different from antibiotics + ctrl group (Fig. S9c, d, f).

Discussion

In this study, we detected serum C4 levels and used the highest upper limit of C4 from healthy controls to identify the pathological increase of C4 in IBD patients. The differences on metabolic and microbial characteristics, therapeutic effects of FMT between BAM and non-BAM group in patients with IBD were analyzed. In order to further verify the clinical findings, we conducted administration of PBAs (CA and CDCA) to exacerbate colitis in the antibiotic-mediated dysbiosis plus DSS-induced acute colitis model, which further confirmed the important impact of imbalanced BA levels on the occurrence and development of IBD, and efficacy has been observed after FMT. To identify as many IBD patients with BAM/BAD as possible, we used the upper limit of C4 as the threshold bases on the relative abundance results of serum metabolomics in healthy controls. Consequently, CD patients with ileal and ileocolonic diseases exhibited excessive colonic BA influx and compensatory elevation in serum C4 levels [4, 5]. The rate of having a history of ileal resection was 33.33% (7/21) in BAM group and 8.24% (7/85) in non-BAM group in this study ($P = 0.005$). Our results showed that CD patients with a history of ileal resection had the highest prevalence trend of BAM, which was consistent with previous studies [16, 33, 34]. The combination of clinical endoscopy scores, symptoms, efficacy, serum metabolome, and fecal microbiome, as well as healthy controls, better characterized C4 concentrations in IBD patients with suspected BAM and BAD.

The reconstruction of gut microbiota and relative metabolism through FMT can bring benefit to many diseases categorized into eight domains in clinical practice, including gastrointestinal disorders[35, 36], infections[37, 38], metabolic disorders[39], microbiota-gut-liver axis[40], microbiota-gut brain axis[41], oncology[42, 43], hematological disorders[44], and other diseases[45] such as chronic obstructive pulmonary disease. In this study, we found that in BAM group, the long-term efficacy of FMT was more obvious, with a sustained higher rate of clinical response (66.67%) and clinical remission (52.38%) even 3 months after FMT. Conversely, in non-BAM group, the rate of clinical response (49.41%) and clinical remission (40.00%) began to decline 3 months after FMT. In IBD patients with BAM, the two main symptoms: diarrhea and abdominal pain improved obviously along with serum C4 decreased significantly after FMT. However, in non-BAM group, the trend of diarrhea and abdominal pain even exhibited an increase trend in some patients. In CD patients with ileal resection, ileal or ileocolonic type, the C4 levels decreased significantly after FMT, along with more obvious and stable clinical response and remission in IBD symptoms. The aforementioned phenomenon may be attributed to the composition and function changes of the differential microbiota and metabolites between BAM and non-BAM groups before and after FMT.

BAs such as CA and CDCA are synthesized from cholesterol in the liver and then enter the intestine, where they are further transformed into various complex BA derivatives under the action of gut microbial BA metabolic enzymes. At the diversity level, both BAM and non-BAM groups exhibited lower richness and Shannon diversity compared to healthy group. Pathogens genera *Escherichia*[46] and *Enterococcus*[47, 48] showed higher proportion in BAM group than non-BAM group. Chen et al. characterized that *Escherichia coli* was the main type of intestinal microbiota in children with active IBD[6]. Compared to BAM group, only 3 OTUs exhibited high relative abundance in non-BAM group, which were also highly enriched in healthy group. After selecting 18

OTUs and the top 4 OTUs based on importance ranking for random forest model to predict the occurrence of BAM in patients with IBD, the AUC reached 0.92 and 0.83 on the test set, respectively, with an accuracy of 86.7% and an F1-score of 0.909 remaining unchanged. The model suggested that top OTUs such as *Alloprevolella* spp. (OTU60), *Eubacterium nodatum* group (OTU226), *Eubacterium brachy* ATCC 33089 (OUT592), and *Stomatobaculum* sp. (OTU715) may play important roles in the occurrence and development of BAM in IBD. Our study identified that OTU226 decreased significantly in BAM group after FMT. In other studies, OTU226 aggravated intestinal mucosal injury in LPS-induced enteritis and were potential periodontal pathogens[49], even can cause brain abscess[50]. OUT592 showed positive correlation with KEGG pathways of amino acid metabolism[51] and digestive system and was associated with glycochenodeoxycholate-3-sulfate, which is also a sensitive probe for the assessment of the hepatic uptake and metabolic capacity of atorvastatin[52]. In this study, OTU592 was defined to increase after FMT in BAM group but decrease in non-BAM group, indicating the specificity of OUT592 in the occurrence and development of BAM in IBD, which was consistent with previous studies[53]. OTU60 have the same trend with OTU592 and also linked with glycochenodeoxycholate-3-sulfate[54, 55]. In BAM group, OTU715 started to show enrichment in response group compared to their baseline levels and a decreasing trend in non-response group, while OTU715 showed opposing trends in non-BAM group. OTU715 can protected mice against kidney bilateral ischemia reperfusion injury[56].

Corresponding to the changes of gut microbiota, in non-BAM group, the lower concentration of C4 in baseline may attribute to the metabolism of Gitogenin and Carpipramine driven by *Actinomycetaceae* sp. and *Megasphaera micronuciformis* in co-interaction network analysis. The impaired deconjugation, transformation, and desulphation activities of gut microbiota led to dysmetabolism of BAs. Total BAs consists of serum BAs and fecal BAs. Normal microbiota enzymatic activity led to a physiological BA pool characterized by a large proportion of secondary BAs and very low levels of primary and sulfated BAs [54]. Duboc et al. found high rate of sulfated BAs in feces of IBD patients, impaired microbiota enzymatic activity may lead to modification in the BA pool composition, with increased sulfated BAs at the expense of secondary BAs, making it easier to become BAM[54]. Recent studies have reported newfound BAs and shown that different modified forms of BAs can serve as microbial codes to regulate various functions of the host. Uncovering this diverse array of bioactive substances in microbiota can help decipher the microbial code, thereby laying the foundation for leveraging them to maintain and improve human health[57]. In our study, we also found the higher level of sulfated BAs such as taurochenodeoxycholate-3-sulfate and glycochenodeoxycholate-3-sulfate in disease groups than healthy group at baseline. When FMT got involved, sulfated BAs such as chenodeoxycholic acid 3-sulfate, and primary BAs such as CA and CDCA still maintained a higher level in non-BAM group than BAM group. In non-BAM group, BA-related metabolism was still disordered and complex after FMT, which may partly explain why the non-BAM group was less effective than BAM after FMT treatment. Interestingly, we also found that DCA increased after FMT in non-BAM, as one of secondary BAs modified by the intestinal microbiota which was traditionally thought to produce beneficial effect, which was recently reported to promote colorectal cancer growth by suppressing CD8 + T cell effector functions[58]. On the one hand, the proportion of microbiota that metabolize primary BAs to secondary BAs was reduced; on the other hand, lower microbiota enzymatic activity led to lower desulphation, resulting the enrichment of sulfated BAs. These alterations contributed to the diminution of the anti-inflammatory effects of secondary BAs on intestinal epithelial cells, concurrently exacerbating chronic inflammation.

In addition, BAs play pleiotropic roles in lipid and glucose metabolism [59–61]. Jiang et al. explained a new way for metformin to improve metabolic disorders such as hyperglycemia and hyperlipidemia by regulating the specific microbiota-BA-gut FXR axis [62]. In our study, differential metabolites identified in BAM, non-BAM, and healthy groups were mostly belonged to Lipids and Lipid-like Molecules superclass. BA metabolism also regulates glycerophosphocholine-related metabolism, which is an important component of biofilm with related metabolism that cannot be ignored in enteritis. In BAM group, we also observed a decrease in the level of Lineolic acids derivatives, which may mediate the rapid improvement of abdominal pain in patients with IBD [63, 64]. Reduced BAs excretion is an independent risk factor for stroke and mortality may result in dyslipidemia [65], dyslipidemia may also be an important modifiable risk factor for contributing to the increased cardiovascular risk in IBD [66].

Given the findings in clinical data, we explored the significant role of primary BAs (CA and CDCA) in exacerbating acute and chronic enteritis in mice model. In acute model, the progressive increase of DAI scores from day 4 and day 5 in CA/CDCA + DSS group underscored the potent effect of CA and CDCA on the inflammatory response compared to DSS group, which was further corroborated by the endoscopic and histopathological findings. The observed persistence of the colitis phenotype

following DSS withdrawal suggested that PBAs may contribute to sustained inflammatory processes probably through the modulation of cytokines, which was consistent with Chen' study [26]. The significant increase in pro-inflammatory cytokines (TNF-α, IL-1β) and the concomitant decrease in the anti-inflammatory cytokine IL-10 in CA/CDCA + DSS groups underscore a potential mechanism by which these BAs exacerbate enteritis. In chronic model, we also found that long-term PBA supplementation can independently induce mild to moderate intestinal injury, especially in antibiotic pretreatment model, which suggested that gut microbiota-dysbiosis may further create a microenvironment for the proinflammatory effects of PBAs.

Importantly, the intervention with FMT from healthy donors demonstrated a remarkable reversal of the BA-induced exacerbation of colitis. The improved histopathological and colonoscopic outcomes following FMT suggested that the gut microbiota plays a crucial role in mitigating the inflammatory effects of PBAs. The upregulation of tight junction proteins (ZO-1, Occludin) and Muc2 post-FMT indicated a restoration of intestinal barrier function, which likely contributed to the observed reduction in colonic and serum inflammatory cytokines. After FMT, the C4 level decrease significantly along with the alterations in CYP7A1, CYP8B1, and CYP27A1 expression, suggested that FMT may influence BA metabolism through gut-liver axis, thereby attenuating the pro-inflammatory effects of PBAs. The antibiotic-depleted microbiota and FMT management showed that the exacerbation of primary BAs on enteritis was regulated by gut microbiota. These findings underscored the therapeutic potential of microbiota-targeted interventions in managing BA-associated enteritis and provided a basis for further exploration of gut microbiota as a strategy to treat BAD or BAM.

This study also had limitations when interpreting the findings. First, although our study provided preliminary evidence for the model's validity, the cohorts remain modest in size and were derived from a single-center and homogeneous population. Such data inadequately capture inter-regional, dietary, and genetic heterogeneity, thereby constraining external generalizability. Second, the current model was constructed from cross-sectional data, precluding the capture of dynamic fluctuations in BA metabolism across the longitudinal disease trajectory. Third, the quantification of key enzymatic genes governing BA synthesis, transport, and microbial biotransformation was not performed. Last, although the untargeted metabolomics platform employed in this study enabled high-throughput screening, it exhibited limited quantitative accuracy for BA subspecies, low-abundance BAs, and stereoisomers. How to ameliorate microbial composition through FMT or supplementation with probiotics, or modulate BA metabolism of gut microbiota using secondary BAs within the vast and complex microbiota-BA regulatory network, may necessitate the design of more rigorous randomized controlled trials and validation in large cohort studies.

Conclusions

This study systematically analyzed the efficacy of FMT in the treatment of IBD by connecting dysbiosis, gut microbiota, serum metabolomics, bile acid dysmetabolism, and gut inflammation, found that IBD patients with BAM were intended to have more obvious improvement in clinical response and remission after FMT treatment, with alleviation in abdominal pain and diarrhea accompanied by decrease of serum C4. Potential metabolites as biomarkers were explored for the diagnosis and treatment of specific subtypes of IBD patients. The pro-inflammatory effects of PBAs were further verified in the animal model, and FMT also reversed PBA-exacerbated colitis in mice. This study provides a multi-dimensional biomarker basis for comprehensive diagnosis and treatment of IBD patients.

Abbreviations

BAs	Bile acids
BAD	Bile acid diarrhea
BAM	Bile acid malabsorption
IBD	Inflammatory bowel disease
FMT	Fecal microbiota transplantation
UC	Ulcerative colitis
CD	Crohn's disease
C4	7α-Hydroxy-4-cholesten-3-one
BAS	Bile acid sequestrant
SeHCAT	^{75}Se-homocholic acid-taurine
CA	Cholic acid
CDCA	Chenodeoxycholic acid
DSS	Dextran sulfate sodium salt
HBI	Harvey Bradshaw index
OTUs	Operational taxonomic units
LEfSe	Linear discriminant analysis of effect size
PCoA	Principal coordinates analysis
AUC	Area under the receiver-operating characteristic curve

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12916-025-04353-y.

Additional file 1: Figures S1-S9. Fig. S1: Metabolic characteristics in BAM, non-BAM and HC group after FMT in neg and post model. Fig. S2: Metabolic characteristics in BAM, non-BAM and HC group before FMT in PLS-DA, OPLS-DA and Volcano analysis. Fig. S3: Microbial characteristics in BAM, non-BAM and HC group before FMT. Fig. S4: 18 differential microbiota at the OTU level between BAM, non-BAM and HC group. Fig. S5: Co-occurrence patterns based on 16S rRNA and LC–MS data using Pearson's correlation coefficient in BAM group and non-BAM group. Fig. S6: Changes of inflammatory cytokines in acute modeling mice and FMT treatment mice. Fig. S7: Primary BAs administration in FMT treatment mice. Fig. S8: Changes of function of intestinal mucosal barrier and indicators of BAs metabolism in FMT treatment mice. Fig. S9: Primary BAs administration in chronic modeling mice

Additional file 2: Tables S1-S3. Table S1: Differential metabolites in BAM and non-BAM group. Table S2: Differential metabolites in BAM group before and 1-week after FMT. Table S3: Differential metabolites in non-BAM group before and 1-week after FMT

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Authors' contributions
G.L., S.Z. and R.W.: Analysis and interpretation of results; raft manuscript preparation. X.W., Y.C. and Q.W.: Data collection. F.Z. and P.L.: Study conception and design. G.L., S.Z. and R.W. contributed equally. All authors read and approved the final manuscript.

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Data availability
AAdditionally, the 16S rRNA sequencing dataset generated from this study has been deposited to the NCBI database and can be accessed via the following link: BioProject: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1114418 (2025). Datasets generated for this study are all included in the article/supplementary material. Data will be made available on a case-by-case basis with a data sharing agreement made between the Second Affiliated Hospital of Nanjing Medical University and requesting institution.

Declarations

Ethics approval and consent to participate
The study was conducted according to the guidelines of the Declaration of Helsinki. It was a part of two registered trials (ClinicalTrials.gov: NCT01790061 and NCT01793831) and approved by the Second Affiliated Hospital of Nanjing Medical University Institutional Ethical Review Board. Written informed consents to participate in this study were provided by patients or their legal guardian.

Consent for publication
Written informed consents for publication were provided by patients or their legal guardian.

Competing interests
Faming Zhang holds patents on an automatic purification system and transendoscopic enteral tubing and devices related to them. The remaining authors declare no conflict of interests.

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