Original Article

Alterations of gut microbiota and metabolites in children with Crohn's disease and their correlation with disease activity

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Background: The disruption of the gut microbiota is a prominent feature seen in children with Crohn's disease (CD), impacting metabolic processes. These factors collectively contribute significantly to the onset and progression of CD. The aim of this study was to assess the variations in gut microbiota and metabolites in children with newly diagnosed CD and those in remission, and to investigate their potential correlation with clinical indexes.

Methods: This was a retrospective study. From June 2018 and March 2024, 57 children with CD admitted to Beijing Children's Hospital were included, and 22 healthy children during the same period were selected as the control group. Their peripheral blood and fecal samples were obtained, and clinical data were collected. Analysis of the fecal microbiota and serum metabolites was conducted using metagenomic sequencing and non-targeted mass spectrometry, respectively, to compare the alteration in children with CD and healthy controls (HCs), and their correlation with clinical indexes.

Results: Analysis of fecal metagenomic sequencing data revealed that the alpha diversity was significantly lower in the newly diagnosed CD group compared to the HC group, whereas it was ameliorated in the CD remission group. The beta diversity showed that the microbial structures of the three groups were obviously separated. *Firmicutes* was identified as the primary altered bacteria in the microbiota. Specifically, the abundance of *Ruminococcus*, *Faecalimonas*, *Blautia*, and *Faecalibacterium* were correlated with clinical indexes such as pediatric Crohn's disease activity index (PCDAI). Metabolomic analysis highlighted differences in lipid metabolism, bile acid (BA) metabolism, amino acid metabolism and energy homeostasis between the CD remission and newly diagnosed CD groups. Notably, the levels of citric acid were correlated with clinical indexes such as PCDAI, which was also potential indicator for identifying clinical activity of pediatric CD patients [area under the curve (AUC) =0.77, specificity =0.64, sensitivity =0.83].

Conclusions: The microbial diversity of children with newly diagnosed CD decreased, but then ameliorated in the remission stage. Some short-chain fatty acids (SCFAs)-producing bacteria, lipid metabolites, and energy homeostasis products were associated with clinical indexes. In particular, citric acid demonstrated specific effectiveness in identify clinical activity of pediatric CD patients, which was a potential biomarker. Further exploring the mechanism of energy homeostasis in CD is beneficial to find new therapeutic targets.

Keywords: Crohn's disease (CD); gut microbiota; metabolites; child

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Introduction

Pediatric inflammatory bowel disease (IBD) is a persistent, non-specific inflammatory disease primarily affecting the gastrointestinal tract, categorized into Crohn's disease (CD), ulcerative colitis (UC), and undifferentiated IBD. Clinical indications of pediatric CD include abdominal pain, weight loss, delayed growth, diarrhea, abdominal mass, intestinal fistula, perianal lesions, as well as systemic symptoms such as fever and anemia (1). The etiology of CD remains uncertain, linked to abnormal immune responses in genetically predisposed individuals reacting to the gut microbiota. The global incidence rates of CD have been steadily rising, which range from 0.1 to 13.9/100,000 for pediatric CD (2), imposing a substantial disease burden worldwide (3).

Disturbance of the gut microbiota plays a significant role in the onset and progression of IBD (4). In pediatric CD, the gut microbiota displays decreased diversity, heightened instability, and changes in relative abundance compared to that of healthy children (5). The gut microbiota's regulatory role largely relies on various metabolites, including bile acids (BAs) and short-chain fatty acids (SCFAs). BAs are synthesized in the liver and undergo biotransformation in the intestine by the gut microbiota, impacting its composition (6). SCFAs are byproducts of dietary fiber fermentation by the gut microbiota, primarily consisting of acetate, propionate, and butyrate. SCFAs not only fuel intestinal epithelial cells and boost intestinal barrier function, but also act as ligands for G protein-coupled receptor, contributing to immune system activation and regulation (7).

Energy homeostasis is also crucial for maintaining the intestinal environment. Mitochondrial dysfunction involves a reduction in electron transport chain expression, a decrease in tricarboxylic acid (TCA) cycle intermediates, alterations in mitochondrial morphology, and an increase in mitochondrial reactive oxygen species, all of which are observed in chronic conditions such as IBD. In the presence of inflammation, the structure of mitochondrial in intestinal epithelial cells becomes distorted, characterized by cristae damage and a reliance on fission due to high dynamin-related protein 1 levels. These changes in mitochondrial structure and energy production lead to a shift towards glycolysis and the suppression of anti-inflammatory mediator expression (8).

Previous studies have indicated that pediatric CD is associated with alterations in the gut microbiota structure, a decrease in SCFAs, and impaired secondary BAs metabolism (9,10). Adult CD has shown a decrease in TCA cycle intermediates (11), yet research specific to pediatric CD is limited. The relationship between the changes in microflora, metabolites, and disease activity in pediatric CD remains unclear. Therefore, the aim of this study was to assess the variations in gut microbiota and metabolites in children with newly diagnosed CD and those in remission, and to investigate their potential correlation with clinical indexes. The goal is to identify specific gut microbiota and metabolite patterns associated with disease remission, with the ultimate objective of utilizing these as potential biomarkers for monitoring disease activity. We present this article in accordance with the STROBE reporting checklist (available at https://tp.amegroups.com/article/view/10.21037/tp-2025-36/rc).

Highlight box

Key findings

- The study found that some short-chain fatty acid (SCFA)-producing bacteria, lipid metabolites, and energy homeostasis products were associated with clinical indexes. Citric acid demonstrated specific effectiveness in identifying Crohn's disease (CD) activity [area under the curve (AUC) =0.77, specificity =0.64, sensitivity =0.83].

What is known and what is new?

- CD in children has been shown to have alterations in the gut microbiota structure, a decrease in SCFAs, and impaired secondary bile acids (BAs) metabolism.
- The relationship between the changes in microflora, metabolites, and disease activity in children with CD is unclear.

What is the implication, and what should change now?

- Citric acid may be considered for clinical application as a biomarker to identify the clinical activity of pediatric CD patients.

Methods

Study design and participants

This was a retrospective study, which recruited children with CD in Beijing Children's Hospital from June 2018 and

March 2024. The study was conducted in accordance with the Declaration of Helsinki and its subsequent amendments. The study was approved by the Institutional Ethics and Review Committee of the Beijing Children's Hospital, Capital Medical University (No. 2022-E-099-Y-01). Informed consent was obtained from the children's parents.

The inclusion criteria were as follows: (I) newly diagnosed with CD according to the European Society of Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) revised Porto criteria (12), or in clinical remission following treatment; and (II) 0–18 years old. The exclusion criteria were as follows: (I) complicated with infectious colitis caused by intestinal tuberculosis, parasitic infection, and so on; and (II) having used antibiotics or probiotics in the past week. Another 20 healthy children in the same period were selected as the control group (healthy control, HC), who had no family history of IBD and did not take antibiotics or probiotics within 2 months before sample collection.

Data and samples collection

Demographic and clinical data were extracted from electronic medical records, including age, sex, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), white blood cells (WBCs), red blood cells (RBCs), hemoglobin (HB), hematocrit (HCT), platelet (PLT), and albumin (ALB). Fecal and serum samples were obtained from children with CD, and fecal samples from healthy children. All fecal and serum samples were stored at –80 ℃ before detection.

Disease assessment

The pediatric Crohn's disease activity index (PCDAI) was used to assess the disease activity, with a PCDAI score of <10 indicating remission and a score of ≥10 indicating activity (13). According to Paris classification, the disease locations were categorized as distal 1/3 ileum ± limited caecum (L1), colon (L2), ileocolon (L3), and upper digestive tract (L4). Disease behaviors included non-stricture and non-penetration (B1), stricture (B2), penetration (B3), both penetration and stricture (B2B3), and perianal disease (P). Growth status was classified as no evidence of growth delay (G0) and growth delay (G1) (14).

DNA extraction, metagenomic sequencing, and annotation

DNA extraction was performed using the FastPure Stool DNA Isolation Kit (Magnetic bead, MJYH, Shanghai, China). DNA concentration and purity measurements were performed with Synergy HTX (Agilent, Santa Clara, CA, USA) and NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA), respectively. DNA integrity verification was conducted through 1% agarose gel electrophoresis. DNA extract was fragmented to an average size of about 400 bp using Covaris M220 (Gene Company Limited, Shanghai, China), followed by paired-end library preparation. Paired-end library was generated with NEXTFLEX Rapid DNA-Seq (Bioo Scientific, Austin, TX, USA).

Paired-end sequencing was performed on Illumina NovaSeq 6000 (Illumina Inc., San Diego, CA, USA) at MyGenostics, Co. Ltd. (Beijing, China) using NovaSeq 6000 S4 Reagent Kit v1.5 (300 cycles) according to the manufacturer' s instructions (Illumina, USA). The metagenomic sequencing data associated with this project have been deposited in the National Center for Biotechnology Information (NCBI) Short Read Archive database.

The raw sequencing reads underwent adapter trimming and quality filtering using fastq (https://github.com/OpenGene/fastp, version 0.20.0), with removal of low-quality reads (length<50 bp or with a quality value <20 or having N bases). Reads were aligned to the human genome through Burrows-Wheeler Aligner) BWA (http://bio-bwa.sourceforge.net, version 0.7.17) and any hit associated with the reads and their paired reads were deleted. The remaining high-quality data were assembled using MEGAHIT (https://github.com/voutcn/megahit, version 1.1.2). Contigs meeting the minimum length threshold of 300 bp were retained as final assembly outputs for downstream analysis. Open reading frames (ORFs) from each assembled contig were predicted using Prodigal (https://github.com/hyattpd/Prodigal, version 2.6.3) and a length ≥100 bp ORFs was retrieved.

CD-HIT (http://weizhongli-lab.org/cd-hit/, version 4.7) was used to construct a non-redundant gene catalog, with 90% sequence identity and 90% coverage. The gene abundance in each sample was quantified using by SOAPaligner (https://github.com/ShujiaHuang/SOAPaligner, version soap2.21 release) with 95% identity. The non-redundant genes were compared with NCBI NR database by DIAMOND (http://ab.inf.uni-tuebingen.de/software/diamond/, version 2.0.13), and the optimal classification was obtained with an e-value cutoff of 1e-5. Similarly, the functional annotation [Gene Ontology (GO),

Kyoto Encyclopedia of Genes and Genomes (KEGG), evolutionary gene Nomenclature, Orthologous Groups (eggnog), Carbohydrate-Active EnZYmes Database (CAZy), Comprehensive Antibiotic ResistDatabase (CARD), Pathogen-Host Interactions (PHI)] of non-redundant genes was obtained.

Difference analysis of abundance and composition of gut microbiota

The alpha diversity index Shannon, Simpson, and Chao1 indices were calculated to measure the community richness and diversity. Principal coordinate analysis (PCoA) was used to investigate the structural variation of microbial communities, and groups were compared using permutational multivariate analysis of variance method. The abundance and composition of gut microbiota in different groups were compared by Wilcoxon rank-sum test or Kruskal-Wallis *H* test. Differential microbiota was annotated by the KEGG database to obtain the participating pathways.

Metabolite extraction and identification

For metabolite extraction, 100 µL serum sample was combined with 400 µL solution (acetonitrile: methanol =1:1 [v:v]) containing 0.02 mg/mL internal standard (L-2-chlorophenylalanine) in a 1.5 mL centrifuge tube. This was then subjected to vortex mixing for 30 seconds and low temperature ultrasound for 30 minutes (5 ℃, 40 KHz). To precipitate proteins, the samples were incubated at –20 ℃ for 30 minutes. Then the samples were centrifuged for 15 minutes (4 ℃, 13,000 g). The supernatant was collected, evaporated to dryness under nitrogen, and then reconstituted with 100 µL solution (acetonitrile: water =1:1). After low-temperature ultrasonication for 5 minutes (5 ℃, 40 KHz), the samples were centrifuged for 10 minutes (4 ℃, 13,000 g). The supernatant was transferred to liquid chromatography-tandem mass spectrometry (LC-MS/MS) vials for subsequent analysis.

As a part of the system conditioning and quality assurance, a pooled quality control (QC) sample was prepared by combining equal volumes of all samples. The QC samples were processed and analyzed identically to the experimental samples. To monitor analytical stability, the QC samples were injected at regular intervals (every 5–15 samples), serving as a representative benchmark for the entire dataset. The LC-MS/MS analysis of sample was performed using a Thermo UHPLC-Exploris 240 system equipped with an ACQUITY HSS T3 column (100×2.1 mm i.d., 1.8 µm; Waters, Milford, MA, USA) at MyGenostics, Co. Ltd. (Beijing, China).

The UHPLC-MS raw data were processed using Progenesis QI software (Waters, USA), which performed baseline filtering, peak identification, peak integral, retention time correction, and peak alignment. The processed data were then exported as a matrix containing sample names, m/z, retention time and peak intensities for subsequent analyses. Metabolite identification was conducted by database search, and the main databases were the HMDB (http://www.hmdb.ca/) and Metlin (https://metlin.scripps.edu/).

Difference analysis of metabolites

For metabolites, principal component analysis (PCA) and orthogonal least partial squares discriminant analysis (OPLS-DA), and 7-cycle interactive validation were used to evaluate the stability of the model. The metabolites with variable importance in the projection (VIP) >1, P<0.05 were determined as significantly different metabolites based on the VIP obtained by the OPLS-DA model and the P value generated by Student's *t*-test. Differential metabolites were annotated using the KEGG database to obtain the participating pathways.

Statistical analysis

Statistical analysis was performed using "R" software version 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria). Continuous variables were presented as mean ± standard deviation, or median (interquartile range) depending on the probability distribution. Data with more than two groups were first analyzed by the Kruskal-Wallis *H* test. If P<0.05, Mann-Whitney *U* test was used for pairwise comparison. Categorical variables were presented as frequency with percentage and compared using the χ^2 test or Fisher's exact test. Spearman correlation analysis was used to evaluate the correlation between differential microbiota and metabolites, and their correlation with clinical indexes such as PCDAI. A P value <0.05 was considered statistically significant. The receiver operating characteristic (ROC) curve was used to evaluate the effectiveness of the altered gut microbiota and metabolites in assessing disease activity.

Results

Clinical characteristics

A total of 57 children with CD were included in this study, including 30 newly diagnosed patients and 27 in remission, with an average age of 11.98±0.37 years. Of them, 35 were boys (61.40%) and 22 were girls (38.60%). The HC group consisted of 22 children, with an average age of 8.64±0.76 years, including 13 boys (59.09%) and 9 girls (40.91%). Among children with CD, the most common disease locations were L3 + L4 (36.84%) and L3 (35.09%), followed by L1 (12.28%), L2 (8.77%), L1 + L4 (5.26%), and L4 (1.75%). The majority (77.19%) of children with CD did not have intestinal stricture or perforation. The proportion of intestinal stricture, perforation, both stricture and perforation, and perianal disease in children with CD was 17.54%, 3.51%, 1.75%, and 24.56%, respectively. Growth delay was observed in 63.16% (36/57) of patients. The median PCDAI score in the newly diagnosed CD group was 30 [20, 40]. Significantly higher levels of ESR (P<0.001), CRP (P<0.001), WBC (P=0.006), and PLT (P=0.001) were noted in the newly diagnosed CD group compared to the remission group, whereas HB (P<0.001), HCT (P=0.002), and ALB (P<0.001) levels decreased significantly (*Table 1*).

Alterations of gut microbiota in children with CD

A total of 22 fecal samples from the HC group, 26 fecal samples from individuals newly diagnosed with CD, and 21 fecal samples from individuals with CD in remission were analyzed. A total of 3,894 microbial genus and 16,082 microbial species were detected. The study compared the alterations in microbial diversity and composition among the three groups. Compared with the HC group, the Chao index (P<0.001) and ACE index (P<0.001) of the newly diagnosed CD group were obviously lower, and there was no difference in Simpson index (P=0.78). In addition, the Chao index (P=0.003) and ACE index (P=0.003) in remission group were improved (*Figure 1A-1C*). The PCoA of beta diversity demonstrated clear separation in microbial structures among the three groups (*Figure 1D*).

Compared to the HC group, the levels of *Faecalibacterium*, *Bacteroides*, *Bifidobacterium*, *Ruminococcus*, and *Clostridium* at the genus level, as well as *Phocaeicola vulgatus*, *Segatella copri*, *Faecalibacterium prausnitzii*, and *Bacteroides ovatus* at the species level, were notably lower in individuals with newly diagnosed CD. Similarly, in the CD remission group, the abundance of *Faecalibacterium*, *Bifidobacterium*, and *Blautia* remained decreased compared to the HC group. Furthermore, in the CD remission group, there was an increase in the levels of *Blautia*, *Clostridium*, *Ruminococcus*, and *Faecalimonas* at the genus level, and *Enterocloster bolteae*, *Faecalimonas umbilicata*, and *Flavonifractor plautii* at the species level, in contrast to the newly diagnosed CD group. Conversely, the abundance of *Thomasclavelia* and *Parabacteroides* at the genus level, along with *Clostridium innocuum* and *Enterococcus faecalis* at the species level, decreased in the CD remission group compared to the newly diagnosed CD group (*Figure 1E-1G*). The changes in microbiota were identified by the KEGG database to explore related pathways. Notably, in the newly diagnosed CD group, there was a significant reduction in the synthesis of secondary metabolites, coenzyme factor synthesis, amino acid synthesis, and carbon metabolism enrichment compared to both the HC and CD remission groups (*Figure 1H*).

Alterations of metabolites in children with CD

A total of 26 serum samples were collected from the newly diagnosed group with CD, whereas 21 serum samples were obtained from the CD group in remission, resulting in the detection of 2,892 serum metabolites. The volcano plot illustrated the altered metabolites between the two groups, with 156 metabolites significantly up-regulated and 364 metabolites significantly down-regulated (*Figure 2A*). Notably, among the top 15 different metabolites based on VIP score, a majority were associated with lipid metabolism, BA metabolism, amino acid metabolism, and energy homeostasis. Specifically, levels of hyodeoxycholic acid, prolyl-leucine, and isocitrate were notably lower in the newly diagnosed CD group compared to the CD group in remission (*Figure 2B*). Furthermore, a metabolic pathway enrichment analysis was performed to pinpoint the pathways contributing to the major differences between the two groups, revealing an increase in lipid metabolism and TCA cycle in the CD group in remission compared to the newly diagnosed CD group (*Figure 2C*).

Correlation between altered microbiota, metabolites, and clinical index

The study examined the relationship between microbiota, metabolites, and clinical indexes such as PCDAI using Spearman rank correlation analysis. The top 50 microbiota

Table 1 Clinical characteristics of pediatric CD and HC

Characteristics	HC (n=22)	Newly diagnosed CD (n=30)	CD in remission (n=27)	P value*
Age (years)	8.64±0.76	11.73±0.474	12.26±0.59	0.49
Sex				
Boy	13 (59.09)	18 (60.00)	17 (62.96)	0.82
Girl	9 (40.91)	12 (40.00)	10 (37.04)	
Disease location				>0.99
L1		3 (10.00)	4 (14.81)	
L2		3 (10.00)	2 (7.41)	
L3		10 (33.33)	10 (37.04)	
L4		1 (3.33)	0 (0.00)	
L1 + L4		2 (6.67)	1 (3.70)	
L3 + L4		11 (36.67)	10 (37.04)	
Disease behavior				
B1		24 (80.00)	20 (74.07)	>0.99
B2		5 (16.67)	5 (18.52)	
B3		1 (3.33)	1 (3.70)	
B2B3		0 (0.00)	1 (3.70)	
P		8 (26.67)	6 (22.22)	0.70
Growth				0.26
G0		9 (30.00)	12 (44.44)	
G1		21 (70.00)	15 (55.56)	
PCDAI		30 [20, 40]	0 [0, 5]	<0.001
ESR (mm/h)		30 [14.75, 49.25]	5.5 [2, 7.75]	<0.001
CRP (mg/L)				<0.001
<10		13 (43.33)	27 (100.00)	
≥10		17 (56.67)	0 (0.00)	
WBC (10^9/L)		7.76 [5.89, 10.46]	6.07 [4.71, 7.00]	0.006
RBC (10^9/L)		5.37±0.80	4.78±0.10	0.50
HB (g/L)		100.64±5.63	129.11±2.97	<0.001
HCT (%)		34.10 [30.43, 38.53]	39.30 [37.7, 41.00]	0.002
PLT (10^9/L)		412.76±19.70	317.78±18.65	0.001
ALB (g/L)		34.38±0.84	42.40±0.73	<0.001
Treatment				
Biologicals			22 (81.48)	
Exclusive enteral nutrition			4 (14.81)	
Mesalazine			1 (3.70)	

Data are presented as mean ± standard deviation or n (%) or median [interquartile range]. *, the data were compared by Student's *t*-test or Wilcoxon rank-sum test or Chi-squared test or Fisher exact test. ALB, albumin; CD, Crohn's disease; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HB, hemoglobin; HC, healthy control; HCT, hematocrit; PCDAI, pediatric Crohn's disease activity index; PLT, platelet; RBC, red blood cell; WBC, white blood cell.

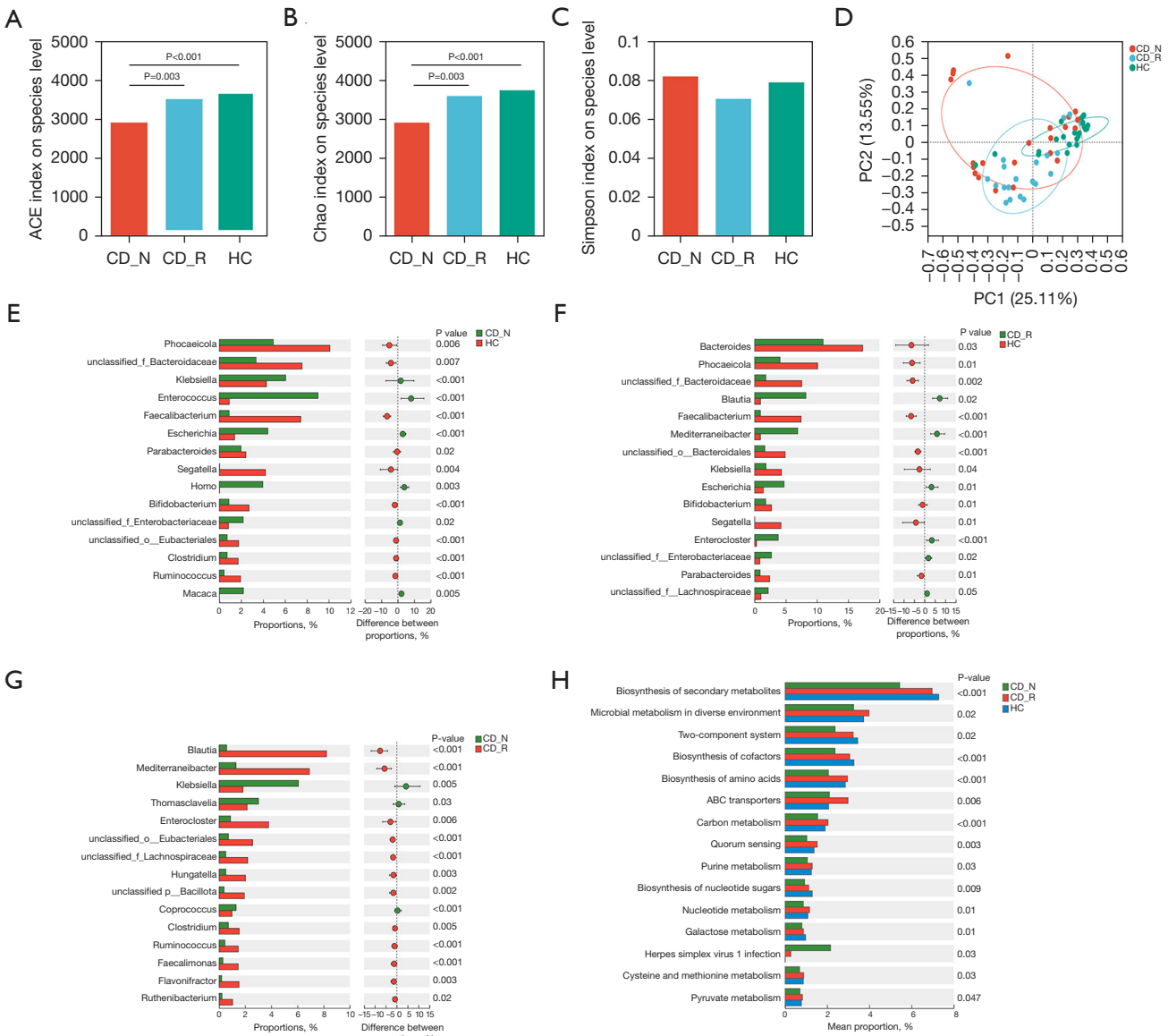

Figure 1 Gut microbiota biodiversity and composition in children with newly diagnosed CD, CD in remission and HC. ACE (A), Chao (B) and Simpson (C) index of gut microbiota at species level. (D) PCoA analysis of HC, CD_N, and CD_R groups. (E-G) Top 15 significantly altered microbial genera and their relative abundances. (H) KEGG enrichment analysis of differential microbiota. CD, Crohn's disease; CD_N, newly diagnosed; CD_R, CD in remission; HC, healthy control; KEGG, Kyoto Encyclopedia of Genes and Genomes; PCoA, principal coordinate analysis.

and metabolites ranked by p-value were shown in the heat map, with positive and negative correlations represented by red and green color, respectively (*Figure 3A,3B*). Notably, *Ruminococcus*, *Faecalimonas*, *Blautia*, and *Faecalibacterium* showed negative correlations with PCDAI (r=–0.62, –0.62, –0.70, and –0.43 respectively), CRP (r=–0.54, –0.54, –0.63, and –0.48 respectively), and ESR (r=–0.60, –0.61, and –0.67 respectively), while being positively correlated with HB (r=0.67, 0.62, 0.72, and 0.47 respectively) and HCT (r=0.52, 0.54, 0.57, and 0.30 respectively). Among the metabolites, citric acid levels were negatively linked to PCDAI, CRP, and ESR (r=–0.56, –0.43, and –0.43 respectively), while being positively correlated with HB and ALB (r=0.57 and 0.41, respectively), all with a significance level of P<0.05.

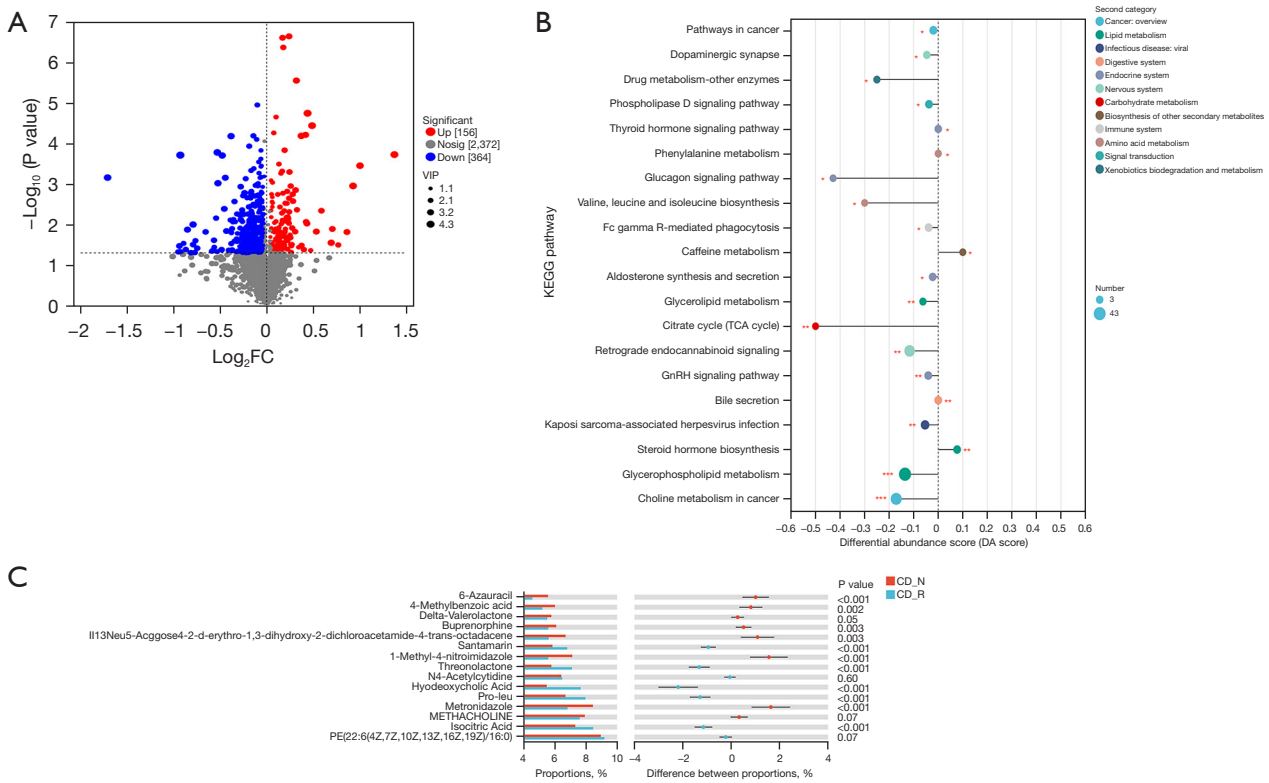

Figure 2 Serum metabolomics in children with newly diagnosed CD and CD in remission. (A) Volcano plot of the altered metabolites. (B) The top 15 altered metabolites ranked by VIP scores and their relative levels. (C) Metabolic pathway enrichment analysis. *, 0.01≤P<0.05; **, 0.001≤P<0.01; ***, P<0.001. CD, Crohn's disease; CD_N, newly diagnosed; CD_R, CD in remission; FC, fold change; KEGG, Kyoto Encyclopedia of Genes and Genomes; VIP, variable importance in the projection.

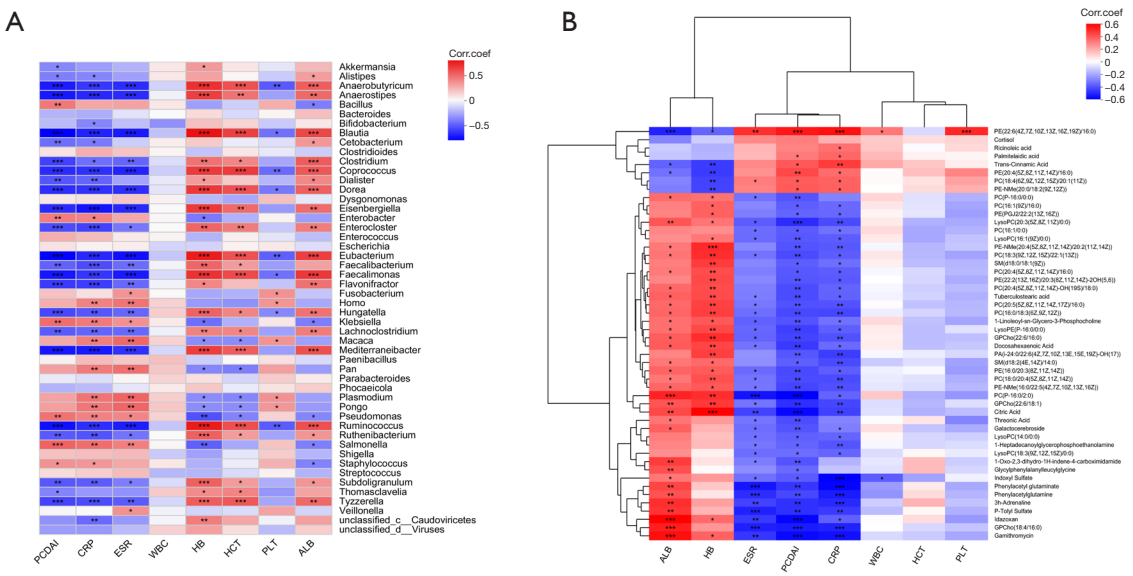

Figure 3 The correlation between altered microbiota (A), metabolites (B), and clinical indexes. *, 0.01≤P<0.05; **, 0.001≤P<0.01; ***, P<0.001. ALB, albumin; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HB, hemoglobin; HCT, hematocrit; PCDAI, Pediatric Crohn's Disease Activity Index; PLT, platelet; WBC, white blood cell.

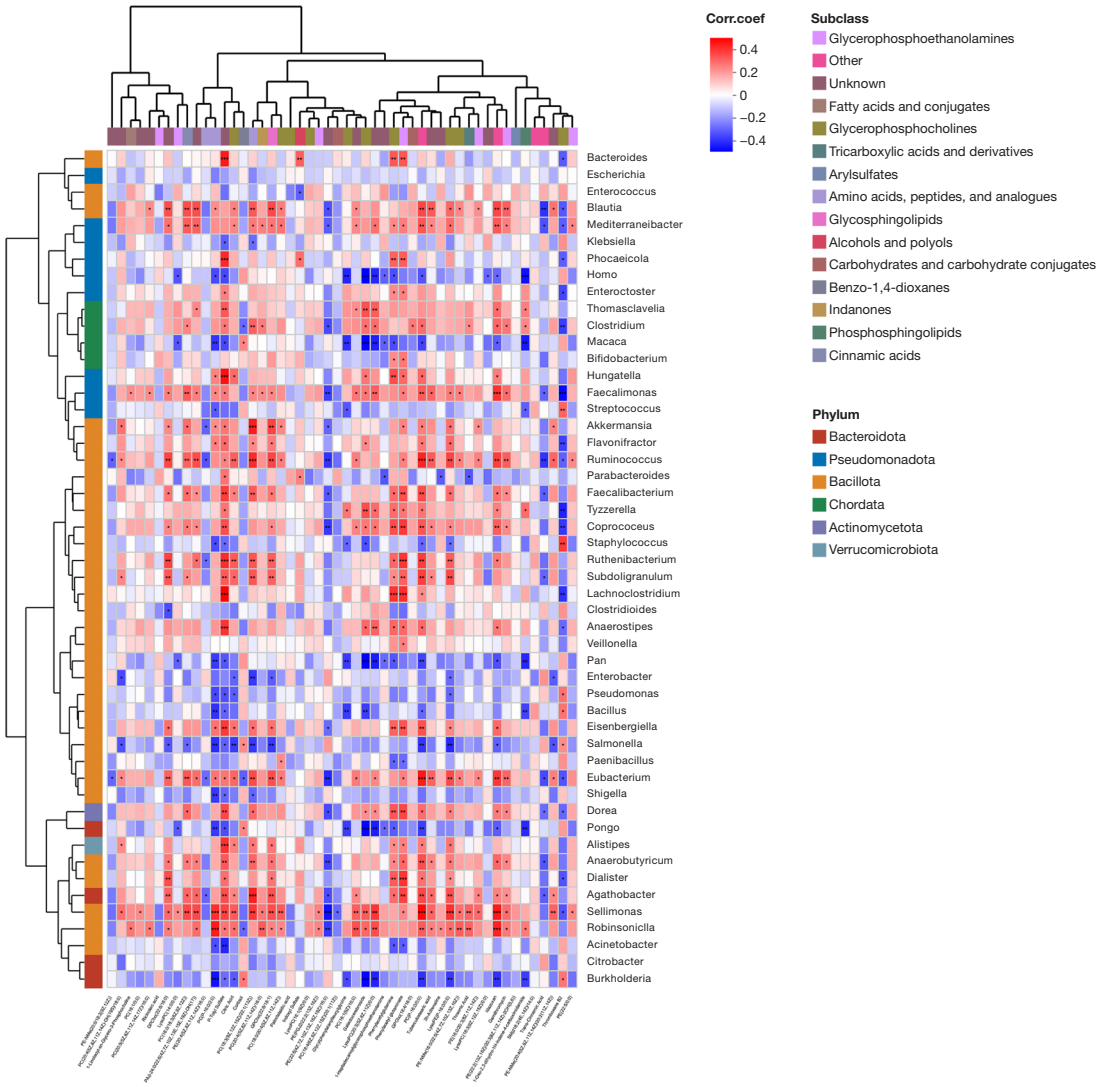

Figure 4 The correlation between microbiota and metabolites. *, 0.01≤P<0.05; **, 0.001≤P<0.01; ***, P<0.001.

Correlation between altered gut microbiota and metabolites

In order to couple the alterations of the gut microbiota and dysmetabolism in the newly diagnosed CD group and the CD in remission group, Spearman rank correlation was calculated between the levels of metabolites and the abundance of microbiota. A total of 50 significant bacteria-metabolite correlations were identified at the genus level (*Figure 4*). Notably, a screening process based on a Spearman correlation co-efficient cutoff of >0.25 or <–0.25 revealed weakly negative correlations between *Ruminococcus*, *Blautia*, *Faecalibacterium*, and citric acid levels (r=0.311, 0.251, and 0.251 respectively), all with a significance level of P<0.05.

Potential biomarkers for identifying clinical activity of children with CD

The efficacy of differential flora and metabolites in assessing pediatric CD activity was examined using ROC curve analysis. The findings revealed that citric acid demonstrated specific effectiveness in identifying CD activity, and the area under curve (AUC) was 0.77 (specificity =0.64, sensitivity =0.83) (*Figure 5*).

Discussion

The complex communication between gut microbiota and metabolites plays a key role in regulating intestinal

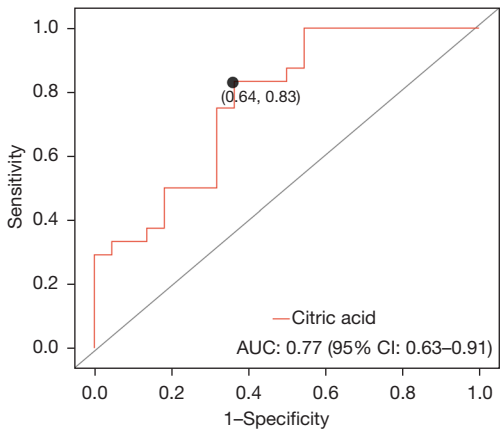

Figure 5 ROC curve of citric acid in identifying clinical activity of children with CD. AUC, area under the curve; CD, Crohn's disease; CI, confidence interval; ROC, receiver operating characteristic.

homeostasis. Through metagenomic sequencing and non-targeted mass spectrometry respectively, our study analyzed the alterations of the gut microbiota and metabolites in children with newly diagnosed CD and CD in remission, as well as their correlation with clinical indexes. This study found potential biomarkers to monitor the clinical activity of pediatric CD, and further elucidated the role of the gut microbiota and metabolites in the activity and remission of CD.

The alpha diversity of the gut microbiota was lower in children with newly diagnosed CD compared to the HC, but showed an improvement in children in remission. Most children in remission received biological therapies, whereas a few were treated with exclusive enteral nutrition or immunosuppressants. Similar to our results, a separate study also observed an increase in alpha diversity of the gut microbiota in children with IBD over time and during disease remission, irrespective of treatment methods (15). *Bacteroidetes* and *Firmicutes* are the predominant bacterial phyla in the human gut, with *Bacteroidetes* mainly producing acetate and propionate, whereas *Firmicutes* mainly produce butyrate. Besides butyrate, other SCFAs are generated by bacteria such as *Bifidobacterium*, which produce acetate and lactate through carbohydrate fermentation (7). Our findings indicated that the levels of *Faecalibacterium*, *Clostridium*, and *Ruminococcus* of the phylum *Firmicutes*, as well as *Parabacteroides* of the phylum *Bacteroidetes*, were lower in the newly diagnosed CD group compared to the HC group. Conversely, the abundance of *Blautia*, *Hungatella*, *Clostridium*, *Ruminococcus*, and *Faecalimonas* was higher in the CD remission group than that in the newly diagnosed CD group. Previous studies have also shown that the most commonly reduced microbiota in pediatric IBD patients are bacteria from the *Firmicutes* phylum, including *Ruminococcus* and *Clostridium* (16,17). In addition, our results revealed associations between *Ruminococcus*, *Faecalimonas*, *Blautia*, and *Faecalibacterium*, and clinical parameters such as PCDAI score. These results suggested that achieving remission in pediatric CD might be connected to the restoration of a microbiota that produces SCFAs.

Primary BAs, synthesized from cholesterol in the liver and secreted into the intestine, consist of cholic acid and chenodeoxycholic acid (CDCA) in humans. These BAs are mostly absorbed in the ileum for enterohepatic circulation, whereas some are transformed by intestinal bacteria into secondary BAs, such as deoxycholic acid (DCA) and lithocholic acid (LCA). CDCA, DCA, and LCA have been identified as ligands of farnesoid X receptor and Takeda G-protein receptor 5, known to mediate anti-inflammatory responses (6). Our results showed that compared to the newly diagnosed CD group, the level of hyodeoxycholic acid in the CD in remission group was significantly higher, suggesting a potential role in inhibiting intestinal inflammation. Additionally, Watanabe *et al.* (18) found that the interaction between hydroxydeoxycholic acid and dextran sulfate sodium (DSS) led to increased fecal BA levels, thus preventing colitis induced by DSS in mice.

Mitochondrial dysfunction has been increasingly linked to the development of IBD according to recent studies (19). Adult CD patients have demonstrated a significant reduction in TCA cycle intermediates, including citrate, aconitate, a-ketoglutarate, succinate, fumarate, and malate compared to HC and UC patients (11). A study performing RNA-Seq analysis of rectal samples showed that compared with the control group, the mitochondria, aerobic TCA cycle and metabolic functions of children and adults with UC decreased significantly (20). Inflammation-induced mitochondrial dysfunction in the intestinal epithelium was found to disrupt metabolic imbalance, leading to diminished stemness and dysfunctional Paneth cells (21). Our results showed that citric acid levels in newly diagnosed children with CD were lower than those in remission, showing promise as an indicator of CD activity. Moreover, isocitrate levels were related to clinical inflammatory indexes such as PCDAI, both being TCA cycle intermediates, highlighting the importance of energy homeostasis in pediatric CD. In addition, the presence of *Blautia*, *Ruminococcus*, and

Faecalibacterium in the TCA cycle was inversely related to citric acid levels, suggesting a potential role for these bacteria in energy metabolism.

The limited sample size of this study may impact the generalizability of these findings, therefore further validation in larger populations is warranted. Furthermore, comparing serum metabolites in healthy children with those diagnosed with pediatric CD could provide valuable insights for future research.

Conclusions

This study showed that the gut microbiota and metabolites changed in children with CD in the initial and remission stages, indicating that they were involved in the occurrence and remission of the disease. Among them, some SCFA-producing bacteria, lipid metabolites, and energy homeostasis products were associated with clinical indexes, as potential biomarkers. In particular, citric acid had a high efficiency in identifying the clinical activity of pediatric CD patients. Future research can further explore the mechanism of energy homeostasis in CD to find new therapeutic targets.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://tp.amegroups.com/article/view/10.21037/tp-2025-36/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tp.amegroups.com/article/view/10.21037/tp-2025-36/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki and its subsequent amendments. The study was approved by the Institutional Ethics and Review Committee of the Beijing Children's Hospital, Capital Medical University (No. 2022-E-099-Y-01). Informed consent was obtained from the children's parents.

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