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Changes in amino acid concentrations and the gut microbiota composition are implicated in the mucosal healing of ulcerative colitis and can be used as noninvasive diagnostic biomarkers

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Abstract

Background Mucosal healing is the therapeutic target for ulcerative colitis (UC). While amino acids (AAs) and the gut microbiota are known to be involved in the pathogenesis of UC, their specific roles in mucosal healing have not been fully defined.

Objectives To longitudinally assess the changes in AA concentrations and the gut microbiota composition in the context of mucosal healing in UC patients, with the aim of identifying new biomarkers with predictive value for mucosal healing in UC patients and providing a new theoretical basis for dietary therapy.

Methods A total of 15 UC patients with infliximab-induced mucosal healing were enrolled. Serum and fecal AA concentrations before and after mucosal healing were determined via targeted metabolomics. A receiver operating characteristic (ROC) curve was plotted to evaluate the value of different AAs in predicting mucosal healing in UC patients. The changes in the composition of the fecal gut microbiota were analyzed via metagenomics, and bioinformatics was used to analyze the functional genes and metabolic pathways associated with different bacterial species. Spearman correlation analyses of fecal AAs with significantly different concentrations and the differentially abundant bacterial species before and after mucosal healing were performed.

Results 1. The fecal concentrations of alanine, aspartic acid, glutamic acid, glutamine, glycine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine were significantly

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decreased after mucosal healing. The serum concentrations of alanine, cysteine and valine significantly increased, whereas that of aspartic acid significantly decreased. Glutamic acid, leucine, lysine, methionine and threonine could accurately predict mucosal healing in UC patients, and the area under the curve (AUC) was > 0.9. After combining the 5 amino acids, the AUC reached 0.96. 2. There were significant differences in the gut microbiota composition before and after mucosal healing in UC, characterized by an increase in the abundance of beneficial microbiota (*Faecalibacterium prausnitzii* and *Bacteroides fragilis*) and a decrease in the abundance of harmful microbiota (*Enterococcus faecalis*). LEfSe analysis identified 57 species that could predict mucosal healing, and the AUC was 0.7846. 3. Amino acid metabolic pathways were enriched in samples after mucosal healing, was associated with the abundance of multiple species, such as *Faecalibacterium prausnitzi*, *Bacteroides fragilis*, *Bacteroides vulgatus* and *Alistipes putredinis*. 4. The fecal concentrations of several AAs were negatively correlated with the abundance of a variety of beneficial strains, such as *Bacteroides fragilis*, uncultured Clostridium sp., Firmicutes bacterium CAG:103, *Adlercreutzia equolifaciens*, *Coprococcus comes* and positively correlated with the abundance of several harmful strains, such as *Citrobacter freundii*, *Enterococcus faec*alis, *Klebsiella aerogenes*, *Salmonella enterica*.

Conclusion Altered concentrations of amino acids and their associations with the gut microbiota are implicated in the mucosal healing of UC patients and can serve as noninvasive diagnostic biomarkers.

Keywords Ulcerative colitis, Mucosal healing, Amino acids, Gut microbiota, Biomarkers

Introduction

Ulcerative colitis (UC) is a chronic, relapsing disease of the colorectum tract, and its pathogenesis is still unclear. The incidence and prevalence of UC have been increasing worldwide in recent years, resulting in a considerable health burden [1, 2]. Endoscopic mucosal healing is the current therapeutic target for UC. Achieving mucosal healing in patients with UC is associated with a higher incidence of sustained clinical hormone-free remission and translates to lower rates of hospitalization and surgery, further improving the quality of life of patients [3, 4]. Colonoscopy is the main examination used to evaluate mucosal healing in UC patients, but it is an invasive examination that increases the risk of infection and perforation and requires miserable bowel preparation [5]. Alternately, diagnostic biomarkers such as high-sensitivity C-reactive protein, the erythrocyte sedimentation rate and fecal calprotectin (FC) are commonly used to evaluate disease activity and predict mucosal healing in a noninvasive manner. However, the low specificity and different cutoff values cause confusion in the clinic. Therefore, it is important to explore new noninvasive biomarkers for predicting mucosal healing in UC patients.

The structural and functional repair of the colon mucosa is the basis for achieving mucosal healing [6]. Amino acids (AAs), microelements, proteins and other materials are required in this process. Some special AAs play a role in reducing intestinal inflammation and restoring intestinal mucosal homeostasis, which may be related to enhancing intestinal barrier integrity, promoting protein synthesis, increasing energy metabolism, and regulating oxidative stress and inflammation [7, 8]. AA concentrations in the serum, feces and urine of patients with UC are altered with the disruption of intestinal mucosal homeostasis. Quantitative metabolomic profiling revealed that AAs were the most significantly altered metabolites in serum and plasma samples from inflammatory bowel disease (IBD) patients compared to healthy individuals [9]. Moreover, metabonomics is rapidly becoming a powerful method to characterize IBD because it reflects the pathophysiological processes involved in the pathogenesis of IBD and thereby may have greater accuracy than other methods [10].

There is a mutual relationship between AAs and the gut microbiota; AAs can promote the growth of the gut microbiota, and the gut microbiota can utilize AAs to synthesize new metabolites. The interplay between the gut microbiota and AAs can have profound effects on host health [11]. Previous studies have confirmed gut microbiota imbalance in patients with IBD, which manifests as reduced bacterial diversity and a lower abundance of Firmicutes and a higher abundance of Proteobacteria; this imbalance plays an important role in the pathogenesis of IBD [12, 13]. Pull et al. [14] reported that intestinal mucosal healing was strongly affected and that the proliferation rate of colonic epithelial stem cells was significantly reduced in germ-free mice. Subsequent in vivo and in vitro experiments have shown that the gut microbiota profoundly affects epithelial barrier function and cell metabolism, proliferation and differentiation; participates in mucosal repair; and maintains intestinal homeostasis in UC [15]. Regretfully, recent studies have focused mainly on comparisons between IBD patients and healthy individuals, and there is still a lack of assessment of the changes in AA concentrations and the gut microbiota composition before and after mucosal healing in UC patients.

Metagenomics or metabolomics alone do not fully reflect the function of the gut microbiota. In recent years, gas chromatography and mass spectrometry

(GC–MS/MS) have been applied to the gut microbiota to systematically characterize the metabolic diversity of symbiotic bacteria and hosts through targeted and untargeted metabolomics combined with metagenomics [16]. Accordingly, we performed targeted metabolomics combined with metagenomics before and after mucosal healing in UC patients to explore new biomarkers for predicting the mucosal healing of UC patients. In addition, we aimed to gain insight into the interplay between AAs and the gut microbiota and provide a new theoretical basis for dietary therapy.

Materials and methods

Ethics statements

In this study, informed consent forms were signed voluntarily by all patients. This study was approved by the Ethics Committee of the First Affiliated Hospital of Kunming Medical University, Kunming, China (2019-L-23).

Patients and sample collection

A cohort of 15 patients aged > 18 years with UC admitted to the First Affiliated Hospital of Kunming Medical University from 2020 to 2022 were enrolled in this study. UC was diagnosed in these patients using the diagnostic criteria of the third European Crohn's and Colitis Organization (ECCO) consensus, and excluded EB, CMV, *Clostridium difficile*, tuberculosis, invasive adhesion E. coli and other common opportunistic infections of UC [17]. The clinical disease activity of UC patients was evaluated via the modified Mayo endoscopic score (MES) score. Fecal and serum samples were collected from patients with moderate to severe UC according to the third ECCO consensus who received infliximab alone and maintained treatment for at least 6 months. UC patients in clinical remission underwent colonoscopy, and mucosal healing was defined as a MES score of 0 [18]. None of these patients used antibiotics, probiotics or enemas at least 3 months before sample collection. Fecal samples were collected before colonoscopy, and serum samples were collected during fasting. All the samples were immediately stored at -80 °C. The participants were willing to provide fecal and serum samples as research samples, and we provided additional assistance to these volunteers, including reductions in examination costs, long-term free health counseling, etc.

Targeted amino acid analysis

The samples were analyzed via GC–MS/MS analysis by Lu-Ming Biotech Co., Ltd. (Shanghai, China).

Sample preparation

A 50 mg fecal sample was mixed with 400 µL of methanol: water (4:1) and ground (60 Hz, 2 min) after 2 min at -20 °C. The mixtures were ultrasonicated at low temperature for 10 min and incubated at -20 °C for 30 min. The samples were centrifuged at 12,000 rpm for 10 min at 4 °C twice, and 500 µL of the supernatant was collected and vortexed for 30 s. One hundred microliters of the supernatant was transferred to a new test tube and dried with a centrifugal freeze dryer. The QC samples were prepared by mixing aliquots of all the samples. Next, 80 µL of 15 mg/mL methoxylamine hydrochloride in pyridine was added. The resulting mixture was vortexed for 2 min and incubated at 37 °C for 90 min. Next, 80 µL BSTFA (with 1% TMCS) and 20 µL n-hexane were added to the mixture, which was vortexed for 2 min and then derivatized at 70 °C for 60 min. The samples were placed at ambient temperature for 30 min before GC–MS/MS analysis. One hundred microliters of serum was added to a 1.5mL Eppendorf tube with 20 µL of 3 ppm succinic acid 2,2,3,3-d4 and 600 µL of methanol: acetonitrile solution (2:1, containing 0.1% formic acid). The mixtures were vortexed for 1 min, ultrasonicated for 10 min, and then incubated at -20 °C. The samples were subsequently centrifuged at 12,000 rpm for 10 min at 4 °C, after which 100 µL of the supernatant was collected. The follow-up procedure was the same as that used for fecal sample processing.

GC–MS/MS analysis

The derivatized samples were analyzed on a Trace1310 gas chromatography system coupled to a TSQ9000 mass spectrometer (Thermo Fisher Scientific, USA). A DB-5MS fused-silica capillary column (30 m×0.25 mm×0.25 µm, Agilent J&W Scientific, Folsom, CA, USA) was utilized to separate the derivatives. Helium (> 99.999%) was used as the carrier gas at a constant flow rate of 1.2 mL/min through the column. The injector temperature was maintained at 300 °C. The injection volume was 1 µL, and the solvent was delayed for 4 min. The initial oven temperature was 50 °C, ramped to 125 °C at a rate of 15 °C/min, held for 2 min, to 210 °C at a rate of 8 °C/min, held for 2 min, to 270 °C at a rate of 11 °C/min, held for 1 min, to 305 °C at a rate of 25 °C/min, and finally held at 305 °C for 3 min. Mass data were acquired in full-scan mode (m/z: 40–600). Selective reaction monitoring by triple quadrupole mass spectrometry was used for quantitative analysis. According to the standard curve, the absolute content of amino acid in the actual sample was obtained.

Receiver operating characteristic (ROC) curve

GraphPad Prism (version 9.5.1) was used for receiver operating characteristic (ROC) curve analyses to determine the AUC values.

Metagenomic shotgun sequencing and analysis

Metagenomic shotgun sequencing and analysis were performed by Novogene Bioinformatics Technology (Beijing, China).

Database building and sequencing

Fecal sample DNA was extracted at Novogene Bioinformatics Technology via the sodium dodecyl sulfate (SDS) method. DNA integrity and quantification were assessed using 1% agarose and a Qubitinstrument. Qualified DNA samples were used for library construction. All the samples were sequenced on an Illumina platform in PE150 mode. The original raw data were preprocessed, and unqualified data were removed to obtain clean data for subsequent analysis.

Annotation of species

DIAMOND software [19] (version 0.9.9.110) was used for alignment of unigene sequences with those of bacterial, archaeal, viral and eukaryotic sequences extracted from the NCBI nonredundant (NR) database (BLASTP, evalue<=1e-5) [20]. In addition to the results of the lowest common ancestor (LCA) annotation strategy and gene abundance table, the abundance of each sample at each taxonomic level was acquired [21, 22]. Alpha diversity (α diversity) was evaluated by determining the Chao1, Shannon and Simpson indices at each taxonomic level with an in-house Perl script. Principal component analysis (PCA) was performed via the R package FactoMineR and factoextra. Beta diversity was visualized via principal coordinate analysis (PCoA) via the Bray–Curtis distance matrix data in R with ggplot2. Metastats and linear discriminant analysis effect size (LEfSe) analysis were used to search for species differences between groups. Metastats analysis is used to perform a permutation test between groups at each taxonomic level and obtain a p value. The Benjamini and Hochberg false discovery rate was subsequently used to correct the p value and obtain a q value [23]. LEfSe analysis was performed with LEfSe software to determine the features most likely to explain differences between groups [24]. All identified biomarkers were verified via random forest (R pROC and random forest packages, Version 2.15.3) with 10-fold cross validation. Finally, receiver operating characteristic (ROC) analysis was conducted to construct a random forest model.

Table 1 Clinical characteristics of the patients

Patient characteristics	Number of patients (%)/Mean(range)
Sex	
Male	7(46.67%)
Female	8(53.33%)
Mean age	43.67(18–68)
Initial modified Mayo score	
Moderate	5(33.33)
severe	10(66.67)
Extent of lesion	
E1	1(6.67)
E2	2(13.33)
E3	12(80.00)

Bioinformatic analysis

Unigenes were annotated using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database [25]. The best BLAST hit results from the alignment results of each sequence were selected for subsequent analysis. Enriched KEGG pathways/modules were identified according to their reporter score from the Z scores of individual orthologs (KOs). Comparative analysis of metabolic pathways was conducted.

Correlation analyses of fecal amino acids and gut microbial species

The Spearman correlations of fecal amino acids with significantly different concentrations and the differentially abundant species identified by LEfSe analysis in the UC and mucosal healing groups were calculated. Heatmaps were hierarchically clustered to represent the species–amino acid–associated patterns on the basis of the correlation distance.

Statistical analysis

The Wilcoxon signed rank test was used to test the paired data. Spearman correlation analysis was performed to evaluate the correlations among differentially abundant bacterial species and fecal amino acids with significantly different concentrations between the two groups. All the statistical analyses were performed with R software, and p values <0.05 were considered statistically significant. The p value of group comparisons that were less than 0.05 was delineated as*. The p value of group comparisons that were less than 0.01 was delineated as**.

Results

Clinical characteristics of the patients

Fifteen UC patients in remission that were treated with infliximab and achieved mucosal healing, as confirmed by colonoscopy, were enrolled in this study. The clinical characteristics are shown in Table 1. The samples were collected for subsequent analysis (Fig. 1).

Five fecal AAs can predict mucosal healing in UC

After mucosal healing in UC patients, the levels of fecal alanine, aspartic acid, glutamic acid, glutamine, glycine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine were significantly decreased ($p<0.05$) (Fig. 2A). The serum concentrations of alanine, cysteine and valine

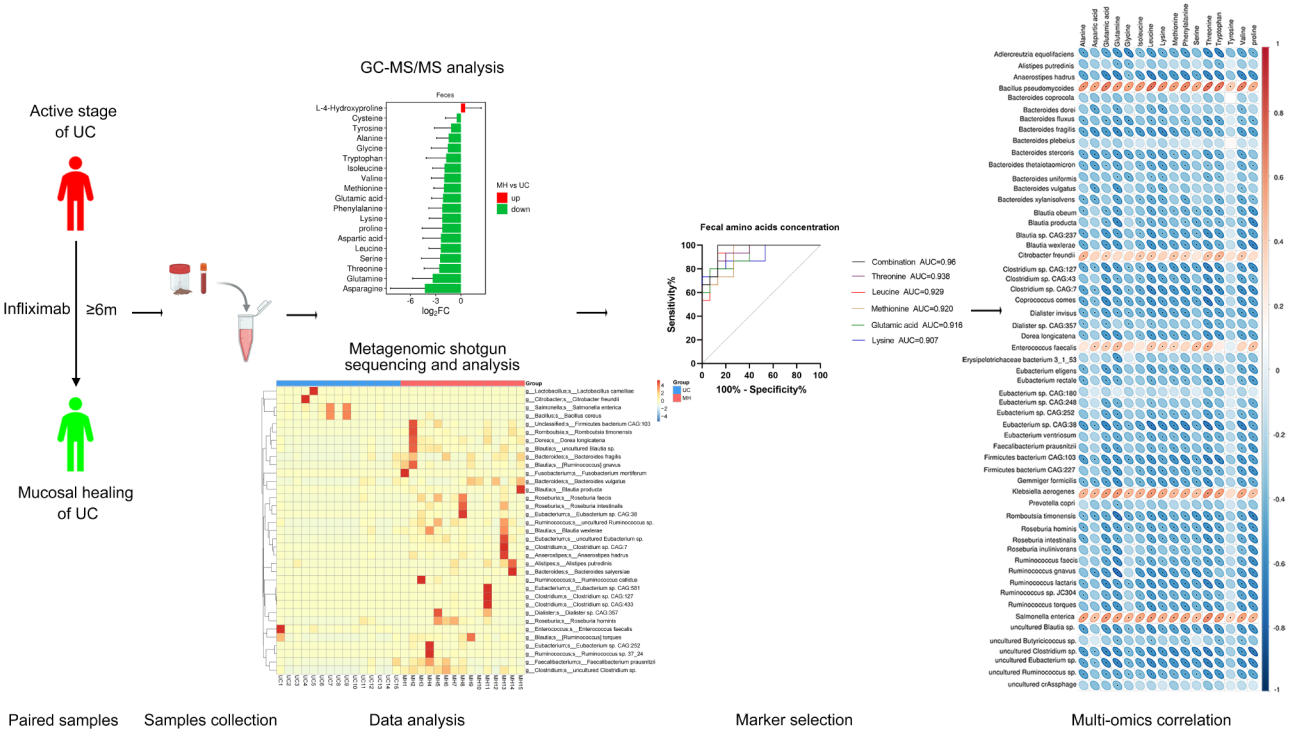

Fig. 1 Workflow of the entire study. Patients with moderate to severe UC who received infliximab ≥ 6 m to achieve mucosal healing were enrolled. All the samples were analyzed via targeted metabolomics combined with metagenomics analysis

significantly increased, and that of aspartic acid significantly decreased ($p<0.05$) (Fig. 2B). Fecal AAs had greater potential for predicting mucosal healing in UC patients than serum AAs. To further evaluate the value of fecal AAs in predicting mucosal healing in UC patients, ROC curves were generated. The results revealed that the 16 kinds of fecal AAs had a certain value in predicting mucosal healing in UC patients, and the areas under the curve (AUCs) were all >0.7 (Table 2). The accuracies of glutamic acid, leucine, lysine, methionine and threonine in the prediction of mucosal healing in UC patients were high, and all the AUCs were >0.9. After the combination of the 5 AAs, the AUC reached 0.96 (95% CI: 0.897-1.000) (Fig. 2C). In brief, our findings indicate that AAs have the potential to predict mucosal healing in UC patients and could be noninvasive diagnostic biomarkers.

Significant differences in the composition of the gut microbiota before and after mucosal healing in UC

Metagenomic sequencing was performed and generated an average of 6591.02 clean reads per fecal sample. The dilution curves of the Core and Pan genes revealed that as the number of samples increased, the number of genes gradually stabilized, suggesting that the sample size of our study was sufficient (Fig. 3A). The biological repeatability of UC and MH samples was good in the inter-sample gene abundance correlation analysis, suggested this results of the study were reliable(Fig. 3B). To assess the difference in bacterial diversity before and after mucosal healing in UC, we aligned sequences for gene count and α diversity. A total of 546,789 genes were identified in the UC patient samples before mucosal healing, and 834,937 genes were identified in the UC patient samples after mucosal healing, among which 487,981 genes were shared between UC and mucosal healing, 346,956 genes were unique to mucosal healing, and 58,808 genes were unique to UC, indicating that the genes were more abundant after mucosal healing (Fig. 3C). At the phylum level, Firmicutes (28.48%), Bacteroidetes (15.20%), Proteobacteria (12.82%) and Ascomycota (5.78%) were dominant in UC, and the abundance of Firmicutes (42.18%) and Bacteroidetes (42.49%) increased significantly after mucosal healing. At the genus level, Bacteroides (12.75%), Enterococcus (5.70%), Saccharomyces (5.39%) and Lactobacillus (3.51%) were dominant in UC, and Bacteroides (32.74%), Faecalibacterium (6.52%), Roseburia (3.57%) and Clostridium (2.94%) were dominant after mucosal healing. At the species level, *Enterococcus faecium* (1.84%) and *Escherichia coli* (1.62%) were the main bacteria in UC, and *Faecalibacterium prausnitzii* (4.73%) was dominant after mucosal healing (Fig. 3D). Alpha diversity reflects the abundance and uniformity of microbial species in a population and can be assessed using the Chao 1, Shannon and Simpson indices. The three indices were significantly different before and after mucosal healing in UC patients, which was indicative of alterations in the gut

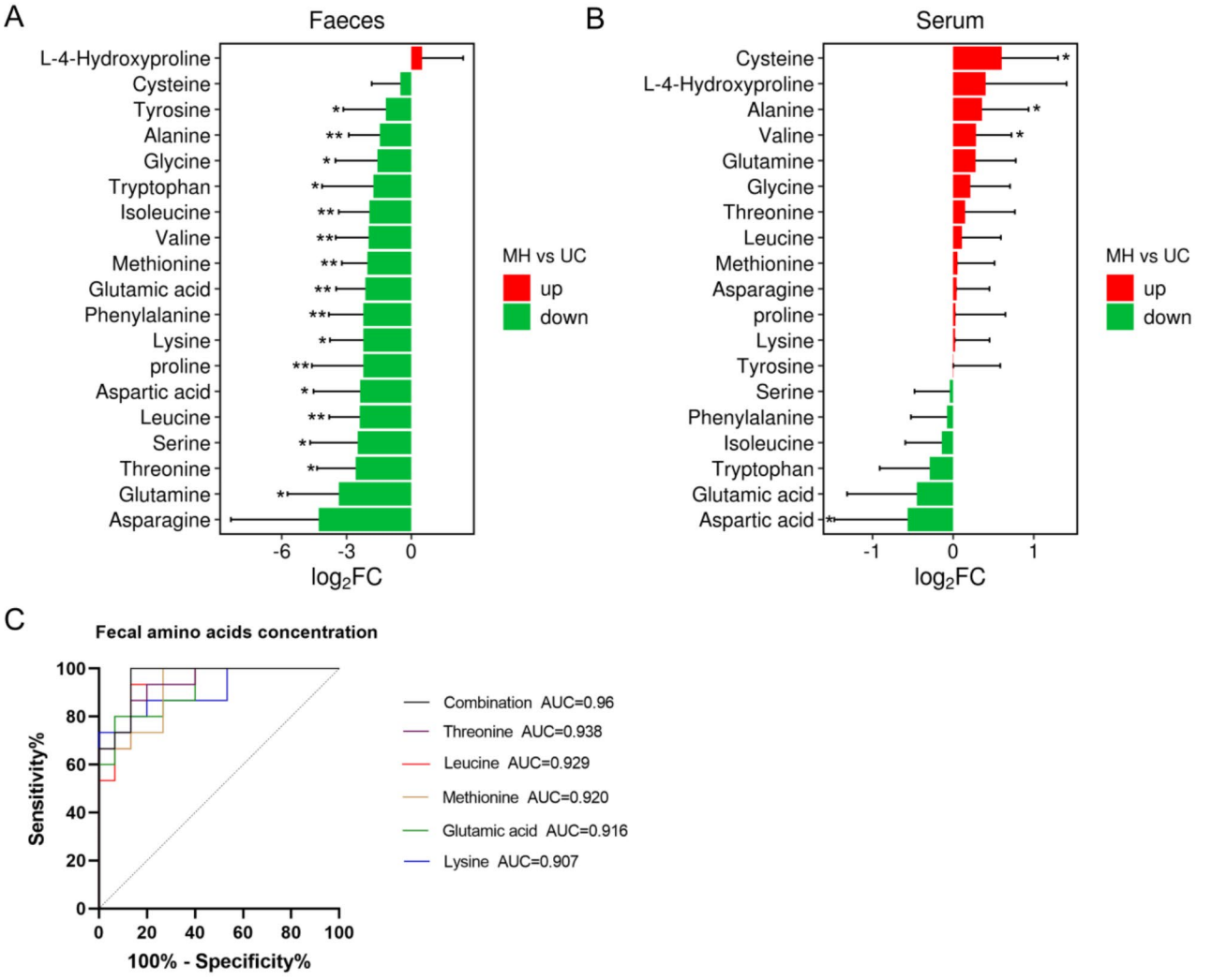

Fig. 2 Changes in serum and fecal AA levels before and after mucosal healing in UC patients and the value of AAs in the prediction of mucosal healing. There were significant changes in various AAs after mucosal healing, and fecal AAs have the potential to predict mucosal healing in UC patients. (**A**): Fecal alanine, aspartic acid, glutamic acid, glutamine, glycine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine levels were significantly decreased after mucosal healing in UC. (**B**): Serum alanine, cysteine and valine levels significantly increased, and aspartic acid levels significantly decreased after mucosal healing in UC patients. (**C**): The AUCs of glutamic acid, leucine, lysine, methionine and threonine for the prediction of mucosal healing in UC patients was > 0.9. After combining the 5 AAs, the AUC reached 0.96

bacterial structure after mucosal healing in UC patients (Chao 1 index, $p<0.001$; Shannon index, $p<0.001$; Simpson index, $p=0.006$) (Fig. 3E). We subsequently used PCA, PCoA and nonmetric multidimensional scaling (NMDS) to evaluate the overall gut microbiota composition in UC patients before and after mucosal healing at the genus and species levels, respectively. Our results revealed some overlap of gut microorganisms at different taxonomic levels before and after mucosal healing in UC patients, but the samples formed distinct clusters, indication the significant differences in the composition of the gut microbiota (Fig. 3F, Fig. S1). Analysis of similarities (ANOSIM) with Bray–Curtis distance (β diversity) also revealed significant differences in the composition of the gut microbiota at different taxonomic levels before and after mucosal healing in UC patients (Fig. S1). Thus, our findings collectively indicated significant differences in the gut microbiota composition before and after mucosal healing in UC patients.

Species-level changes in the gut microbiota composition before and after mucosal healing in UC

Metastats and LEfSe analyses were used to assess the microbial abundance at different taxonomic levels before and after mucosal healing in UC. The results revealed that the gut microbiota differed significantly after mucosal healing in UC at different taxonomic levels (Fig. S2). At the species level, we observed a marked increase in

Table 2 AUCs of fecal AAs in predicting mucosal healing in UC patients

Amino acid	AUC	95%CI
Alanine	0.836	0.686–0.985
Aspartic acid	0.880	0.757-1.000
Glutamic acid	0.916	0.819-1.000
Glutamine	0.876	0.732-1.000
Glycine	0.742	0.559–0.925
Isoleucine	0.840	0.700–0.980
Leucine	0.929	0.839-1.000
Lysine	0.907	0.799-1.000
Methionine	0.920	0.826-1.000
Phenylalanine	0.871	0.744–0.998
Proline	0.813	0.653–0.974
Serine	0.889	0.752-1.000
Threonine	0.938	0.858-1.000
Tryptophan	0.778	0.614–0.942
Tyrosine	0.716	0.520–0.911
Valine	0.880	0.759-1.000

the abundance of microbial species closely associated with UC, such as *Faecalibacterium prausnitzii*, *Bacteroides fragilis* and *Anaerostipes hadrus*. The abundances of some microbial species, such as *Enterococcus faecalis*, *Lactobacillus camelliae* and *Citrobacter freundii* decreased (Fig. 4A). LEfSe analysis was further applied and identified 57 species that were associated with mucosal healing, of which 10 species whose abundance was increased in UC samples before mucosal healing and 47 species whose abundance was increased in UC samples after mucosal healing (LEfSe: LDA>4.0, $p<0.05$). (Fig. 4B). *Faecalibacterium prausnitzii* is a representative species of Faecalibacterium and has a low abundance in individuals with obesity, type 2 diabetes and colorectal cancer. Previous studies have shown that *Faecalibacterium prausnitzii* is an anti-inflammatory symbiotic bacterium associated with inflammation and is depleted in CD [26, 27]. *Bacteroides fragilis* was obviously more abundant in UC samples after mucosal healing. The human commensal *Bacteroides fragilis* has evolved beneficial immunomodulatory properties that induce the production of IL-10 by CD4+Foxp3+regulatory T cells and prevent experimental colitis [28]. In contrast, *Enterococcus faecalis* aggravated experimental colitis and induced colitis in IL-10 knockout mice [29, 30], and the abundance of *Enterococcus faecalis* was significantly reduced in UC samples after mucosal healing in our study. In our study, other unstudied strains that may be involved in mucosal healing in UC were also found, such as R*oseburia faecis*, Clostridium_sp_CAG_127, Eubacterium_sp_CAG_252, Clostridium_sp_CAG_7, Ruminococcus_lactaris, Eubacterium_sp_CAG_38, Dialister_sp_CAG_357, Clostridium_sp_CAG_43, Ruminococcus_sp_JC304 and *Eubacterium ventriosum*. We further built a random forest model to explore the potential use of the gut microbiota for the noninvasive prediction of mucosal healing. The results revealed that the AUC of the combination of significantly altered bacterial species in the prediction of mucosal healing in UC patients was 0.7846 (Fig. 4C). Thus, our results suggest the great potential of these microbial-based classifiers as noninvasive biomarkers for the early prediction of mucosal healing.

AA metabolism was enriched after mucosal healing

Next, we annotated the unigenes with those in the KEGG database. The results revealed that AA metabolism, the metabolism of cofactors and vitamins, glycan biosynthesis and metabolism, and the biosynthesis of other secondary metabolites increased significantly after mucosal healing (Fig. 5A). Many AA metabolic pathways, including glycine, serine, threonine, cysteine, methionine, alanine, aspartic acid, and glutamic acid metabolism and phenylalanine, tyrosine, lysine, and leucine biosynthesis, were enriched after mucosal healing (Fig. 5B). We further identified species contributing predominantly to AA metabolic pathways. *Faecalibacterium prausnitzi*, *Bacteroides fragilis*, *Bacteroides vulgatus* and *Alistipes putredinis* were the leading bacterial species involved in the above pathways (Fig. 5C). Overall, our findings indicate that AA metabolism and the gut microbiota are closely related and participate in mucosal healing in UC.

Putative correlations of fecal AAs with the bacterial species during mucosal healing in UC

There is a close relationship between AAs and the gut microbiota, which plays an important role in the pathophysiological processes of many diseases. To further understand the relationship among fecal AAs and the gut microbiota in the mucosal healing of UC patients, we calculated the Spearman correlations of fecal AAs with significantly different concentrations and distinctively abundant bacterial species between the two groups and generated a heatmap to highlight the species and amino acid-associated patterns. Our results revealed that fecal AAs were negatively correlated with a variety of beneficial strains, such as *Bacteroides fragilis*, uncultured Clostridium sp., Firmicutes bacterium CAG:103, *Adlercreutzia equolifaciens* and *Coprococcus comes*. However, these AAs were positively correlated with several harmful strains, such as *Citrobacter freundii*, *Enterococcus faecalis*, *Klebsiella aerogenes*,*Salmonella enterica* (Fig. 5D). So far studies about *Bacillus pseudomycoides* had focused on plants, there was no relevant studies related to IBD. Our results further demonstrate the close relationship between AAs and the gut microbiota in the process of mucosa healing in UC.

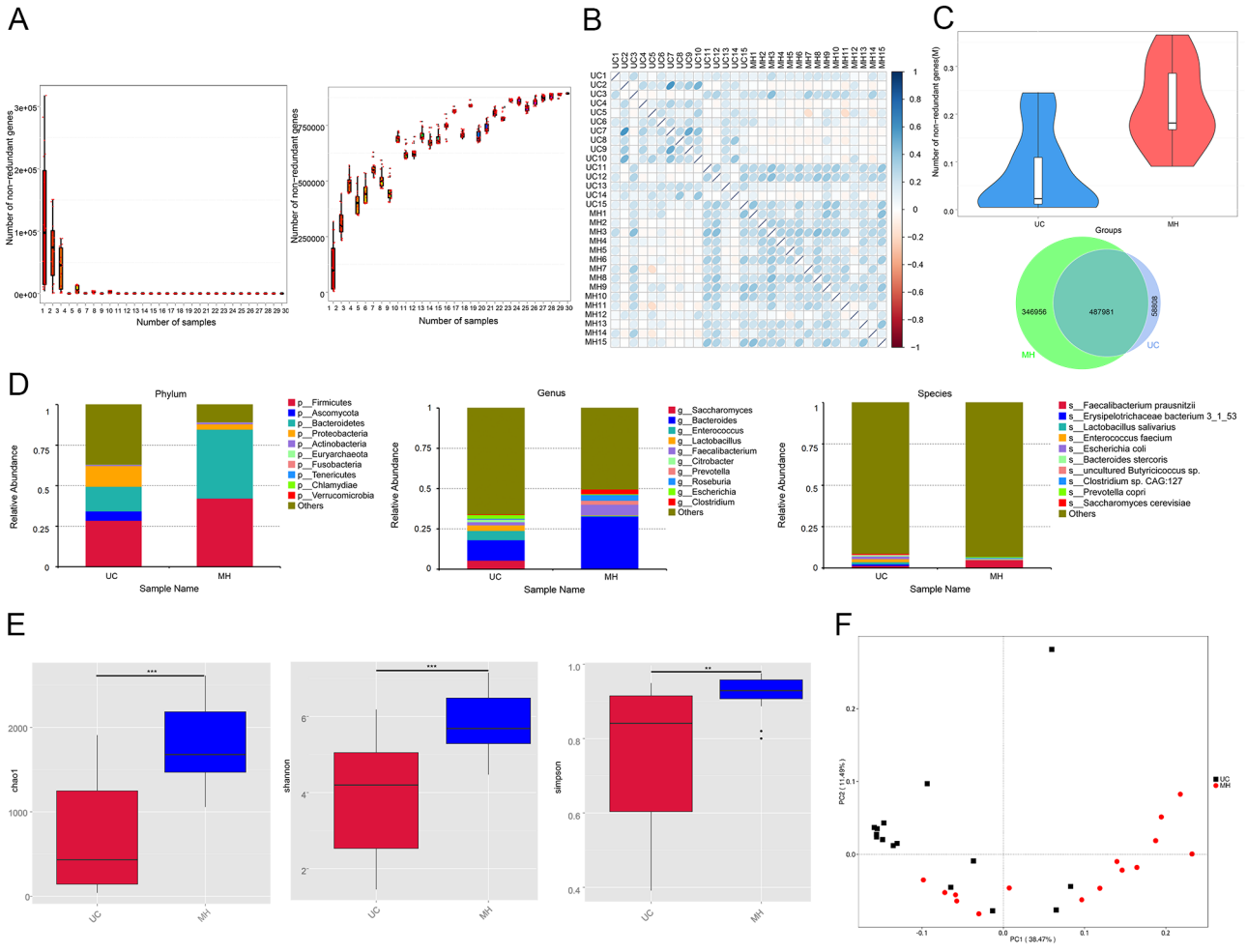

Fig. 3 Gut microbiota composition before and after mucosal healing in UC. Significant differences in the gut microbiota composition existed before and after mucosal healing in UC patients. (**A**): The dilution curves of the core and pan genes revealed that the sample size of our study was sufficient. (**B**): The inter-sample gene abundance correlation analysis showed results of the study were reliable. (**C**): The genes were more abundant after mucosal healing in UC. (**D**): At different taxonomic levels (phylum, genus, and species), the gut microbiota composition changed significantly after mucosal healing in UC patients. (**E**): The Chao 1 index, Shannon index and Simpson index (α diversity) at the species levels were determined, indicating alterations in the gut bacterial composition after mucosal healing in UC patients. (**F**): PCoA of the Bray–Curtis distance of the discovery cohort on the basis of gut metagenomic species profiles revealed some overlap of gut microorganisms before and after mucosal healing in UC patients, but the samples formed distinct clusters, indicated the significant differences in the composition of the gut microbiota

Discussion

Mucosal healing is a therapeutic target for UC that can lead to better clinical outcomes. Intestinal repair is a process involving intestinal epithelial cells, immune and microbial interactions [31]. The process of mucosal healing is triggered by a chain of energetically expensive mechanisms to restore the continuity of tissue after mucosal damage. AAs, as the basic elements of protein synthesis, fuel the anabolism of bioactive compound precursors, interact with the gut microbiota to participate in mucosal healing in UC and maintain intestinal homeostasis. Our study revealed significant changes in AA concentrations and the gut microbiota composition before and after mucosal healing in UC patients, and these altered AAs and bacterial species have predictive value for mucosal healing in UC patients. Moreover, our findings indicate that AA metabolism and the gut microbiota are closely related and participate in mucosal healing in UC.

A prospective intention-to-diagnose pilot study revealed that the concentrations of 9 fecal amino acids (alanine, citrulline, glutamine, leucine, lysine, phenylalanine, serine, tyrosine and valine) differed significantly between IBD patients and non-IBD patients. No significant differences were detected in the serum [32]. Fecal leucine, alanine and tyrosine levels were greater in UC patients than in healthy individuals and were positively correlated with disease severity [33]. In our study, the concentrations of 16 fecal AAs decreased significantly after mucosal healing in UC, whereas the serum

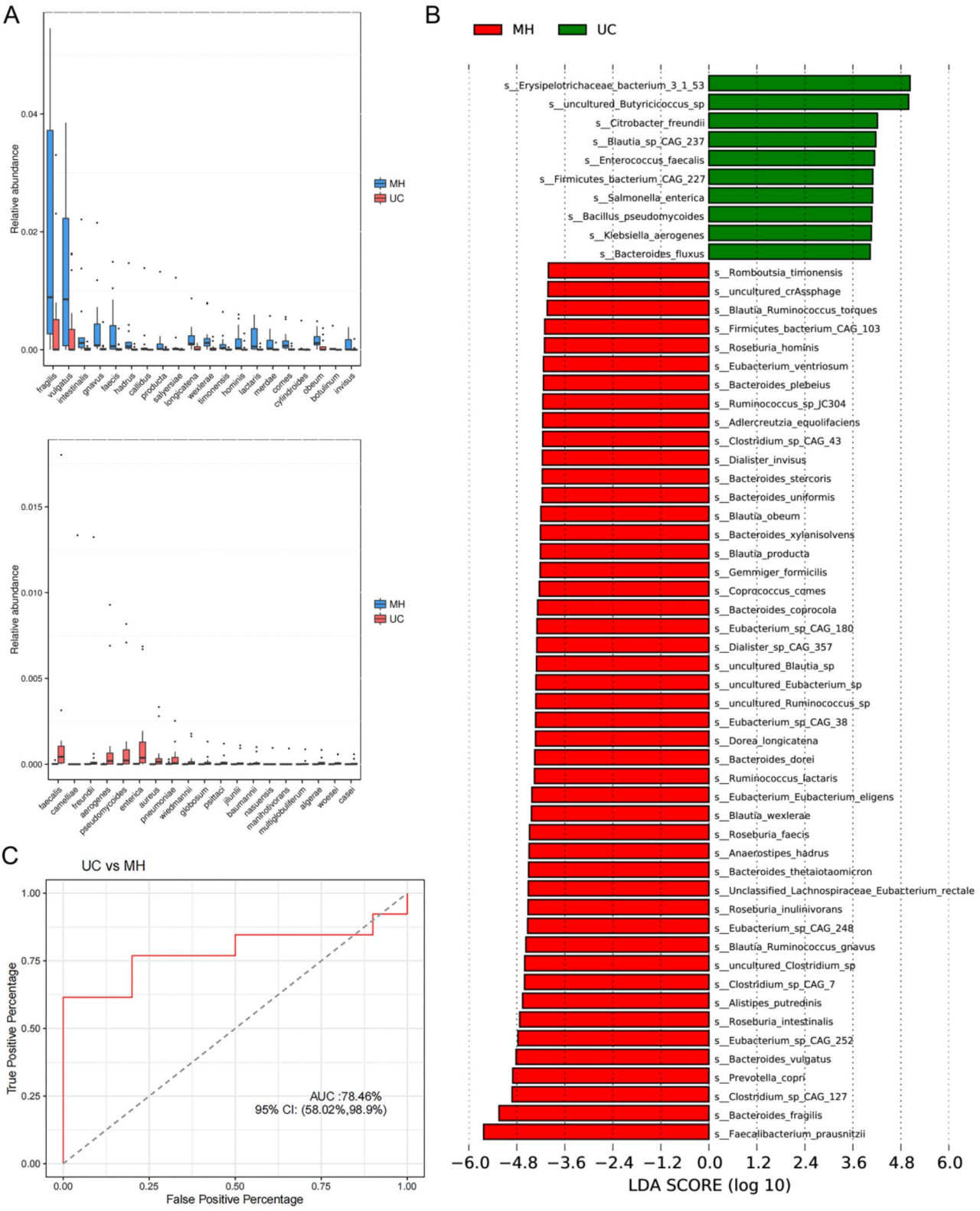

Fig. 4 Species-level changes in microbial community composition after mucosal healing in UC. (**A**): The relative abundance of microbial species significantly differed before and after mucosal healing in UC patients. (**B**): Fifty-seven species that were more abundant in UC samples after mucosal healing compared to those before mucosal healing were identified via LEfSe analysis. (**C**): ROC analysis revealed that the AUC for the 57 species discriminating mucosal healing from UC was 0.7846

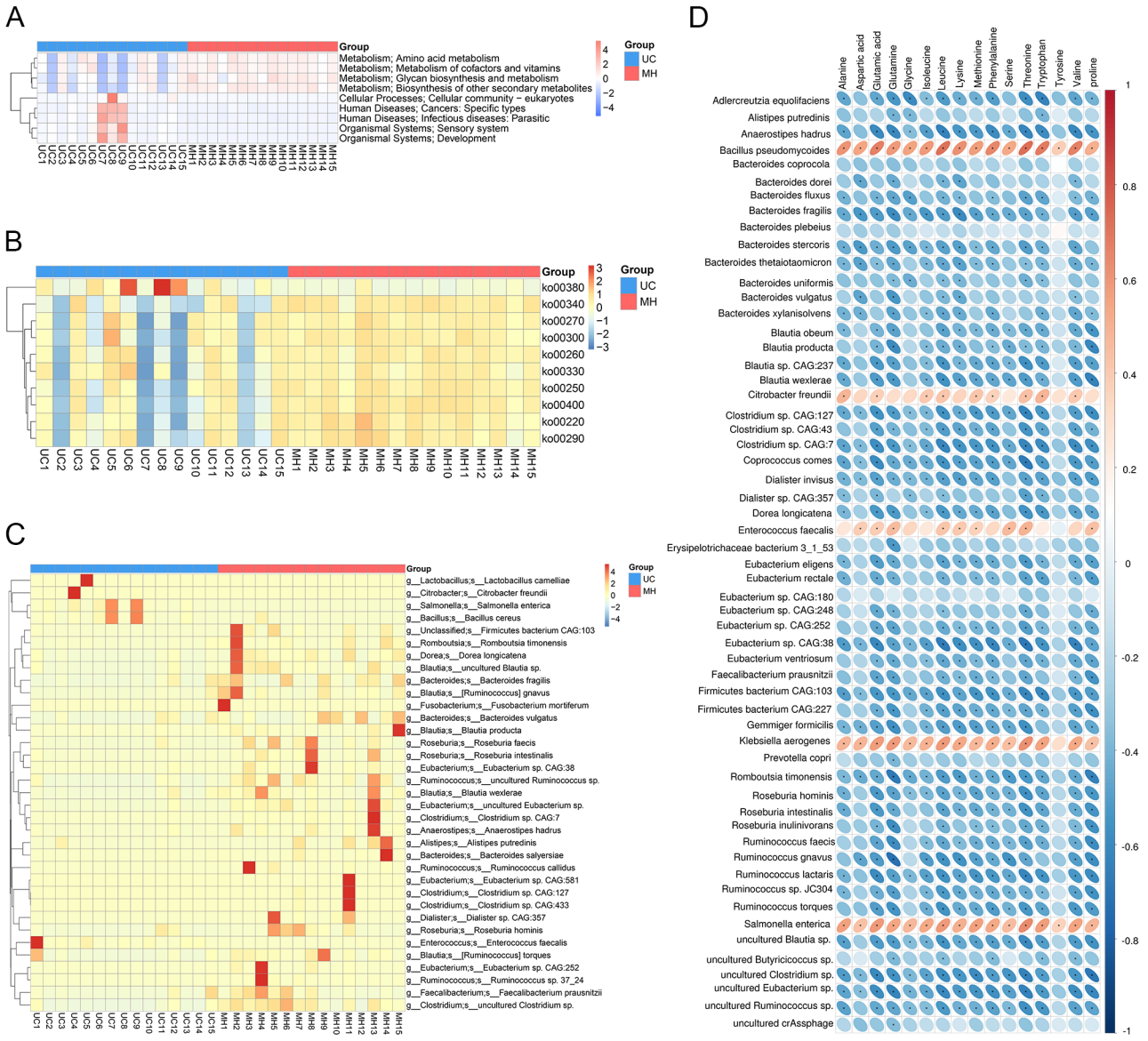

Fig. 5 Relationship between AAs and the gut microbiota in the mucosal healing of UC patients. (**A**): AA metabolism increased significantly after mucosal healing. (**B**): Ten AA metabolic pathways were obviously enriched after mucosal healing. (**C**): AA metabolic pathway characterized by the top 35 bacterial species. (**D**): Putative correlations among fecal AAs with different bacterial species during mucosal healing in UC patients

concentrations of alanine, cysteine and valine increased and that of aspartic acid decreased, suggesting that AAs participate in the process of mucosal healing in UC. Hypotheses regarding potential mechanisms for altered fecal amino acid concentrations in patients with IBD include malabsorption, metabolic changes, gut microbiota disorders and colonic leakage. Because essential AAs cannot be synthesized in the body and must be obtained from the diet, malabsorption and colonic leakage may be responsible for lower concentrations in the serum and higher concentrations in the feces. However, there was no significant difference in serum levels before and after mucosal healing in UC patients in our study, and malabsorption and colonic leakage did not seem to fully explain the changes in feces. The KEGG analysis revealed that the metabolic pathways of several AAs were enriched after mucosal healing, and previous studies have reported that various AA levels are decreased in the colonic mucosa of IBD patients [34]. Changes in intestinal metabolism and gut microbiota disorders may be the most possible explanation [35], suggesting that AAs interact with the gut microbiota and participate in the metabolic processes of intestinal epithelial cells, thus promoting the mucosal healing of UC. During the pathophysiology of UC, AA metabolism in the colon changes in response to injury to provide feedback on tissue damage

and promote repair after injury. If AA metabolism disorders cannot be corrected in a timely manner, the repair ability of the colonic mucosa will be hindered, exacerbating tissue damage and thus promoting the progression of UC. AAs are considered as the building blocks for protein synthesis and play major roles in other processes, such as cell signaling, gene expression, intracellular protein turnover, reproduction, oxidative stress and immunity [36]. At present, many studies have focused on glutamine, glutamic acid, arginine, sulfur-containing amino acids (methionine and cysteine), tryptophan, glycine and histidine, which may play beneficial roles in IBD through anti-inflammatory and anti-apoptotic effects and maintaining intestinal barrier integrity [37], suggesting that restoring AA metabolism though adjusting dietary structure may be a promising therapeutic method.

In addition to being involved in the mucosal healing of UC, AAs also showed a superior ability to predict mucosal healing. Compared with serum amino acid levels, fecal amino acid levels are more sensitive and specific for predicting mucosal healing. Recent studies have shown that the serum and fecal amino acid compositions can be used to distinguish IBD patients from healthy individuals with high sensitivity [38]. Another previous study revealed that, compared with those of healthy individuals, the fecal histidine, tryptophan, phenylalanine, leucine, tyrosine and valine contents of UC patients were significantly greater, and the AUCs for the diagnosis of UC were all greater than 0.75 [35]. Our study revealed that the AUCs of fecal glutamate, leucine, lysine, methionine and threonine for the prediction of mucosal healing in UC patients were 0.916, 0.929, 0.907, 0.920 and 0.938, respectively. After combination, the AUC reached 0.96. These findings underscore the potential of this noninvasive stool test to be applied in clinic to predict the mucosal healing of UC patients.

The treatment of IBD has entered a new era of biological agents. Although a wide variety of biologics are currently available in the clinic, infliximab is widely used as it was the first biologic to be approved for the treatment of UC [39]. In view of the effect of the drug on gut microbiota, only patients who exhibited mucosal healing after infliximab treatment were included in this study. α-Diversity analysis, PCA, PCoA and NMDS collectively revealed significant differences in the gut microbiota composition before and after mucosal healing in UC patients. Furthermore, Metastats and LEfSe analysis identified several species involved in UC and mucosal healing, including *Faecalibacterium prausnitzii*, *Bacteroides fragilis* and *Enterococcus faecalis*. *Lactobacillus rhamnosus* and *Faecalibacterium prausnitzii* can partially restore the function of the intestinal barrier by increasing the levels of Occludin and E-cadherin, which may be associated with microbial-induced downregulation of nuclear transcription factor-kappa B [40]. *Faecalibacterium prausnitzii* supernatant may reduce the severity of DSS-induced colitis in mice by enhancing intestinal barrier function via paracellular permeability regulation, suggesting the potential role of *Faecalibacterium prausnitzii* in the treatment of IBD [41]. EXL01, derived from *Faecalibacterium prausnitzii*, is currently in Phase I clinical trial for treatment of CD, expected to be a therapeutic candidate. In addition to that of *Faecalibacterium prausnitzii*, the abundance of *Bacteroides fragilis* also increased significantly after mucosal healing in UC patients. After mucosal healing, these enriched bacteria, represented by *Faecalibacterium prausnitzii*, are associated with short-chain fatty acid (SCFA) production [42]. The gut microbiota composition of UC patients changed after mucosal healing. For example, the abundance of Clostridium species involved in SCFA production was decreased in UC patients but increased after mucosal healing. The results suggested that the diversity of the gut microbiota was restored, the abundance of beneficial microbes increased, the abundance of harmful microbes decreased, and the production of a variety of metabolites involved in mucosal healing in UC patients increased. On the basis of these findings, it may be hypothesized that these bacterial species are linked to mucosal healing, is the direction of future precise treatment.

When the metabolic pathways in the KEGG database were assessed, carbohydrate and amino acid metabolism were found to be enriched in multiple genes from these taxa, which was consistent with previous studies [43]. The biosynthesis of various AAs increased after mucosal healing in UC. AA metabolism in IBD was found to be significantly suppressed in a previous study [44], suggesting that AA metabolism is involved in mucosal healing in UC, corresponding with the targeted metabolomics results. Further analysis revealed that these changes in AA metabolism were closely related to a variety of gut microbiota, such as *Faecalibacterium prausnitzii*, *Bacteroides fragilis*, *Bacteroides vulgatus*, and *Alistipes putredinis*. In the context of inflammation, an increase in threonine, serine, proline, and cysteine contents by dietary supplementation could promote mucin synthesis, restore balance in the gut microbiota composition, promote the growth of symbiotic bacteria, including Bacteroides and Lactobacillus, and thus favor colonic protection and mucosal healing [45]. Additionally, leucine supplementation increased the Firmicutes: Bacteroidetes ratio [46]. All of the above studies revealed a close relationship between AAs and the gut microbiota. To visualize this relationship, we conducted correlation analyses of distinctively abundant species and AAs and showed that the concentrations of fecal AAs were negatively correlated with the abundance of a variety of beneficial strains, such as A*dlercreutzia equolifaciens*,*Bacteroides*

fragilis, but positively correlated with the abundance of several harmful strains, such as *Citrobacter freundii*, *Enterococcus faecalis*. Intestinal diseases are characterized by gut microbiota dysbiosis and metabolic disorders, and gut metabolites may also manipulate the metabolic state of intestinal epithelial cells to induce an immune response in the gut. AAs can not only be used for the synthesis of the gut microbiota but also be degraded to produce metabolites through different pathways. The latter may affect colon epithelial cell metabolism and maintain homeostasis by altering intestinal barrier function, immunity, reactive oxygen species production, oxygen consumption, SCFA production, and colon epithelial cell activity and proliferation. The mechanism by which AAs and the gut microbiota are involved in mucosal healing in UC needs further study.

In summary, we assessed fecal AA concentrations and the gut microbiota composition before and after mucosal healing in patients with UC in the present prospective case–control study. The strengths of this study include the prospective design in which all patients were treated with infliximab and evaluated via endoscopy, which is currently the gold standard for treating UC. In addition, targeted metabolomics and metagenomics analyses were combined to evaluate the process of mucosal healing in UC. A limitation of this study was the small number of patients included. Moreover, AAs in the colonic mucosa were not detected because of the screening and sampling of noninvasive markers. AA metabolism in intestinal epithelial cells was not directly analyzed, although it was indirectly reflected by the serum and fecal concentrations of AAs. In conclusion, both AAs and the gut microbiota are implicated in the mucosal healing of UC and can be noninvasive diagnostic biomarkers; however, a large sample of patients is needed to verify the accuracy and sensitivity of these biomarkers, and further research is needed to explore the underlying mechanism involved, to provide new theoretical basis for individual therapy.

Supplementary Information

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Supplementary Material 1

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Author contributions
JW, JKN and YLM conceived and designed the project. MJL, JMR and FRZ recruited patients and collected specimens. JW, CZ and YLW conducted experiments. JHQ, RW and JKN analyzed and interpreted the data. JW and MJL drafted the manuscript. All authors reviewed the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This study was approved by the Ethics Committee of the First Affiliated Hospital of Kunming Medical University, Kunming, China. The patients/participants provided written informed consent to participate in this study.

Competing interests
The authors declare no competing interests.

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References

1. Ng SC, Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. Lancet. 2017;390(10114):2769–78.
2. Du L, Ha C. Epidemiology and Pathogenesis of Ulcerative Colitis. Gastroenterol Clin North Am. 2020;49(4):643–54.
3. Bromke MA, Neubauer K, Kempiński R, Krzystek-Korpacka M. Faecal Calprotectin in Assessment of Mucosal Healing in adults with inflammatory bowel disease: a Meta-analysis. J Clin Med. 2021;10(10).
4. Lopetuso LR, Petito V, Zambrano D, Orlando D, Dal Lago A, Serrichhio L, et al. Gut microbiota: a Key Modulator of Intestinal Healing in Inflammatory Bowel Disease. Dig Dis. 2016;34(3):202–9.
5. Crispin A, Birkner B, Munte A, Nusko G, Mansmann U. Process quality and incidence of acute complications in a series of more than 230,000 outpatient colonoscopies. Endoscopy. 2009;41(12):1018–25.
6. Boal Carvalho P, Cotter J. Mucosal Healing in Ulcerative Colitis: a Comprehensive Review. Drugs. 2017;77(2):159–73.
7. Vidal-Lletjós S, Beaumont M, Tomé D, Benamouzig R, Blachier F, Lan A. Dietary protein and amino acid supplementation in inflammatory bowel disease course: what impact on the Colonic Mucosa? Nutrients. 2017;9(3):310.
8. Zheng X, Zhu Y, Zhao Z, Chu Y, Yang W. The role of amino acid metabolism in inflammatory bowel disease and other inflammatory diseases. Front Immunol. 2023;14:1284133.
9. Schicho R, Shaykhutdinov R, Ngo J, Nazyrova A, Schneider C, Panaccione R, et al. Quantitative metabolomic profiling of serum, plasma, and urine by (1)H NMR spectroscopy discriminates between patients with inflammatory bowel disease and healthy individuals. J Proteome Res. 2012;11(6):3344–57.
10. Patti GJ, Yanes O, Siuzdak G, Innovation. Metabolomics: the apogee of the omics trilogy. Nat Rev Mol Cell Biol. 2012;13(4):263–9.
11. McCann JR, Rawls JF. Essential amino acid metabolites as chemical mediators of host-microbe interaction in the gut. Annu Rev Microbiol. 2023;15(77):479-497.
12. Sokol H, Seksik P. The intestinal microbiota in inflammatory bowel diseases: time to connect with the host. Curr Opin Gastroenterol. 2010;26(4):327–31.
13. Lee M, Chang EB. Inflammatory Bowel diseases (IBD) and the Microbiome-searching the crime scene for clues. Gastroenterology. 2021;160(2):524–37.
14. Pull SL, Doherty JM, Mills JC, Gordon JI, Stappenbeck TS. Activated macrophages are an adaptive element of the colonic epithelial progenitor niche

necessary for regenerative responses to injury. Proc Natl Acad Sci U S A. 2005;102(1):99–104.
15. Filidou E, Kolios G. Probiotics in intestinal mucosal healing: a new therapy or an old friend? Pharmaceuticals (Basel). 2021;14(11):1181.
16. Knight R, Vrbanac A, Taylor BC, Aksenov A, Callewaert C, Debelius J, et al. Best practices for analysing microbiomes. Nat Rev Microbiol. 2018;16(7):410–22.
17. Magro F, Gionchetti P, Eliakim R, Ardizzone S, Armuzzi A, Barreiro-de Acosta M, et al. Third European evidence-based Consensus on diagnosis and management of Ulcerative Colitis. Part 1: definitions, Diagnosis, extra-intestinal manifestations, pregnancy, Cancer Surveillance, surgery, and Ileo-anal Pouch disorders. J Crohns Colitis. 2017;11(6):649–70.
18. Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. N Engl J Med. 1987;317(26):1625–9.
19. Buchfink B, Xie C, Huson DH. Fast and sensitive protein alignment using DIAMOND. Nat Methods. 2015;12(1):59–60.
20. Karlsson FH, Tremaroli V, Nookaew I, Bergström G, Behre CJ, Fagerberg B, et al. Gut metagenome in European women with normal, impaired and diabetic glucose control. Nature. 2013;498(7452):99–103.
21. Karlsson FH, Fåk F, Nookaew I, Tremaroli V, Fagerberg B, Petranovic D, et al. Symptomatic atherosclerosis is associated with an altered gut metagenome. Nat Commun. 2012;3:1245.
22. Feng Q, Liang S, Jia H, Stadlmayr A, Tang L, Lan Z, et al. Gut microbiome development along the colorectal adenoma-carcinoma sequence. Nat Commun. 2015;6:6528.
23. White JR, Nagarajan N, Pop M. Statistical methods for detecting differentially abundant features in clinical metagenomic samples. PLoS Comput Biol. 2009;5(4):e1000352.
24. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. Genome Biol. 2011;12(6):R60.
25. Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K. KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Res. 2017;45(D1):D353–61.
26. Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermúdez-Humarán LG, Gratadoux JJ, et al. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc Natl Acad Sci U S A. 2008;105(43):16731–6.
27. Sartor RB, Wu GD. Roles for intestinal Bacteria, viruses, and Fungi in Pathogenesis of Inflammatory Bowel diseases and therapeutic approaches. Gastroenterology. 2017;152(2):327–e394.
28. Chu H, Khosravi A, Kusumawardhani IP, Kwon AH, Vasconcelos AC, Cunha LD, et al. Gene-microbiota interactions contribute to the pathogenesis of inflammatory bowel disease. Science. 2016;352(6289):1116–20.
29. Fan TJ, Goeser L, Lu K, Faith JJ, Hansen JJ. Enterococcus faecalis glucosamine metabolism exacerbates experimental colitis. Cell Mol Gastroenterol Hepatol. 2021;12(4):1373–89.
30. Balish E, Warner T. Enterococcus faecalis induces inflammatory bowel disease in interleukin-10 knockout mice. Am J Pathol. 2002;160(6):2253–7.
31. Peterson LW, Artis D. Intestinal epithelial cells: regulators of barrier function and immune homeostasis. Nat Rev Immunol. 2014;14(3):141–53.
32. Bosch S, El Hassani ME, Brizzio Brentar S, Ayada M, Bakkali I, Jansen A. Fecal amino acid profiles exceed accuracy of serum amino acids in diagnosing Pediatric Inflammatory Bowel Disease. J Pediatr Gastroenterol Nutr. 2020;71(3):371–5.
33. Lins Neto MÁF, Verdi GMX, Veras AO, Veras MO, Caetano LC, Ursulino JS, USE OF METABOLOMICS TO THE DIAGNOSIS OF INFLAMMATORY BOWEL DISEASE. Arq Gastroenterol. 2020;57(3):311–5.
34. Balasubramanian K, Kumar S, Singh RR, Sharma U, Ahuja V, Makharia GK, et al. Metabolism of the colonic mucosa in patients with inflammatory bowel diseases: an in vitro proton magnetic resonance spectroscopy study. Magn Reson Imaging. 2009;27(1):79–86.
35. Bosch S, Struys EA, van Gaal N, Bakkali A, Jansen EW, Diederen K, et al. Fecal amino acid analysis can discriminate De Novo Treatment-Naïve Pediatric Inflammatory Bowel Disease from controls. J Pediatr Gastroenterol Nutr. 2018;66(5):773–8.
36. Bao X, Feng Z, Yao J, Li T, Yin Y. Roles of dietary amino acids and their metabolites in Pathogenesis of Inflammatory Bowel Disease. Mediators Inflamm. 2017;2017:6869259.
37. Liu Y, Wang X, Hu CA. Therapeutic potential of amino acids in inflammatory bowel disease. Nutrients. 2017;9(9):920.
38. Hisamatsu T, Okamoto S, Hashimoto M, Muramatsu T, Andou A, Uo M, et al. Novel, objective, multivariate biomarkers composed of plasma amino acid profiles for the diagnosis and assessment of inflammatory bowel disease. PLoS ONE. 2012;7(1):e31131.
39. Chang S, Hudesman D. First-line biologics or small molecules in inflammatory bowel disease: a practical guide for the Clinician. Curr Gastroenterol Rep. 2020;22(2):7.
40. Laval L, Martin R, Natividad JN, Chain F, Miquel S, Desclée de Maredsous C, et al. Lactobacillus rhamnosus CNCM I-3690 and the commensal bacterium Faecalibacterium prausnitzii A2-165 exhibit similar protective effects to induced barrier hyper-permeability in mice. Gut Microbes. 2015;6(1):1–9.
41. Carlsson AH, Yakymenko O, Olivier I, Håkansson F, Postma E, Keita AV, et al. Faecalibacterium prausnitzii supernatant improves intestinal barrier function in mice DSS colitis. Scand J Gastroenterol. 2013;48(10):1136–44.
42. Lewis K, Lutgendorff F, Phan V, Söderholm JD, Sherman PM, McKay DM. Enhanced translocation of bacteria across metabolically stressed epithelia is reduced by butyrate. Inflamm Bowel Dis. 2010;16(7):1138–48.
43. Sun M, Wu W, Liu Z, Cong Y. Microbiota metabolite short chain fatty acids, GPCR, and inflammatory bowel diseases. J Gastroenterol. 2017;52(1):1–8.
44. Morgan XC, Tickle TL, Sokol H, Gevers D, Devaney KL, Ward DV, et al. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. Genome Biol. 2012;13(9):R79.
45. Faure M, Mettraux C, Moennoz D, Godin JP, Vuichoud J, Rochat F, et al. Specific amino acids increase mucin synthesis and microbiota in dextran sulfate sodium-treated rats. J Nutr. 2006;136(6):1558–64.
46. Song B, Zheng C, Zha C, Hu S, Yang X, Wang L, et al. Dietary leucine supplementation improves intestinal health of mice through intestinal SIgA secretion. J Appl Microbiol. 2020;128(2):574–83.

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