Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology) in press
www.jzus.zju.edu.cn; www.springer.com/journal/11585
E-mail: jzus_b@zju.edu.cn

Research Article

https://doi.org/10.1631/jzus.B2400330

Pediatric inflammatory bowel disease in mother-child pairs: clinical risk factors and gut microbiota characteristics

Cunzheng ZHANG[1,2*], Ruqiao DUAN[1,2*], Nini DAI[3*], Yuzhu CHEN[1,2], Gaonan LI[1,2], Xiaoang LI[1,2], Xiaolin JI[1,2], Xuemei ZHONG[4], Zailing LI[3✉], Liping DUAN[1,2✉]

[1]*Department of Gastroenterology, Peking University Third Hospital, Beijing 100191, China*
[2]*Beijing Key Laboratory for Helicobacter Pylori Infection and Upper Gastrointestinal Diseases, Beijing 100191, China*
[3]*Department of Pediatrics, Peking University Third Hospital, Beijing 100191, China*
[4]*Department of Gastroenterology, Children's Hospital Capital Institute of Pediatrics, Beijing, 100020, China*

Abstract: Objective: The risk factors and the role of mother-child gut microbiota in pediatric inflammatory bowel disease (PIBD) remain unclear. We aimed to explore the clinical risk factors associated with PIBD, analyze the gut microbiota characteristics of children and their mothers, and examine the correlation of microbial composition in mother-child pairs. Methods: We conducted a case-control study including children with PIBD and their mothers as the case group, as well as healthy children and their mothers as the control group. Questionnaires were used to collect information such as family illness history, and maternal and early-life events. Fecal samples were collected from the children and mothers for microbiota 16S rRNA sequencing, to analyze the composition and its potential association with PIBD. Results: A total of 54 pairs of cases and 122 pairs of controls were recruited. A family history of autoimmune disease and antibiotic use during pregnancy were associated with an increased risk of PIBD, and a higher education level of the father was associated with a decreased risk of PIBD. Children with IBD and mothers exhibited different gut microbiota compared to healthy children and mothers. Similarity was observed in the gut microbiota of mothers and children in the same groups. Some bacterial biomarkers of mothers discovered in this study had the power to predict PIBD in their offspring. Conclusion: PIBD is influenced by maternal risk factors and has unique gut microbiota characteristics. Mother-child gut microbiota are closely related, suggesting transmission and influence of gut microbiota between mothers and children. This study provides a potential pathogenesis of PIBD and a basis for developing targeted interventions.

Key words: Inflammatory bowel disease; Risk factors; Gut microbiota; Mother-child pairs

unedited

1 Introduction

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder of the gastrointestinal tract, including the two main forms, Crohn's disease (CD) and ulcerative colitis (UC). It is widely recognized that IBD is associated with a complex interplay between genetic susceptibility, environmental factors, and microbial dysbiosis, all of which contribute to dysregulated immune responses (Le Berre et al., 2023; Dolinger et al., 2024). IBD can occur at any age, with disease onset under 17 years defined as pediatric IBD (PIBD)

✉ Liping DUAN, duanlp@bjmu.edu.cn
Zailing LI, topbj163@sina.com
* The three authors contributed equally to this work
Cunzheng ZHANG, https://orcid.org/0009-0005-6119-574X;
Ruqiao DUAN, https://orcid.org/0000-0001-8496-0364;
Nini DAI, https://orcid.org/0000-0003-0429-1645;
Liping DUAN, https://orcid.org/0000-0001-6886-6888;
Zailing LI, https://orcid.org/0009-0004-6513-2431

Received June 25, 2024; Revision accepted Aug. 26, 2024;
Crosschecked xxx. xx, 20xx; Published online xxx. xx, 20xx

© Zhejiang University Press 2024

(Nameirakpam et al., 2020). Approximately 25% of patients with IBD have PIBD, and its incidence and prevalence continue to increase globally (Nameirakpam, et al., 2020; Kuenzig et al., 2022). Studies have shown that patients who develop IBD during childhood have more severe disease courses and a higher risk of complications compared with adult patients (Bouhuys et al., 2023). Therefore, it is imperative to identify the risk factors that may affect PIBD and to explore its pathogenesis.

Compared with adult IBD, PIBD is thought to be more influenced by maternal and early-life events, due to lower cumulative exposure to environmental factors after birth (Jezernik et al., 2020). Various prenatal, perinatal, and postnatal determinants have been reported that may modulate the risk of developing IBD, such as maternal diet, antibiotics exposure, delivery mode, feeding patterns, and weaning time (Piovani et al., 2019; Velosa et al., 2022). However, the specific mechanisms of these associations are still poorly understood, and one hypothesis is that the effects are mediated by alterations in the gut microbiota of both mothers and offspring.

It has been established that the gut microbiota exerts a significant influence on the occurrence and development of IBD. Multiple studies have reported alteration of microbial composition and presence of specific strains in stool and colon samples from patients with IBD (Pittayanon et al., 2020). There are many sources of the gut microbiota, and one important source is the maternal microbiota which is vertically transmitted to offspring, thus determining the first colonizers in the human digestive system (Wang et al., 2020). Mother-to-infant microbial transmission takes place from the beginning of pregnancy to a very long time after delivery and is influenced by various intrinsic and extrinsic factors, including a set of prenatal, perinatal, and postnatal factors mentioned above (Xiao and Zhao, 2023). Perturbations of a mother's gut microbiota may be associated with poor development of the offspring's immune system, leading to persistent and long-term susceptibility to certain diseases (Koren et al., 2024). However, little is known about whether microbiota-sharing between mothers and offspring persists over much longer periods of time, and whether it is associated with disease development. To the best of our knowledge, no studies have investigated the gut microbial compositions in IBD mother-child pairs and also evaluated correlations between them.

Consequently, the present study aimed to investigate the clinical risk factors linked to PIBD and to analyze the gut microbiota profiles of both affected children and their mothers. By adopting a case-control study design, we hoped to unravel the interplay between genetic, environmental, and microbial factors that contribute to the development of PIBD, and explore potential microbial biomarkers to predict IBD in children.

Unedited

2 Methods

2.1 Study design and population

This was a multicenter case-control study. Between July 2022 and December 2023, eligible children with a confirmed diagnosis of PIBD were recruited from pediatric outpatient clinics and wards of Peking University Third Hospital and Children's Hospital Capital Institute of Pediatrics in China. Controls were randomly selected from the same hospitals (individuals without PIBD or other gastrointestinal diseases).

The inclusion criteria were: (1) children under 17 years old; (2) a diagnosis of PIBD confirmed by doctors based on a combination of clinical, endoscopic, radiological, and histopathological criteria; and (3) mothers who cooperated with the questionnaire and signed an informed consent form (children over 8 years old also needed to sign an informed consent form). The exclusion criteria were: (1) children with any serious physical or mental illness other than PIBD; (2) mothers with any serious physical or mental illness during preconception, pregnancy, or lactation period; and (3) mothers or children with a history of antibiotic, probiotic, or prebiotic use within the previous 3 months.

2.2 Questionnaire and fecal-sample collection

The following information was extracted from both the case and control groups using standardized questionnaires administered by trained investigators: demographics such as age, gender, and living environment;

family-related factors like having siblings, passive smoke inhalation, family history of PIBD and autoimmune disease, and parental education level; as well as specific details related to the mother's preconception, pregnancy, or lactation periods, including medication history and any past illnesses. Additionally, we inquired about the early-life experiences of the children, focusing on feeding patterns and antibiotic use. For the case group, we also collected detailed information on their PIBD.

Fecal samples were collected from both children and their mothers in the case and control groups using Stool Nucleic Acid Collection and Preservation Tubes containing DNA preservation solution (Norgen, ON, Canada), after guidance by trained personnel. Following collection, the samples were immediately stored at a temperature of -80°C in a freezer.

2.3 Fecal 16S rRNA amplicon sequencing

After thawing the fecal samples, we extracted the total microbial DNA using the QIAamp PowerFecal Pro DNA Kit (Qiagen, NRW, Germany), and measured the DNA concentration. We amplified the V3-V4 hypervariable region of the bacterial 16S rRNA gene using primers 5'-CCTAYGGGRBGCASCAG-3' and 5'-GGACTACNNGGGTATCTAAT-3'. The PCR products were purified using magnetic beads and mixed at the same concentration. Finally, we detected the cDNA, and recovered the target bands.

2.4 Data statistics and fecal microbiota sequencing analysis

Descriptive statistics were used to summarize the general demographic characteristics of all participants. Continuous variables were reported as median and interquartile range [*M* (Q1, Q3)], and the Mann-Whitney U test was used to compare the two groups. Categorical variables were analyzed in terms of number and percentage (*n*, %), and the Chi-square test was used to compare the two groups. We conducted binary logistic regression analyses to identify risk factors associated with PIBD. Each variable underwent univariate analysis first, and the candidate variables with $P<0.05$ in univariate analyses were chosen for inclusion in the multivariate model. The results were expressed by odds ratios (OR) and 95% confidence intervals (95% CI). All statistical analyses were performed using SPSS software version 26.0 (SPSS Inc., IL, USA). Statistical significance was set at $P<0.05$.

After library construction and qualification, we performed sequencing using the NovaSeq6000 platform (Illumina, CA, USA). After trimming barcodes and primer sequences, we assembled the data using FLASH (V1.2.11) (Magoč and Salzberg, 2011), filtered it using fastp (V0.23.1) (Bokulich et al., 2013), and removed chimeras after comparison with the Silva database (Edgar et al., 2011). Next, denoising was performed using the DADA2 module of QIIME2 (V202202) software to generate amplicon sequence variants (ASVs). Species phylogeny and annotation were conducted using the QIIME2 software, with the Silva 138.1 database. Normalization was performed based on the sample with the lowest data volume, and α-diversity and β-diversity were calculated. We used the Wilcoxon test to examine α-diversity and intra-group β-diversity, and used PERMANOVA to test inter-group β-diversity. We then created a Venn diagram with a threshold of occurrence frequency >10% in each group. Linear discriminant analysis effect size (LEfSe) analysis was used to identify statistically different bacteria between the two groups, with a threshold of lg(LDA-score)>3 (Segata et al., 2011). Phylogenetic investigation of communities by reconstruction of unobserved states 2 (PICRUSt2) was used to predict metabolic pathways of gut microbiota (Douglas et al., 2020), which were then statistically analyzed using a non-paired T test. FEAST was employed to trace the sources of gut microbiota (Shenhav et al., 2019). Abundance matrices were plotted at the ASV level. We used the Spearman correlation test to calculate the correlation between different ASVs and clinical variables, and conducted random forest analysis using the random Forest (V4.7-1.1) R package. Receiver operating characteristic (ROC) curves were plotted using the pROC (V1.16.2) R package and cluster analysis was performed at the genus level to determine the gut type of each sample (Arumugam et al., 2011).

Unedited

3 Results

3.1 General demographic characteristics of the study population

A total of 176 mother-child pairs were recruited for the study, including 54 cases and 122 controls. Demographic characteristics of patients with PIBD and controls are shown in Table 1. In the case group, there were 18 UC (33.3%), 28 CD (51.9%), and 8 unclassified IBD cases (14.8%). There were no significant differences in age, gender, place of birth, or living area between the two groups. Table S1 presents the disease classification, severity, and laboratory-test results for the PIBD group.

3.1 Clinical risk factors for children with IBD

Variables associated with PIBD are shown in Table 2. Our univariate analyses revealed that a family history of autoimmune disease (unadjusted OR, 6.90; 95% CI, 1.75–27.14), antibiotic use in pregnancy (unadjusted OR, 10.44; 95% CI, 2.14–50.98), and antibiotic use during lactation (unadjusted OR, 6.12; 95% CI, 1.15–32.63) were all associated with an increased risk of PIBD. Conversely, a higher education level for either the mother (unadjusted OR, 0.45; 95% CI, 0.23–0.87) or the father (unadjusted OR, 0.31; 95% CI, 0.15–0.61) was correlated with a reduced PIBD risk. Upon incorporating these factors into our multivariate model, we determined that a family history of autoimmune disease (adjusted OR, 5.62; 95% CI, 1.21–26.10), antibiotic using during pregnancy (adjusted OR, 5.54; 95% CI, 1.01–30.41), and a higher education level of the father (adjusted OR, 0.34; 95% CI, 0.14–0.84) remained statistically significant factors.

unedited

Table 1 Demographic characteristics of the study population

	PIBD (*N* = 54)	Controls (*N* = 122)	*P* value
PIBD subtype			
CD	28 (51.9%)	–	
UC	18 (33.3%)	–	
IBDU	8 (14.8%)	–	
Age	12 (10.75–14)	11 (10–13)	0.060
Gender			0.941
Male	26 (48.1%)	58 (47.5%)	
Female	28 (51.9%)	64 (52.5%)	
Place of birth			0.308
Beijing	32 (59.3%)	82 (67.2%)	
Other	22 (40.7%)	40 (32.8%)	
Living area			0.964
Urban area	45 (83.3%)	102 (83.6%)	
Rural area	9 (16.7%)	20 (16.4%)	

PIBD, pediatric inflammatory bowel disease; CD, Crohn's disease; UC, ulcerative colitis; IBDU, IBD unclassified.

Table 2 Clinical risk factors for pediatric inflammatory bowel disease

Variables	PIBD (*N* = 54)	Controls (*N* = 122)	Unadjusted		Adjusted [a]	
			OR (95% CI)	*P* value	OR (95% CI)	*P* value
Personal history for children						
Antibiotic use	45 (83.3%)	112 (91.8%)	0.45 (0.17–1.17)	0.101		
Appendectomy or tonsillectomy	1 (1.9%)	3 (2.5%)	0.75 (0.08–7.36)	0.804		
Family-related factors						
Having siblings	26 (48.1%)	50 (41.0%)	1.34 (0.70–2.55)	0.377		
Passive smoke inhalation	22 (40.7%)	55 (45.1%)	0.84 (0.44–1.60)	0.593		
Family history of IBD	2 (3.7%)	0 (0%)	–	–		
Family history of autoimmune diseases	8 (14.8%)	3 (2.5%)	**6.90 (1.75–27.14)**	**0.006**	**5.62 (1.21–26.10)**	**0.028**
Education level of mother						
No degree	26 (48.1%)	36 (29.5%)	1			
Bachelor's degree or above	28 (51.9%)	86 (70.5%)	**0.45 (0.23–0.87)**	**0.018**	0.79 (0.32–1.94)	0.602
Education level of father						
No degree	26 (48.1%)	27 (22.1%)	1			
Bachelor's degree or above	28 (51.9%)	95 (77.9%)	**0.31 (0.15–0.61)**	**0.001**	**0.34 (0.14–0.84)**	**0.020**
Prenatal, perinatal and postnatal factors						
Preconception antibiotic use	2 (3.7%)	8 (6.6%)	0.55 (0.11–2.67)	0.457		
Preconception body mass index						
<18.5	5 (9.3%)	18 (14.8%)	1			
18.5–23.9	40 (74.1%)	89 (73.0%)	1.62 (0.56–4.66)	0.373		
≥24	9 (16.7%)	15 (12.3%)	2.17 (0.59–7.85)	0.242		
Antibiotic use in pregnancy	8 (14.8%)	2 (1.6%)	**10.44 (2.14–50.98)**	**0.004**	**5.54 (1.01–30.41)**	**0.049**
Age ≥35y at delivery	7 (13.0%)	15 (12.3%)	1.06 (0.41–2.78)	0.902		
Premature delivery or birth asphyxia	5 (9.3%)	3 (2.5%)	4.05 (0.93–17.60)	0.062		
Caesarean section	27 (50.0%)	59 (48.4%)	1.07 (0.56–2.03)	0.841		
Breastfeeding	48 (88.9%)	118 (96.7%)	0.27 (0.07–1.00)	0.051		
Refrigerated breast milk feeding	13 (24.1%)	44 (36.1%)	0.56 (0.27–1.16)	0.119		
Lactating antibiotic use	5 (9.3%)	2 (1.6%)	**6.12 (1.15–32.63)**	**0.034**	4.10 (0.66–25.24)	0.132
Postnatal depression or anxiety	3 (5.6%)	17 (13.9%)	0.36 (0.10–1.30)	0.119		

PIBD, pediatric inflammatory bowel disease; OR, odds ratios; CI, 95% confidence intervals
Statistically significant results were shown in bold ($P<0.05$)
[a] Variables with $P<0.05$ in univariate analyses were included in the multivariate model

unedited

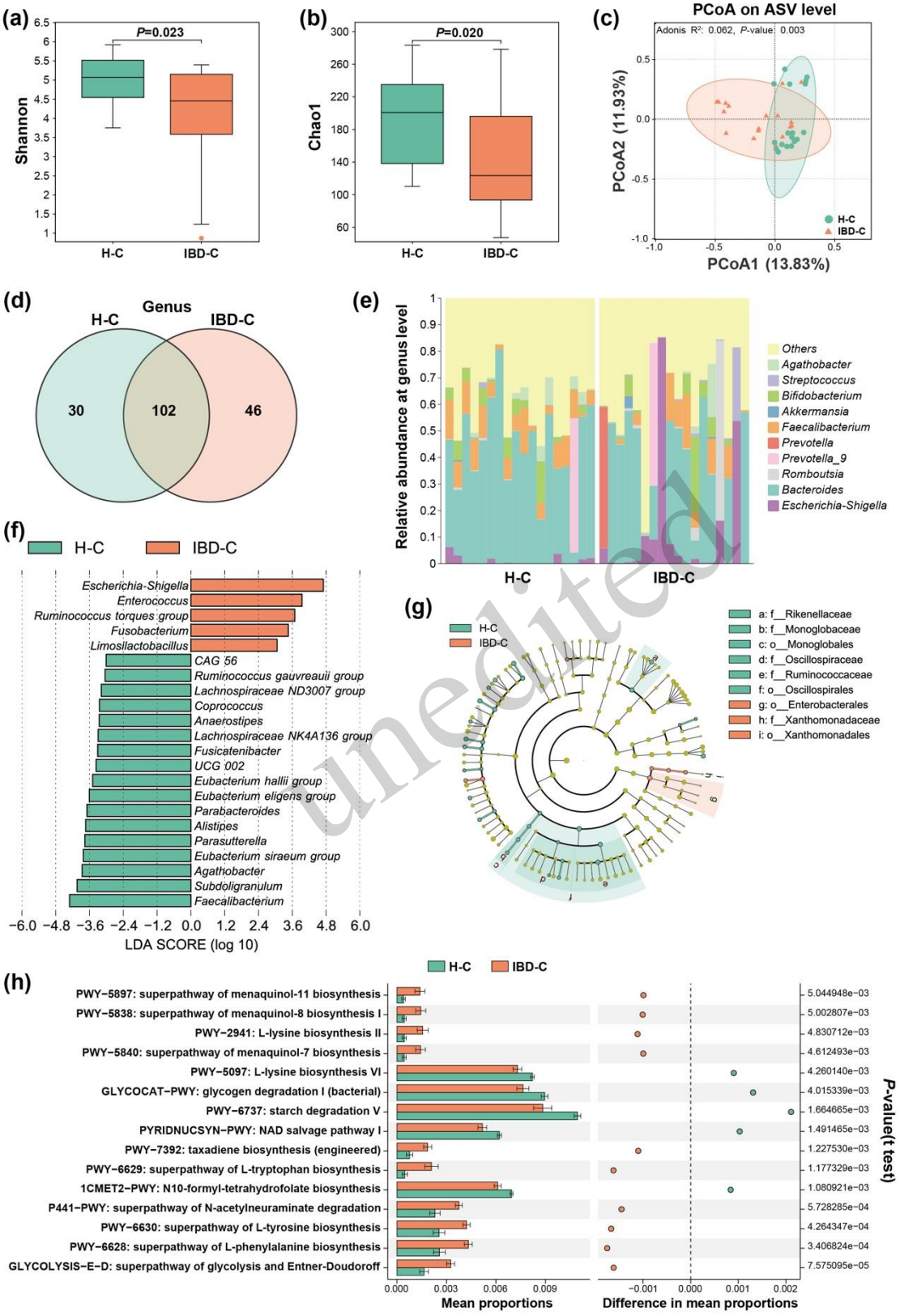
3.2 Differences in gut microbiota between children with IBD and healthy children

We randomly chose 18 mother-child pairs from both the case and control groups, and their fecal samples underwent 16S rRNA amplicon sequencing. In the subsequent results, we defined the groups as IBD-C (children with IBD), IBD-M (mothers of children with IBD), H-C (healthy children), and H-M (mothers of healthy children).

First, we compared the α-diversity of gut microbiota between the IBD-C and H-C groups, and found a significantly lower level of α-diversity in the IBD-C group, as shown by the Shannon index, Chao1 index, Observed ASVs, and Simpson index (Figs. 1a-1b, Fig. S1a). Principal Coordinates Analysis (PCoA) based on Bray-Curtis distance revealed that the gut microbiota of the IBD-C and H-C groups could be clearly distinguished (Fig. 1c), and the IBD-C group exhibited higher β-diversity within the group (Fig. S1b), indicating lower similarity between samples. Furthermore, we conducted LEfSe analyses at the phylum, genus, and ASV levels to explore differences between the two groups. Among eight shared phyla, Proteobacteria and Fusobacteriota were significantly enriched in the IBD-C group (Figs. S1c-S1e). Among 102 shared genera, the IBD-C group showed the highest LDA effect sizes for *Escherichia-Shigella* and *Enterococcus*, while the H-C group exhibited the highest LDA effect sizes for *Faecalibacterium* and *Subdoligranulum* (Figs. 1d-1g). Observations at the ASV level indicated that, besides some differing genera, potential species with alleviating effects on IBD such as *Bacteroides* (species unknown 1), *Bifidobacterium adolescentis*, and *Blautia obeum* showed significantly lower abundance in the IBD-C group (Figs. S1f-S1g). Further prediction of functional differences in gut microbiota between the two groups using PICRUSt2 revealed a series of different metabolic pathways. The Entner-Doudoroff pathway and certain amino acid synthesis pathways were found to be enriched in the IBD-C group, while pathways such as NAD salvage and N10-formyl-tetrahydrofolate synthesis were depleted. Additionally, pathways involved in the synthesis of potentially pro-inflammatory compounds, such as menaquinol and taxadiene (a precursor of paclitaxel), were enriched in the gut microbiota of the IBD-C group (Fig. 1h).

We also conducted a comparison of the gut microbiota between children with CD (CD-C) and UC (UC-C) subtypes, which revealed no significant differences in α-diversity, community composition, or β-diversity between these two groups (Figs. 2a-2c, Figs S2a-S2f). Both CD-C and UC-C were notably distinct from the healthy controls (H-C) (Fig. 2c). Nonetheless, the LEfSe analysis for the IBD subtypes showed that the *Sutterella* and *Ruminococcus torques group* exhibited the highest LDA effect size in CD-C, whereas the *Ruminococcus gnavus group* and *Veillonella* had the highest LDA effect size in UC-C (Fig. 2d, Fig. S2g). Moreover, to account for potential differences between pediatric patients with active and remitting phases of IBD, we compared the gut microbiota of children with active IBD (IBD-C-A), children with remissive IBD (IBD-C-R), and those with H-C. The results indicated that the α-diversity in IBD-C-A was significantly less compared to H-C, while no significant differences were observed between IBD-C-R and H-C (Fig. 2e, Fig. S2h). The stacked bar plot results demonstrated that the gut microbiota composition of IBD-C-R was more similar to H-C than to IBD-C-A (Figs. 2f-2g). PCoA analysis revealed a significant difference between IBD-C-A and H-C, with a higher explained variance than IBD-C-R (Fig. 2h). The β-diversity of IBD-C-A was significantly higher than that of the other two groups (Fig. S2i). At the genus level, the LEfSe analysis results indicated enrichment of *Faecalitalea*, *Flavonifractor*, and *Colidextribacter* in the IBD-C-R group, while *Streptococcus* and *Clostridium sensu stricto 1* were enriched in the IBD-C-A group (Figs. 2i-2g).

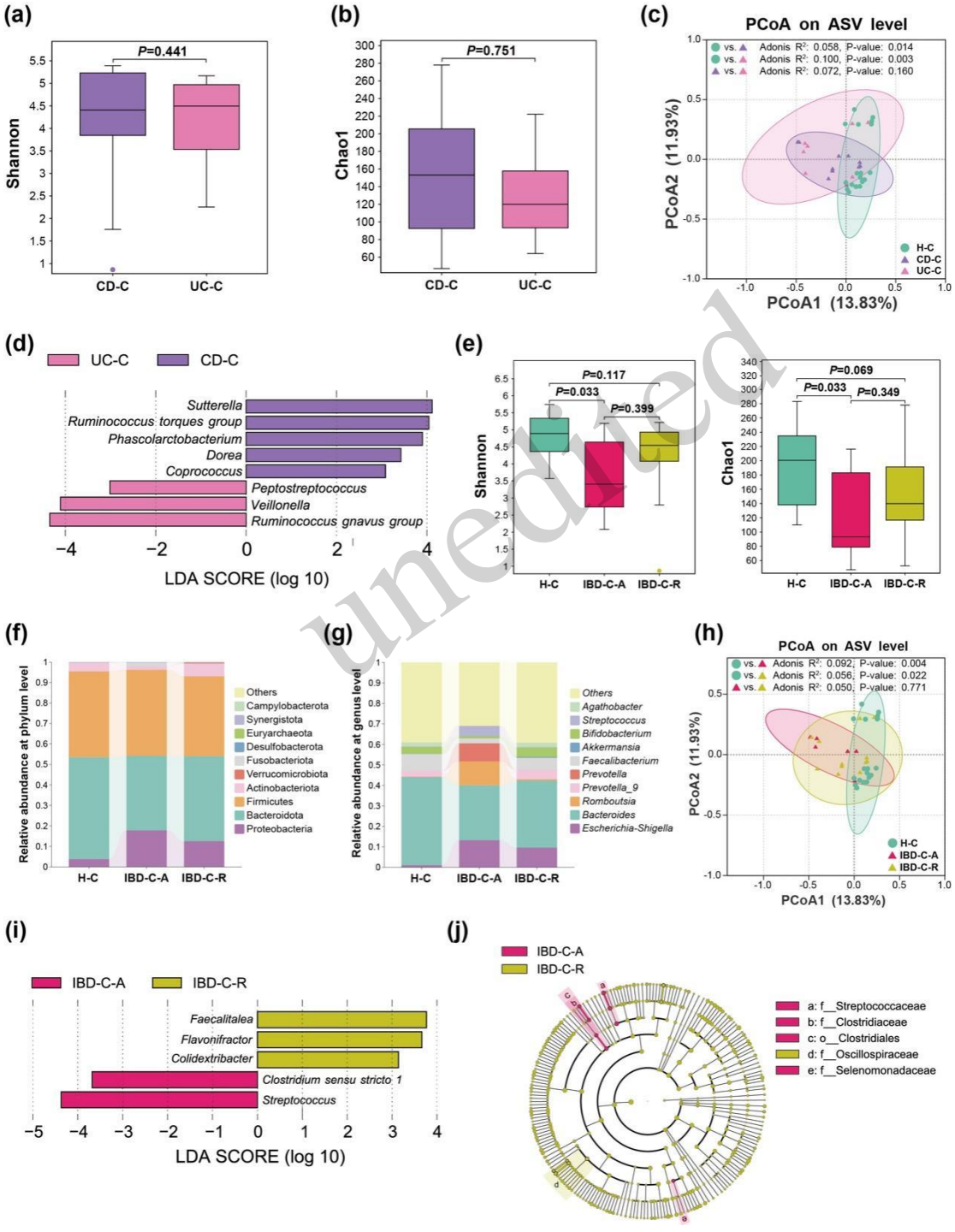
Unedited



unedited

Fig. 1 Characteristics and functions of the gut microbiota in children. (a) Shannon index of gut microbiota in healthy children (H-C) and children with inflammatory bowel disease (IBD-C). (b) Chao1 index of gut microbiota in the two

groups. (c) Principal Coordinates Analysis (PCoA) of gut microbiota in the two groups based on Bray-Curtis distance, with ellipses representing 95% confidence intervals. (d) Venn diagram at the genus level showing overlapping and unique components in the two groups. (e) Stacked bar chart of the top 10 abundant genera. (f) Different genera with LDA scores (Log10)>3 and *P*<0.05 in the two groups. (g) Cladogram of different genera in the two groups, with each ring representing a taxonomic level (from phylum to genus) and the diameter of each circle representing its relative abundance. (h) Relative abundance (with 95% confidence intervals) of the top 15 different functional pathways in the gut microbiota of the two groups. Error bar indicates standard deviation.



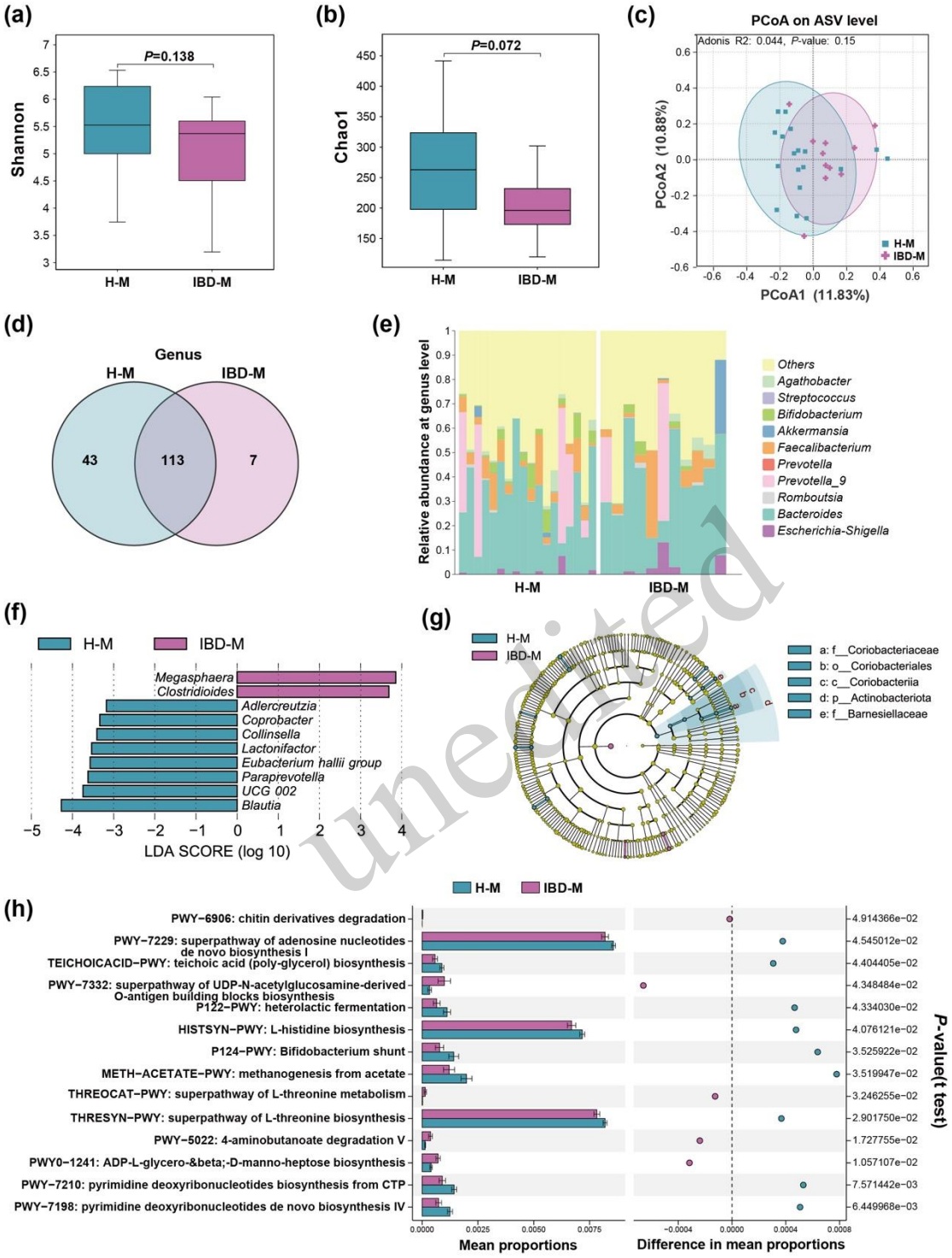
unedited

Fig. 2 Gut microbiota characteristics of children with IBD (for different subtypes or disease stages). (a). Shannon index of gut microbiota in children with CD (CD-C) and children with UC (UC-C). (b). Chao1 index of gut microbiota in both groups. (c). Principal Coordinates Analysis (PCoA) of gut microbiota in CD-C, UC-C, and healthy children (H-C) based on Bray-Curtis distance, with ellipses representing 95% confidence intervals. (d). Different genera with LDA scores (Log10)>3 and *P*<0.05 in CD-C and UC-C. (e). The Shannon and Chao1 indexes of gut microbiota in healthy children (H-C) and children with active IBD (IBD-C-A) or remitting IBD (IBD-C-R). (f). Stacked bar chart of the top 10 abundant phyla. (g). Stacked bar chart of the top 10 abundant genera. (h). PCoA of gut microbiota in IBD-C-A, IBD-C-R, and H-C based on Bray-Curtis distance, with ellipses representing 95% confidence intervals. (i). Different genera with LDA scores (Log10)>3 and *P*<0.05 in the IBD-C-A and IBD-C-R groups. (j). Cladogram of different genera in the IBD-C-A and IBD-C-R groups, with each ring representing a taxonomic level (from phylum to genus) and the diameter of each circle representing its relative abundance.

3.3 Differences in gut microbiota between mothers of children with IBD and mothers of healthy children

We further examined the gut microbiota composition in mothers of children with IBD and healthy controls. The α-diversity of gut microbiota showed no significant differences between the IBD-M and H-M groups in the Shannon index, Chao1 index, Observed ASVs, or Simpson index, although there was a lower trend in the IBD-M group (Figs. 3a-3b, Fig. S3a). PCoA based on Bray-Curtis distance revealed that the gut microbiota of the IBD-M and H-M groups were not significantly distinguishable (Fig. 3c). The IBD-M group exhibited higher β-diversity within the group, as calculated by Bray-Curtis distance and Weighted Unifrac distance (Fig. S3b). LEfSe analyses indicated some significant differences at the phylum, genus, and ASV levels. Among 10 shared phyla, the abundance of Actinobacteriota was significantly reduced in the IBD-M group (Figs. S3c-S3e). Among the 113 shared genera, *Megasphaera* and *Clostridioides* were predominantly higher in the IBD-M group, whereas *Blautia* and *UCG-002* (belonging to Oscillospiraceae) were more prevalent in the H-M group (Figs. 3d-3g). At the ASV level, species like *Bacteroides dorei*, *Parabacteroides merdae*, *Blautia* (spices unknown), and *Bifidobacterium longum* were significantly diminished in the IBD-M group, while species like *Bacteroides fragilis* and *Clostridiales bacterium CCNA10* were significantly enriched (Figs. S3f-S3g). Furthermore, using PICRUSt2 to predict functional differences in gut microbiota between the two groups revealed that pathways enriched in the IBD-M group were often those involved in degradation pathways of organic compounds, such as the 4-aminobutanoate (GABA) and chitin derivatives, while synthesis pathways of certain amino acids and nucleotides were depleted (Fig. 3h).

Unedited



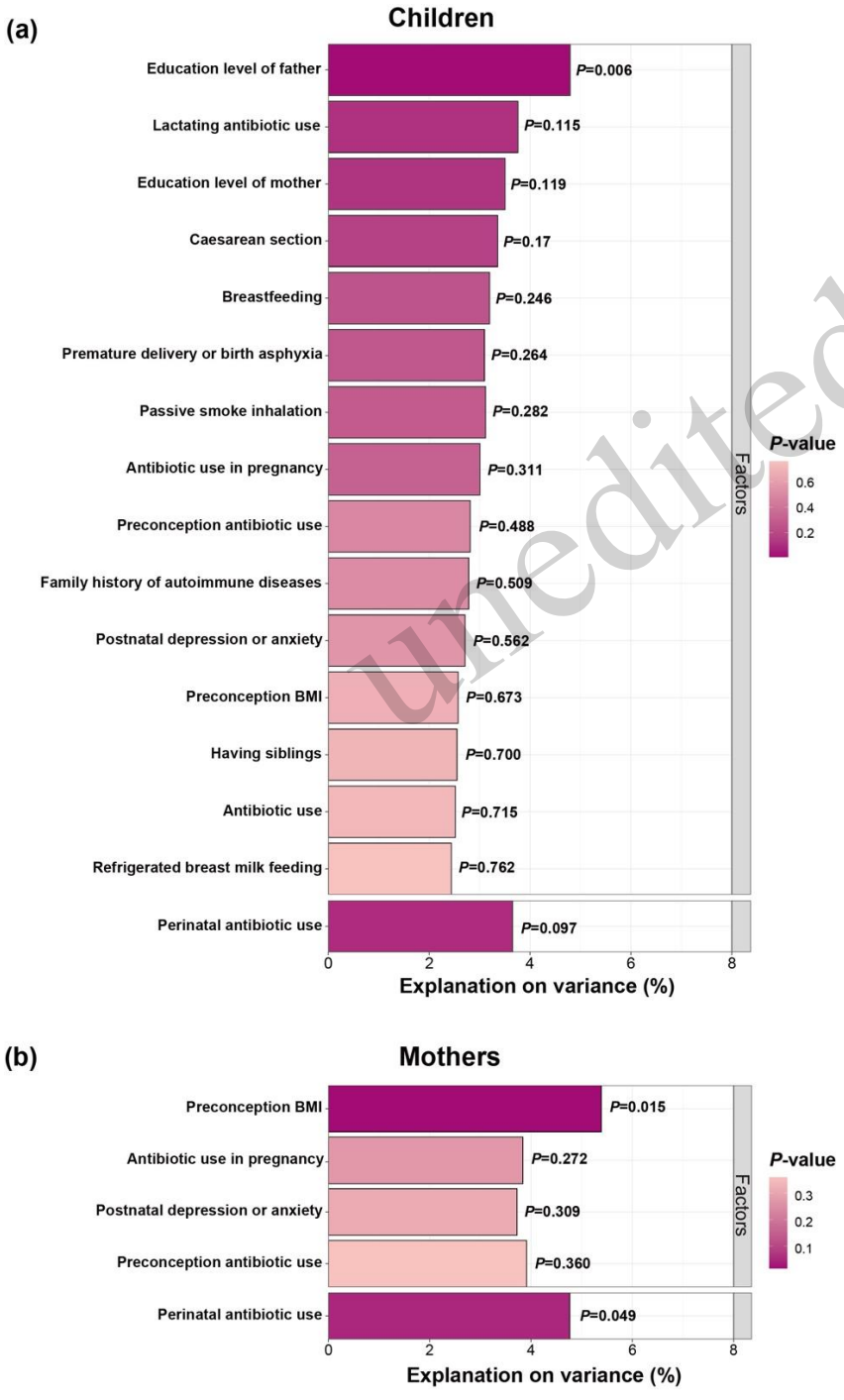
unedited

Fig. 3 Characteristics and functions of the gut microbiota in mothers. (a). Shannon index of gut microbiota in mothers of healthy children (H-M) and mothers of children with inflammatory bowel disease (IBD-M). (b). Chao1 index of gut microbiota in the two groups. (c). Principal Coordinates Analysis (PCoA) of gut microbiota in the two groups based on Bray-Curtis distance, with ellipses representing 95% confidence intervals. (d). Venn diagram at the genus level, showing overlapping and unique components in the two groups. (e). Stacked bar chart of the top 10 abundant genera. (f). Different genera with LDA scores (Log10)>3 and *P*<0.05 in the two groups. (g). Cladogram of different genera in the two groups, with each ring representing a taxonomic level (from phylum to genus) and the diameter of each circle representing its relative abundance. (h). Relative abundance (with 95% confidence intervals) of the top 15 different functional pathways in

the gut microbiota of the two groups. Error bar indicates standard deviation.

3.4 Clinical risk factors explain the variation in children's and mothers' gut microbiota

Through PERMANOVA analysis, we found that the father's education level exerted the most significant influence on children's gut microbiota. Moreover, perinatal maternal antibiotic use, encompassing preconception, pregnancy, and lactating periods, potentially contributed to variations in children's gut microbiota (Fig. 4a). Results from PERMANOVA analysis indicated that preconception body mass index (BMI) and perinatal antibiotic use significantly influenced the variations in mothers' gut microbiota (Fig. 4b).



Unedited

Fig. 4 Explanation of the variation in children's and mothers' gut microbiota by clinical risk factors. (a). PERMANOVA analyses were used to calculate the proportion of variation in children's gut microbiota explained by perinatal and family risk factors, based on Bray-Curtis distance. Specifically, maternal perinatal antibiotic use was integrated as a variable in the analysis. (b). PERMANOVA analyses were used to calculate the proportion of variation in mothers' gut microbiota explained by perinatal risk factors, based on Bray-Curtis distance. Specifically, maternal perinatal antibiotic use was integrated as a variable in the analysis.

3.5 Enterotype analysis of mother-child pairs in the case and control groups

In addition, the gut microbiota of both children and mothers were analyzed for enterotypes, resulting in classification of the four groups into two enterotypes (Fig. 5a). Enterotype 1 was distinguished by a high abundance of *Bacteroides* and *Faecalibacterium*, whereas Enterotype 2 featured a predominance of *Escherichia-Shigella* and *Enterococcus* (Figs. 5b-5c). Notably, only the IBD-C group exhibited both enterotypes. Among children with IBD presenting Enterotype 2, 40% had mothers with a history of perinatal antibiotic exposure, compared to just 16.13% among those presenting as Enterotype 1; and among PIBD sufferers with Enterotype 1, 64.52% of the fathers had a Bachelor's degree or above, while no fathers had Bachelor's degree or above in children with Enterotype 2. This further indicates that mothers' perinatal antibiotic exposure and familial factors may influence the development of PIBD by altering the gut microbiota of children.

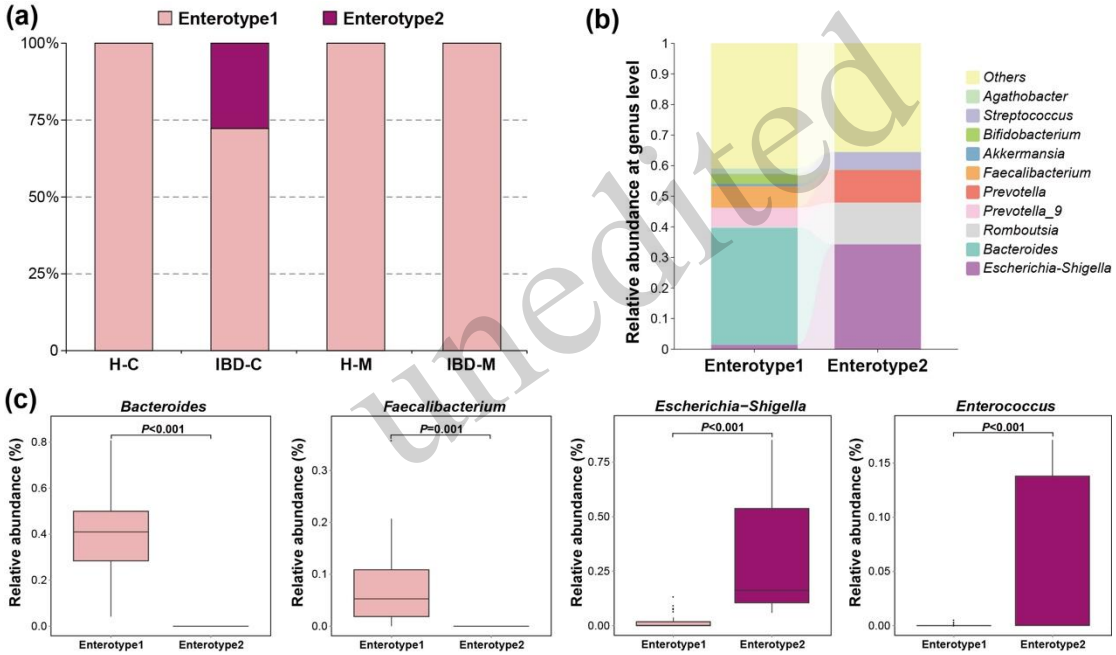


Fig. 5 Enterotype analysis of the four groups. (a). Gut microbiota of healthy children (H-C) and their mothers (H-M), as well as children with inflammatory bowel disease (IBD-C) and their mothers (IBD-M) could be classified into two enterotypes. (b). Stacked column plots at the genus level for Enterotype 1 and Enterotype 2. (c). Representative genus with the highest abundance for Enterotype 1 and Enterotype 2.

3.6 Gut microbiota characteristics of mother-child pairs exhibited a higher degree of similarity

To investigate the similarity of gut microbiota between mothers and their children, we first used a Venn diagram for observation and found that the number of shared ASVs among mothers and children in the same group surpassed that of mothers and children from different groups (Fig. 6a). Utilizing FEAST for microbiota tracing, we discovered that 89.11% of ASVs for children in the H-C group possibly originated from their mothers, whereas 57.80% of ASVs for children in the IBD-C group possibly originated from their mothers (Fig. 6b). Notably, despite the fact that intergenerational microbiota transmission was lower in the case group compared to the control group, nearly all high-abundance strains were effectively shared between mothers and children in both groups (Fig. 6c). There were also no significant differences in gut microbiota similarity be-

unedited

tween mother-child pairs in the IBD-C group and the H-C group (Fig. 6d). The overall microbial composition exhibited significantly greater similarity between mothers and their children than between mothers and unrelated children, as evidenced by metrics such as Bray-Curtis distance, Jaccard distance, and Weighted UniFrac distance. Additionally, the overall microbial similarity between mothers was significantly higher than that between children (Fig. 6e).

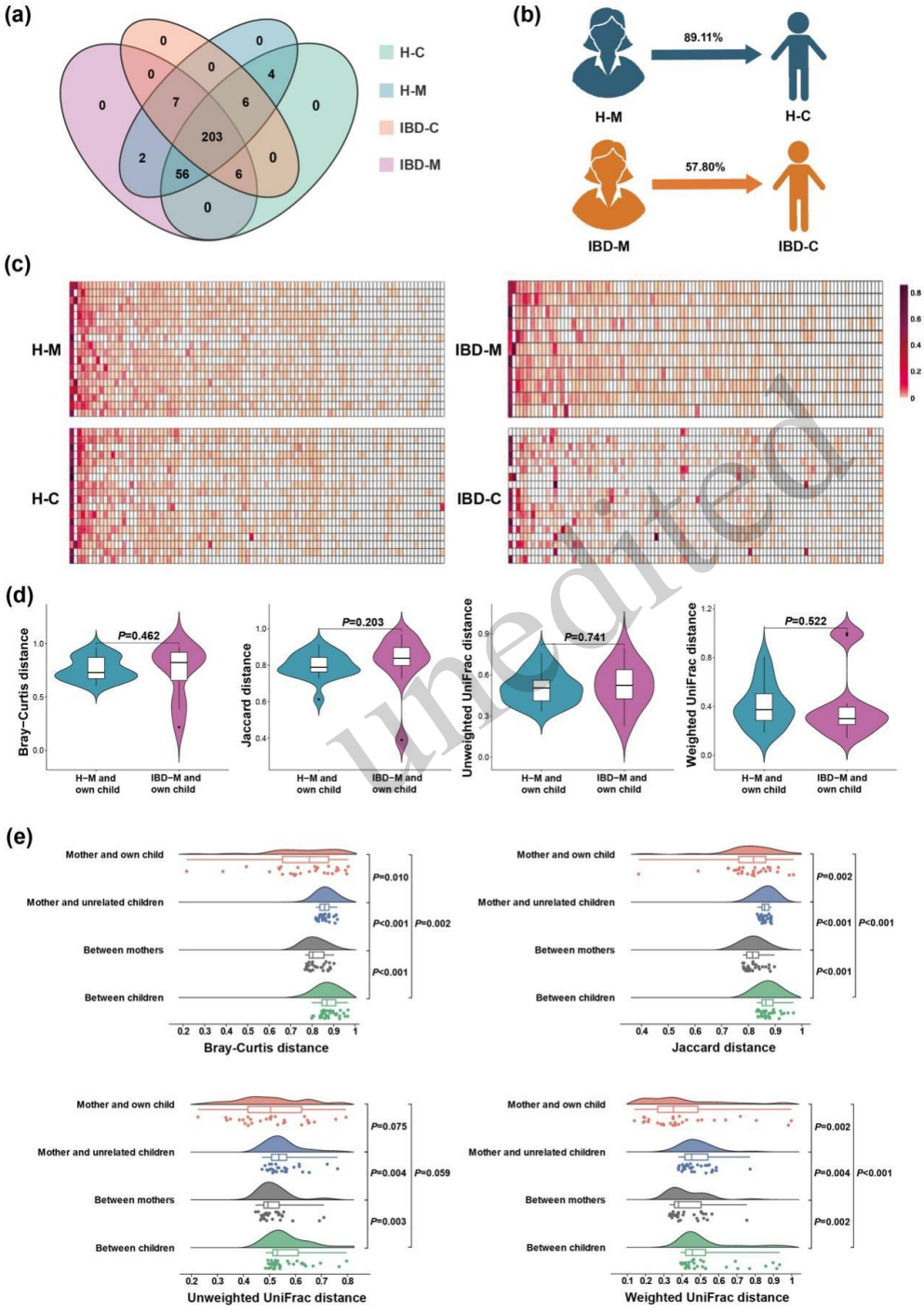
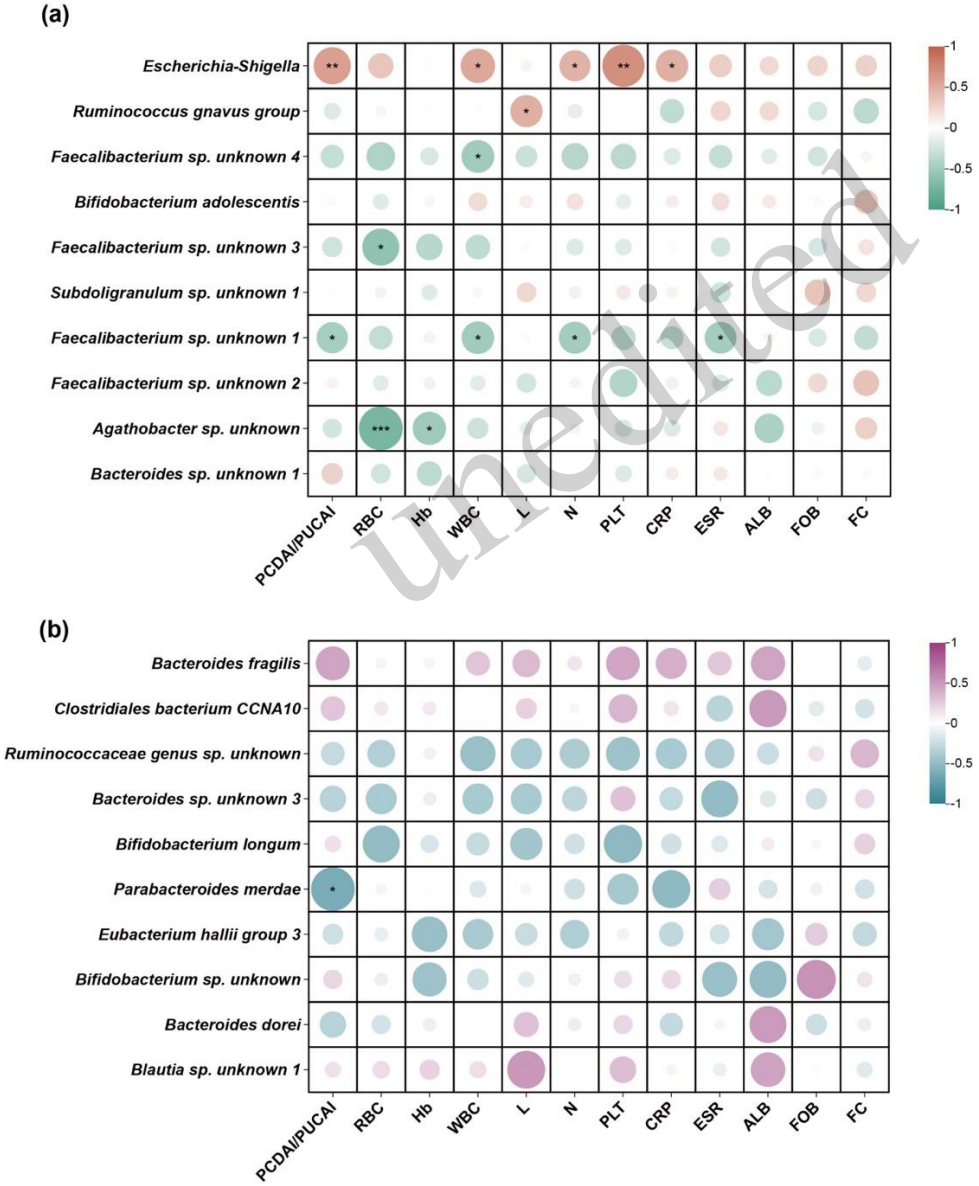


Fig. 6 Degree of similarity between mothers and children. (a). Venn diagram at the ASV level showing overlapping and unique components between healthy children (H-C) and their mothers (H-M), as well as between children with inflammatory bowel disease (IBD-C) and their mothers (IBD-M). (b). FEAST analysis indicating the transmission ratio of gut microbiota between mothers and children within the same group. (c). Similarity of gut microbiota between mothers and

children at the ASV level. (d). Comparison of Bray-Curtis, Jaccard, Unweighted UniFrac, and Weighted UniFrac distances between H-M and IBD-M mothers and their own children. (e). Comparison of Bray-Curtis, Jaccard, Unweighted UniFrac, and Weighted UniFrac distances between mothers and their own children, mothers and unrelated children, and all mothers and all children.

3.7 Gut microbiota of mothers had biomarkers for PIBD

We performed Spearman correlation analyses between the top 10 differentially abundant ASVs of children's and mothers' gut microbiota and clinical variables. The differential ASVs in children were significantly correlated with multiple clinical indicators. Overall, PCDAI/PUCAI showed a positive correlation with *Escherichia-Shigella* and a negative correlation with *Faecalibacterium* (species unknown 1) (Fig. 7a). In mothers, the differential ASVs of gut microbiota showed a negative correlation between PCDAI/PUCAI and *Parabacteroides merdae* (Fig. 7b).



Uncorrected

Fig. 7 Correlation between gut microbiota of children and mothers and clinical indicators. (a). Correlation between the top 10 differentially abundant ASVs in children and clinical indicators. (b). Correlation between the top 10 differentially abundant ASVs in mothers and clinical indicators. PCDAI, pediatric Crohn's disease activity index; PUCAI, pediatric

ulcerative colitis activity index; RBC, red blood cell; Hb, hemoglobin; WBC, white blood cell; L, lymphocyte; N, neutrophil; PLT, platelet; CRP, C-reactive protein, ESR, erythrocyte sedimentation rate; ALB, albumin; FOB, fecal occult blood; FC, fecal calprotectin. *P<0.05, **P<0.01, ***P<0.001.

Given the correlation between the children's and mothers' gut microbiota and clinical indicators of children's disease, we were further able to identify potential biomarkers for distinguishing between the IBD-C and H-C groups using a random forest approach. In the gut microbiota of children, the top five ASVs with the highest mean decrease in the Gini index were *Lachnospiraceae bacterium GAM79*, *Blautia obeum*, *Blautia* (species unknown 3), *Escherichia-Shigella*, and *Bacteroides* (species unknown 1) (Fig. 8a). In the gut microbiota of mothers, the top five ASVs with the highest mean decrease in the Gini index were identified as *Blautia* (species unknown 1), Ruminococcaceae (genus species unknown), *Bacteroides fragilis*, *Eubacterium hallii group 3*, and Enterobacteriaceae (genus species unknown) (Fig. 8b).

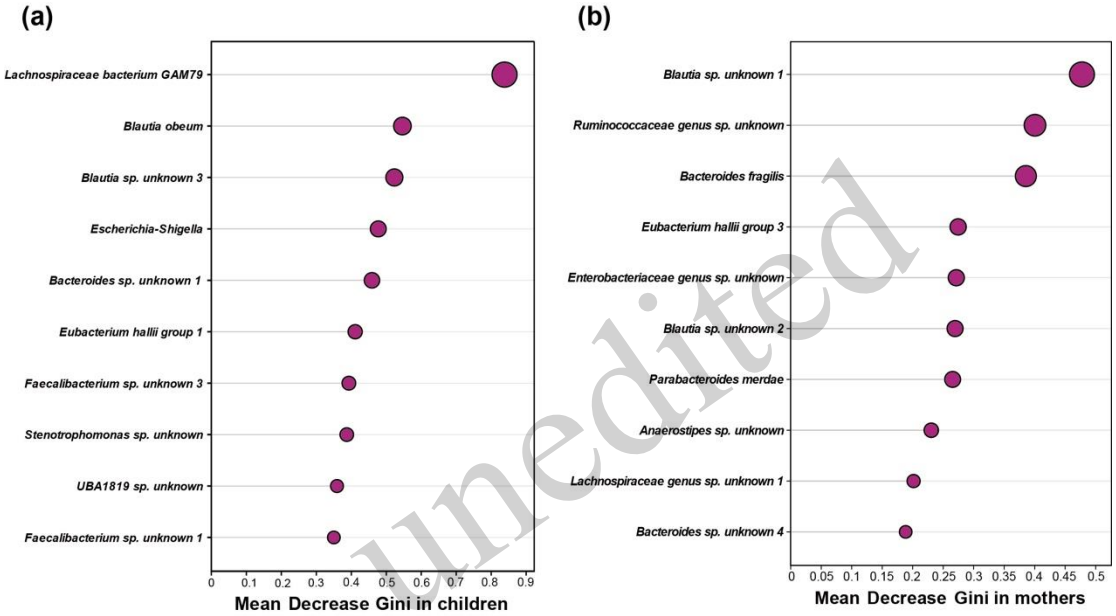
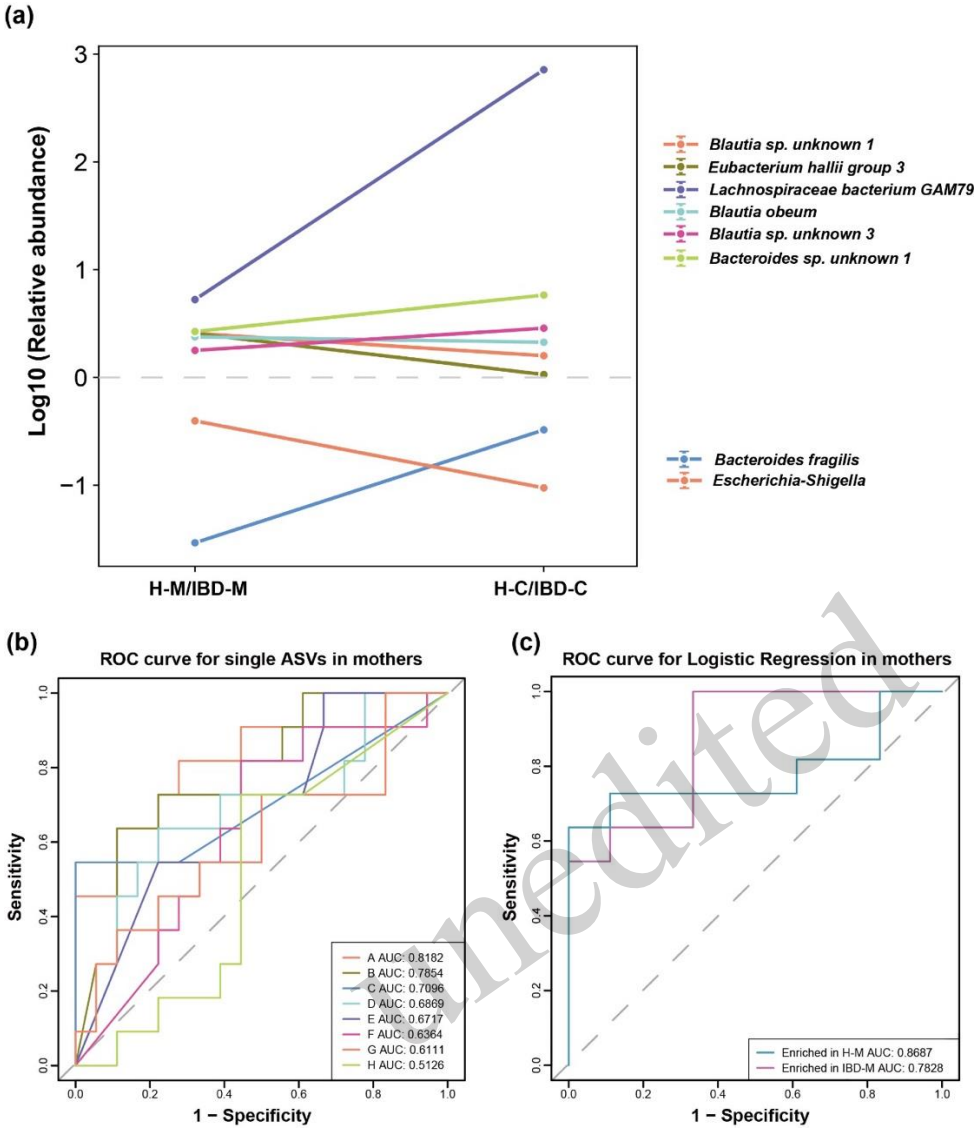


Fig. 8 Random forest analysis of the gut microbiota of children and mothers. (a). ASVs identified by the random forest method to distinguish healthy children (H-C) from children with inflammatory bowel disease (IBD-C). (b). ASVs identified by the random forest method to distinguish mothers of healthy control children (H-M) from mothers of children with inflammatory bowel disease (IBD-M).

Next, we compared the abundance of the top five potential bacterial biomarkers with the highest mean decrease in the Gini index in the gut microbiota of children and mothers. Although there was less overlap in potential biomarkers between children and mothers, eight of ten biomarkers showed a consistent fold-change of abundance (Fig. 9a), suggesting that mothers' gut microbiota may influence the development of PIBD in offspring by modulating the abundance of certain bacteria. To investigate this, we constructed ROC curves that would show the ability of these eight bacterial biomarkers in mothers' microbiota to distinguish between the IBD-C and H-C groups; the highest value for the area under the curve (AUC) was 0.8182 (Fig. 9b). After performing logistic regression on the biomarkers enriched in the H-M group, including *Blautia* (species unknown 1), *Eubacterium hallii group 3*, *Blautia obeum*, *Lachnospiraceae bacterium GAM79*, *Blautia* (species unknown 3), and *Bacteroides* (species unknown 1), as well as the biomarkers enriched in the IBD-M group (*Bacteroides fragilis* and *Escherichia-Shigella*), the ROC curve exhibited an AUC of 0.8687 for the H-M enriched biomarkers and an AUC of 0.7828 for the IBD-M enriched biomarkers (Fig. 9c).

Unedited



Unedited

Fig. 9 Biomarkers in the gut microbiota of mothers. (a). We selected the top 5 biomarkers with the highest Mean Decrease Gini in the gut microbiota of children and mothers separately, resulting in a total of 10 biomarkers. These biomarkers were further analyzed using the following approach: the average abundance of the biomarkers in mothers of healthy children (H-M) was compared to that in mothers of children with inflammatory bowel disease (IBD-M) by calculating the ratio. Subsequently, a logarithmic transformation was applied which positioned the biomarkers enriched in the IBD-M group below the x-axis, while the depleted biomarkers were positioned above the x-axis. The same transformation was performed for healthy children (H-C) and children with inflammatory bowel disease (IBD-C). If both values of a biomarker are located on the same side of the x-axis, this indicates that the biomarker is simultaneously enriched/depleted in the IBD-C and IBD-M groups. Eight biomarkers with consistent change trends are presented. (b). Eight biomarkers were selected and ROC curves were used to predict the risk of pediatric inflammatory bowel disease (PIBD) in children. A: *Blautia sp. unknown 1*; B: *Eubacterium hallii group 3*; C: *Bacteroides fragilis*; D: *Blautia obeum*; E: *Lachnospiraceae bacterium GAM79*; F: *Blautia sp. unknown 3*; G: *Escherichia−Shigella*; H: *Bacteroides sp. unknown 1*. (c). Logistic regression analysis was conducted for biomarkers enriched in the H-M and IBD-M groups separately, to evaluate their predictive ability for the risk of PIBD in children.

4 Discussion

To the best of our knowledge, this is the first study that explores the gut microbiota composition of both children with IBD and their mothers, along with their intergenerational correlation. Our findings reveal that a family history of autoimmune disease and antibiotic use in pregnancy are positively associated with PIBD risk, while a higher education level of the father is protective. IBD-C exhibits reduced α-diversity compared to H-C, with enrichment of Proteobacteria, Fusobacteriota, *Escherichia-Shigella*, and *Enterococcus*, and depletion of *Faecalibacterium*. IBD-M shows an increased abundance of *Clostridioides* and reduced levels of *Bacteroides dorei* and *Parabacteroides merdae*. Functional alterations in gut microbiota were also observed in both children with IBD and their mothers. The gut microbiota in the IBD-C group exhibits two enterotypes. Intriguingly, the gut microbiota structures of mothers and children within the same group exhibit greater similarity. Some potential bacterial biomarkers of mothers discovered in this study, such as *Bacteroides fragilis* and *Eubacterium hallii*, demonstrate the ability to predict PIBD in offspring.

Exploring risk factors for PIBD is paramount not only for understanding its complex pathogenesis but also for identifying individuals at risk who may benefit from early intervention. In a large meta-analysis assessing the impact of different types of prenatal, perinatal, and postnatal exposure on IBD, researchers identified prenatal antibiotic exposure, tobacco smoke, and early life otitis media as risk factors associated with IBD, and breastfeeding as a protective factor for IBD (Agrawal et al., 2021). Our findings indicate that a family history of autoimmune disease is associated with an increased risk of PIBD, consistent with Mari's finding that first-degree relatives of patients with IBD have a higher risk of autoimmune disease (Mari et al., 2020), which partly reflects the role of family genetic predisposition in the development of IBD. In our study, antibiotic use during pregnancy is shown to be another risk factor for PIBD. Our previous meta-analysis demonstrated that antibiotic exposure is associated with an increased risk of PIBD, and that exposure in the first year of life leads to a relatively higher risk estimate (Duan et al., 2024). Furthermore, antibiotic exposure in pregnancy has been reported to affect the risk of PIBD in offspring. In a Swedish cohort study, antibiotics during pregnancy were associated with very-early-onset IBD in children (Örtqvist et al., 2019). Another nationwide study from Denmark found that exposure to ≥3 courses of antibiotics during pregnancy increased IBD risk in offspring (Agrawal et al., 2023). It is biologically plausible that antibiotics can change the mother's gut microbiota, which in turn affects the offspring's gut microbiota and immune-system development, increasing their susceptibility to IBD. A higher education level of the father appears to be a protective factor for PIBD, and education level is often related to other factors that may affect IBD, such as diet, family income, and social living environment (Krishna et al., 2020).

Previous studies have indicated that PIBD patients exhibit alterations in their gut microbiota (Wang et al., 2021; D'adamo et al., 2023). In our study, we observed significant changes in the gut microbiota of children with IBD, particularly in the population of Proteobacteria and Fusobacteriota, which were prominently enriched in the IBD-C group. Proteobacteria comprises various pathogenic microbiota such as *Escherichia coli*, *Escherichia-Shigella*, *Vibrio cholerae*, and *Pseudomonas aeruginosa*, often found to be enriched in individuals with IBD (Mukhopadhya et al., 2012). Notably, enrichment of Fusobacteriota, primarily attributed to *Fusobacterium*, is strongly associated with colitis and colorectal cancer pathogenesis (Liu et al., 2020; Chen et al., 2023). Moreover, we discovered that the IBD-C group displayed enriched synthesis pathways for menaquinol and taxadiene, which may contribute to the onset of inflammation (Khorsand et al., 2022; Iwamuro et al., 2023). Conversely, the IBD-C group demonstrated depleted N10-formyl-tetrahydrofolate biosynthesis, which has been shown to have anti-inflammatory effects(Pathikkal et al., 2022). We also discovered that the community composition of IBD-C-R and H-C are more similar than that of IBD-C-A and H-C, and the composition of IBD-C-R is enriched with *Clostridium sensu stricto 1*, which has been demonstrated to influence IBD activity (Leibovitzh et al., 2022), aligning with previous research findings (Chen et al., 2024).

Consistent with our hypothesis, the gut microbiota of IBD-M exhibit substantial differences compared to H-M, despite both groups being composed of healthy individuals. IBD-M is characterized by an increased

Unedited

abundance of opportunistic pathogens like *Clostridioides* (Dong et al., 2023), while beneficial bacteria such as *Bacteroides dorei* (Sun et al., 2023) and *Parabacteroides merdae* (Qiao et al., 2022) are significantly diminished. The functional pathways enriched in IBD-M are associated with inflammatory compounds, including chitin derivatives (Lee et al., 2014) and O-antigen (Lehri et al., 2024), which are known to be components of pathogenic bacteria. Derivatives of GABA can promote colitis through the GABA receptor A (Wang et al., 2022). The involvement of these specific bacteria in these functional pathways suggests vertical transmission that influences the development of PIBD in offspring. Additionally, we found that the factors contributing to the differences in gut microbiota between children and mothers may stem from the perinatal period or from certain familial factors, such as the education level of the father and perinatal maternal antibiotic use. These findings align with our analyses of risk factors for PIBD, indicating that these factors likely participate in PIBD development through their impact on the gut microbiota.

We further identified two distinct Enterotypes within the IBD-C group. Enterotype 1 is characterized by a dominance of *Bacteroides*, while Enterotype 2 is characterized by a dominance of *Escherichia-Shigella*. It has been demonstrated that the *Bacteroides*-Enterotype in children with PIBD is associated with disease remission, whereas the *Escherichia-Shigella*-Enterotype is associated with disease progression (Chen, et al., 2024). Additionally, the IBD-C group exhibited elevated levels of *Enterococcus*, which can impede intestinal stem-cell proliferation through the production of tyramine (Li et al., 2024). Interestingly, within Enterotype 2, there is a higher proportion of maternal perinatal antibiotic use history and fathers with no degree. These findings provide supportive evidence for the underlying factors contributing to the variation observed in this Enterotype.

Furthermore, we analyzed the association between the gut microbial composition of children and their mothers, and the results show that there are high similarities in fecal microbiota of mother-child pairs, especially in the control group. This may be the result of a combined effect of long-term maternal microbial imprinting throughout pregnancy and microbial transmission driven by shared social environment later in life (Valles-Colomer et al., 2023). The mother-offspring vertical transmission of microbiota is the earliest and most important factor influencing microbial structure in infants (Asnicar et al., 2017). Based on metagenomic profiling of the microbiome from 25 mother-infant pairs across multiple body sites, Ferretti et al. found that the maternal gut was the largest source of colonizing microbiota in the gastrointestinal tract of healthy infants (Ferretti et al., 2018). The large number of strains shared at birth confirms maternal microbiome seeding of the infant's gut, and in addition, strain-sharing remains significant in senior individuals, with one study showing that non-cohabiting mother-offspring pairs still share significantly more strains than children do with unrelated mothers (Valles-Colomer, et al., 2023). This is consistent with our finding that the closest microbiome proximity is between mothers and their own children. Emerging evidence indicates that the early colonized microbiota is an essential driver of a range of processes, including development, immunity, and metabolism, ultimately affecting host health in the long term (Tian et al., 2023). Any factors that affect the maternal microbiota can also disrupt optimal microbial acquisition for infants, leading to an increased lifetime risk for various diseases (Martino et al., 2022). Based on the above, we propose a hypothesis that perturbation of maternal gut microbiota during pregnancy due to various factors (such as antibiotic exposure, mother-to-child vertical transmission of microbiota, and microbial alterations during the critical developmental window) are associated with greater susceptibility to IBD later in life. Interventions that favorably modify microbial composition would provide opportunities for overcoming microbial imbalances in early life, for example giving probiotic supplements to mothers (Korpela et al., 2018). In addition, the high microbial similarities in mother-child pairs in our findings may be due to the fact that gut microbes can be co-acquired due to shared environmental factors. However, the cross-sectional nature of the study prevented us from elucidating these mechanisms and providing a longitudinal view. Additional large cohort studies are needed to determine mother-child pairs in chronological order and to evaluate alterations in the gut microbiota of mothers and children mechanistically.

We have identified eight bacterial biomarkers that exhibit shared patterns of variation between the case and control groups. The typical vertical transmission of gut microbiota underscores the significant predictive value of maternal biomarkers for PIBD risk in offspring. The function of *Bacteroides fragilis*, which we found to be

Uncorrected

enriched in IBD-M, has been extensively studied. A subset of *Bacteroides fragilis* secretes *Bacteroides fragilis* toxin, known as enterotoxigenic *Bacteroides fragilis* (ETBF); and this toxin could disrupt the colonic epithelial barrier by cleaving E-cadherin (Sears et al., 2014), leading to chronic colitis (Rhee et al., 2009). Another subset of *Bacteroides fragilis* acts as a commensal microbe, providing metabolic benefits (Chan et al., 2019). ETBF reveals a high transmission capacity from mother to offspring (Valles-Colomer, et al., 2023). It establishes colonization within the lamina propria and persists into adulthood, further antagonizing beneficial bacteria and disrupting intestinal homeostasis (Hill et al., 2024). *Blautia*, which exhibits decreased abundance in both IBD-M and IBD-C, encompasses potential beneficial bacteria with anti-inflammatory properties, that improve the host's gut homeostasis, inhibit colonization by pathogens, and influence lipid metabolism (Benítez-Páez et al., 2020; Liu et al., 2021). The vertical transmission of *Blautia* depends on breastfeeding (Qi et al., 2022). Maternal overweight and stress are associated with decreased abundance of *Blautia*, making offspring more susceptible to conditions like colitis and cognitive abnormalities (Sun et al., 2021; Buchenauer et al., 2023). *Eubacterium hallii* showed reduced abundance in mothers' and children's gut microbiota in the IBD group. It plays a role in producing butyrate and propionate, supporting intestinal integrity, and suppressing pro-inflammatory factors to prevent intestinal inflammation (Mukherjee et al., 2020). *Eubacterium hallii* is also recognized as an early producer of butyrate in the infant gut (Pham et al., 2016). Additionally, *Escherichia-Shigella*, which is enriched in the IBD-C group and exhibits higher abundance in IBD-M, is a pathogen consistently found in the gut microbiota of individuals with disease (Baltazar-Díaz et al., 2022; Zhao et al., 2022). It induces colitis by penetrating epithelial cells to induce apoptosis in infected macrophages and release IL-1β (Mirsepasi-Lauridsen Hengameh et al., 2019).

Our study has significant strengths. It serves as a pilot study to simultaneously investigate the microbiota of mother-child pairs and their relationship with the development of IBD in children, filling the gap in current research on maternal gut microbiota and subsequent IBD. The study has also identified potential biomarkers for PIBD susceptibility, which suggests that modulating the abundance of bacterial biomarkers in the maternal gut microbiota during the perinatal period may be an effective preventive strategy against disease development in offspring.

However, there were some limitations that warrant discussion for future applications. First, due to the low incidence of PIBD and the relatively small sample size, clinical stratified analyses based on PIBD subtype and severity were not feasible. Additionally, it was challenging to collect fecal samples from mothers and children simultaneously, which resulted in insufficient data for gut microbiota analyses. Second, as with other case-control studies and questionnaire surveys, the results are inherently prone to recall bias and should be interpreted with caution. Further large-scale cohort studies will be necessary. Lastly, 16S rRNA sequencing cannot provide sequences at a high resolution like shotgun sequencing.

5 Conclusions

Our study identified familial and perinatal risk factors for PIBD and elucidated differences in gut microbiota structure, composition, and functionality between PIBD children and their mothers compared to healthy controls. The risk factors partially explain the differences observed in the gut microbiota. We observed remarkable similarities in gut microbiota between mothers and children. The gut microbiota of children and mothers is associated with the occurrence and severity of PIBD, and mothers' bacterial biomarkers effectively predict PIBD, independently or in combination. Our findings suggest a potential intergenerational influence of maternal microbiota on IBD development in children. Further large cohort studies are warranted to determine mother-child pairs in chronological order and evaluate gut microbiota alterations in mothers and children mechanistically.

Unedited

Data availability statement

The 16S rRNA sequencing data of gut microbiota has been deposited into the Sequence Read Archive (SRA) database, and can be accessed at https://dataview.ncbi.nlm.nih.gov/object/PRJNA1125871?reviewer=aqc33j7ucfhvouvknbnl9r7sv8. Other research data can be obtained from the authors upon reasonable request.

Acknowledgments

Some of the figures were generated using https://www.chiplot.online/. This work was supported by the National Key R&D Program of China (No. 2021YFA1301300, 2019YFA0905600, 2023YFC2706503) and the National Natural Science Foundation of China (No. 82170557, 82000510).

Author contributions

Liping DUAN, Cunzheng ZHANG and Ruqiao DUAN proposed the scientific question. Cunzheng ZHANG and Ruqiao DUAN designed the study. Liping DUAN and Zailing LI obtained funding. Cunzheng ZHANG and Yuzhu CHEN conducted bioinformatics analyses. Cunzheng ZHANG, Ruqiao DUAN, Nini DAI, Gaonan LI, Xiaoang LI, Xiaolin JI, and Xuemei ZHONG collected clinical samples and obtained patient information through consent forms and questionnaire. Cunzheng ZHANG and Ruqiao DUAN analyzed the data. Cunzheng ZHANG and Ruqiao DUAN wrote the draft of the manuscript. Liping DUAN performed project management and conducted critical revisions of the manuscript.

Compliance with ethics guidelines

Cunzheng ZHANG, Ruqiao DUAN1, Nini DAI, Yuzhu CHEN, Gaonan LI, Xiaoang LI, Xiaolin JI, Xuemei ZHONG, Zailing LI, and Liping DUAN declare no competing interests.

This study was approved by the Institutional Review Board of Peking University Third Hospital (2022-652-02). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). Informed consent was obtained from all patients for being included in the study.

Unedited

References

Agrawal M, Sabino J, Frias-Gomes C, et al., 2021. Early life exposures and the risk of inflammatory bowel disease: Systematic review and meta-analyses. *EClinicalMedicine*, 36:100884. https://doi.org/10.1016/j.eclinm.2021.100884

Agrawal M, Poulsen G, Colombel JF, et al., 2023. Maternal antibiotic exposure during pregnancy and risk of ibd in offspring: A population-based cohort study. *Gut*, 72(4):804-805. https://doi.org/10.1136/gutjnl-2022-327724

Arumugam M, Raes J, Pelletier E, et al., 2011. Enterotypes of the human gut microbiome. *Nature*, 473(7346):174-180. https://doi.org/10.1038/nature09944

Asnicar F, Manara S, Zolfo M, et al., 2017. Studying vertical microbiome transmission from mothers to infants by strain-level metagenomic profiling. *mSystems*, 2(1) https://doi.org/10.1128/mSystems.00164-16

Baltazar-Díaz TA, González-Hernández LA, Aldana-Ledesma JM, et al., 2022. Escherichia/shigella, scfas, and metabolic pathways-the triad that orchestrates intestinal dysbiosis in patients with decompensated alcoholic cirrhosis from western mexico. *Microorganisms*, 10(6) https://doi.org/10.3390/microorganisms10061231

Benítez-Páez A, Gómez Del Pugar EM, López-Almela I, et al., 2020. Depletion of blautia species in the microbiota of obese children relates to intestinal inflammation and metabolic phenotype worsening. *mSystems*, 5(2) https://doi.org/10.1128/mSystems.00857-19

Bokulich NA, Subramanian S, Faith JJ, et al., 2013. Quality-filtering vastly improves diversity estimates from illumina amplicon sequencing. *Nat Methods*, 10(1):57-59. https://doi.org/10.1038/nmeth.2276

Bouhuys M, Lexmond WS, Van Rheenen PF, 2023. Pediatric inflammatory bowel disease. *Pediatrics*, 151(1) https://doi.org/10.1542/peds.2022-058037

Buchenauer L, Haange SB, Bauer M, et al., 2023. Maternal exposure of mice to glyphosate induces depression- and anxiety-like behavior in the offspring via alterations of the gut-brain axis. *Sci Total Environ*, 905:167034. https://doi.org/10.1016/j.scitotenv.2023.167034

Chan JL, Wu S, Geis AL, et al., 2019. Non-toxigenic bacteroides fragilis (ntbf) administration reduces bacteria-driven chronic colitis and tumor development independent of polysaccharide a. *Mucosal Immunol*, 12(1):164-177. https://doi.org/10.1038/s41385-018-0085-5

Chen H, Tong T, Lu SY, et al., 2023. Urea cycle activation triggered by host-microbiota maladaptation driving colorectal tumorigenesis. *Cell Metab*, 35(4):651-666.e657. https://doi.org/10.1016/j.cmet.2023.03.003

Chen W, Wang D, Deng X, et al., 2024. Bile acid profiling as an effective biomarker for staging in pediatric inflammatory bowel disease. *Gut Microbes*, 16(1):2323231. https://doi.org/10.1080/19490976.2024.2323231

D'adamo GL, Chonwerawong M, Gearing LJ, et al., 2023. Bacterial clade-specific analysis identifies distinct epithelial responses in inflammatory bowel disease. *Cell Rep Med*, 4(7):101124. https://doi.org/10.1016/j.xcrm.2023.101124

Dolinger M, Torres J, Vermeire S, 2024. Crohn's disease. *Lancet*, 403(10432):1177-1191.

https://doi.org/10.1016/s0140-6736(23)02586-2
Dong D, Su T, Chen W, et al., 2023. Clostridioides difficile aggravates dextran sulfate solution (dss)-induced colitis by shaping the gut microbiota and promoting neutrophil recruitment. *Gut Microbes*, 15(1):2192478. https://doi.org/10.1080/19490976.2023.2192478
Douglas GM, Maffei VJ, Zaneveld JR, et al., 2020. Picrust2 for prediction of metagenome functions. *Nat Biotechnol*, 38(6):685-688. https://doi.org/10.1038/s41587-020-0548-6
Duan R, Zhang C, Li G, et al., 2024. Antibiotic exposure and risk of new-onset inflammatory bowel disease: A systematic review and dose-response meta-analysis. *Clin Gastroenterol Hepatol*, https://doi.org/10.1016/j.cgh.2024.02.010
Edgar RC, Haas BJ, Clemente JC, et al., 2011. Uchime improves sensitivity and speed of chimera detection. *Bioinformatics*, 27(16):2194-2200. https://doi.org/10.1093/bioinformatics/btr381
Ferretti P, Pasolli E, Tett A, et al., 2018. Mother-to-infant microbial transmission from different body sites shapes the developing infant gut microbiome. *Cell Host Microbe*, 24(1):133-145.e135. https://doi.org/10.1016/j.chom.2018.06.005
Hill CA, Casterline BW, Valguarnera E, et al., 2024. Bacteroides fragilis toxin expression enables lamina propria niche acquisition in the developing mouse gut. *Nat Microbiol*, 9(1):85-94. https://doi.org/10.1038/s41564-023-01559-9
Iwamuro M, Tanaka T, Kagawa S, et al., 2023. Collagenous colitis in a patient with gastric cancer who underwent chemotherapy. *Cureus*, 15(5):e39466. https://doi.org/10.7759/cureus.39466
Jezernik G, Mičetić-Turk D, Potočnik U, 2020. Molecular genetic architecture of monogenic pediatric ibd differs from complex pediatric and adult ibd. *J Pers Med*, 10(4) https://doi.org/10.3390/jpm10040243
Khorsand B, Asadzadeh Aghdaei H, Nazemalhosseini-Mojarad E, et al., 2022. Overrepresentation of enterobacteriaceae and escherichia coli is the major gut microbiome signature in crohn's disease and ulcerative colitis; a comprehensive metagenomic analysis of ibdmdb datasets. *Front Cell Infect Microbiol*, 12:1015890. https://doi.org/10.3389/fcimb.2022.1015890
Koren O, Konnikova L, Brodin P, et al., 2024. The maternal gut microbiome in pregnancy: Implications for the developing immune system. *Nat Rev Gastroenterol Hepatol*, 21(1):35-45. https://doi.org/10.1038/s41575-023-00864-2
Korpela K, Salonen A, Vepsäläinen O, et al., 2018. Probiotic supplementation restores normal microbiota composition and function in antibiotic-treated and in caesarean-born infants. *Microbiome*, 6(1):182. https://doi.org/10.1186/s40168-018-0567-4
Krishna M, Salako A, Fofanova T, et al., 2020. Parental education may differentially impact pediatric inflammatory bowel disease phenotype risk. *Inflamm Bowel Dis*, 26(7):1068-1076. https://doi.org/10.1093/ibd/izz225
Kuenzig ME, Fung SG, Marderfeld L, et al., 2022. Twenty-first century trends in the global epidemiology of pediatric-onset inflammatory bowel disease: Systematic review. *Gastroenterology*, 162(4):1147-1159.e1144. https://doi.org/10.1053/j.gastro.2021.12.282
Le Berre C, Honap S, Peyrin-Biroulet L, 2023. Ulcerative colitis. *Lancet*, 402(10401):571-584. https://doi.org/10.1016/s0140-6736(23)00966-2
Lee IA, Kamba A, Low D, et al., 2014. Novel methylxanthine derivative-mediated anti-inflammatory effects in inflammatory bowel disease. *World J Gastroenterol*, 20(5):1127-1138. https://doi.org/10.3748/wjg.v20.i5.1127
Lehri B, Atkins E, Scott TA, et al., 2024. Investigation into the efficiency of diverse n-linking oligosaccharyltransferases for glycoengineering using a standardised cell-free assay. *Microb Biotechnol*, 17(6):e14480. https://doi.org/10.1111/1751-7915.14480
Leibovitzh H, Lee SH, Xue M, et al., 2022. Altered gut microbiome composition and function are associated with gut barrier dysfunction in healthy relatives of patients with crohn's disease. *Gastroenterology*, 163(5):1364-1376.e1310. https://doi.org/10.1053/j.gastro.2022.07.004
Li C, Zhang P, Xie Y, et al., 2024. Enterococcus-derived tyramine hijacks α(2a)-adrenergic receptor in intestinal stem cells to exacerbate colitis. *Cell Host Microbe*, 32(6):950-963.e958. https://doi.org/10.1016/j.chom.2024.04.020
Liu H, Hong XL, Sun TT, et al., 2020. Fusobacterium nucleatum exacerbates colitis by damaging epithelial barriers and inducing aberrant inflammation. *J Dig Dis*, 21(7):385-398. https://doi.org/10.1111/1751-2980.12909
Liu X, Mao B, Gu J, et al., 2021. Blautia-a new functional genus with potential probiotic properties? *Gut Microbes*, 13(1):1-21. https://doi.org/10.1080/19490976.2021.1875796
Magoč T, Salzberg SL, 2011. Flash: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*, 27(21):2957-2963. https://doi.org/10.1093/bioinformatics/btr507
Mari A, Khoury T, Ahamad HS, et al., 2020. Autoimmune diseases in first- and second-degree relatives of patients with inflammatory bowel diseases: A case-control survey in israel. *Minerva Med*, 111(2):107-114. https://doi.org/10.23736/s0026-4806.19.06389-4
Martino C, Dilmore AH, Burcham ZM, et al., 2022. Microbiota succession throughout life from the cradle to the grave. *Nat Rev Microbiol*, 20(12):707-720. https://doi.org/10.1038/s41579-022-00768-z
Mirsepasi-Lauridsen Hengameh C, Vallance Bruce A, Krogfelt Karen A, et al., 2019. Escherichia coli pathobionts associated with inflammatory bowel disease. *Clinical Microbiology Reviews*, 32(2):10.1128/cmr.00060-00018. https://doi.org/10.1128/cmr.00060-18
Mukherjee A, Lordan C, Ross RP, et al., 2020. Gut microbes from the phylogenetically diverse genus eubacterium and their various contributions to gut health. *Gut Microbes*, 12(1):1802866. https://doi.org/10.1080/19490976.2020.1802866
Mukhopadhya I, Hansen R, El-Omar EM, et al., 2012. Ibd-what role do proteobacteria play? *Nat Rev Gastroenterol Hepatol*, 9(4):219-230. https://doi.org/10.1038/nrgastro.2012.14

Uncorrected

Nameirakpam J, Rikhi R, Rawat SS, et al., 2020. Genetics on early onset inflammatory bowel disease: An update. *Genes Dis*, 7(1):93-106. https://doi.org/10.1016/j.gendis.2019.10.003

Örtqvist AK, Lundholm C, Halfvarson J, et al., 2019. Fetal and early life antibiotics exposure and very early onset inflammatory bowel disease: A population-based study. *Gut*, 68(2):218-225. https://doi.org/10.1136/gutjnl-2017-314352

Pathikkal A, Puthusseri B, Divya P, et al., 2022. Folate derivatives, 5-methyltetrahydrofolate and 10-formyltetrahydrofolate, protect beas-2b cells from high glucose–induced oxidative stress and inflammation. *In Vitro Cellular & Developmental Biology - Animal*, 58(5):419-428. https://doi.org/10.1007/s11626-022-00691-w

Pham VT, Lacroix C, Braegger CP, et al., 2016. Early colonization of functional groups of microbes in the infant gut. *Environ Microbiol*, 18(7):2246-2258. https://doi.org/10.1111/1462-2920.13316

Piovani D, Danese S, Peyrin-Biroulet L, et al., 2019. Environmental risk factors for inflammatory bowel diseases: An umbrella review of meta-analyses. *Gastroenterology*, 157(3):647-659.e644. https://doi.org/10.1053/j.gastro.2019.04.016

Pittayanon R, Lau JT, Leontiadis GI, et al., 2020. Differences in gut microbiota in patients with vs without inflammatory bowel diseases: A systematic review. *Gastroenterology*, 158(4):930-946.e931. https://doi.org/10.1053/j.gastro.2019.11.294

Qi C, Zhou J, Tu H, et al., 2022. Lactation-dependent vertical transmission of natural probiotics from the mother to the infant gut through breast milk. *Food & Function*, 13(1):304-315. https://doi.org/10.1039/D1FO03131G

Qiao S, Liu C, Sun L, et al., 2022. Gut parabacteroides merdae protects against cardiovascular damage by enhancing branched-chain amino acid catabolism. *Nat Metab*, 4(10):1271-1286. https://doi.org/10.1038/s42255-022-00649-y

Rhee KJ, Wu S, Wu X, et al., 2009. Induction of persistent colitis by a human commensal, enterotoxigenic bacteroides fragilis, in wild-type c57bl/6 mice. *Infect Immun*, 77(4):1708-1718. https://doi.org/10.1128/iai.00814-08

Sears CL, Geis AL, Housseau F, 2014. Bacteroides fragilis subverts mucosal biology: From symbiont to colon carcinogenesis. *J Clin Invest*, 124(10):4166-4172. https://doi.org/10.1172/jci72334

Segata N, Izard J, Waldron L, et al., 2011. Metagenomic biomarker discovery and explanation. *Genome Biol*, 12(6):R60. https://doi.org/10.1186/gb-2011-12-6-r60

Shenhav L, Thompson M, Joseph TA, et al., 2019. Feast: Fast expectation-maximization for microbial source tracking. *Nat Methods*, 16(7):627-632. https://doi.org/10.1038/s41592-019-0431-x

Sun X, Chen Z, Yu L, et al., 2023. Bacteroides dorei bdx-01 alleviates dss-induced experimental colitis in mice by regulating intestinal bile salt hydrolase activity and the fxr-nlrp3 signaling pathway. *Front Pharmacol*, 14:1205323. https://doi.org/10.3389/fphar.2023.1205323

Sun Y, Xie R, Li L, et al., 2021. Prenatal maternal stress exacerbates experimental colitis of offspring in adulthood. *Front Immunol*, 12:700995. https://doi.org/10.3389/fimmu.2021.700995

Tian M, Li Q, Zheng T, et al., 2023. Maternal microbe-specific modulation of the offspring microbiome and development during pregnancy and lactation. *Gut Microbes*, 15(1):2206505. https://doi.org/10.1080/19490976.2023.2206505

Valles-Colomer M, Blanco-Míguez A, Manghi P, et al., 2023. The person-to-person transmission landscape of the gut and oral microbiomes. *Nature*, 614(7946):125-135. https://doi.org/10.1038/s41586-022-05620-1

Velosa M, Hochner H, Yerushalmi B, et al., 2022. Pre- and perinatal factors predicting inflammatory bowel disease: A population-based study with fifty years of follow-up. *J Crohns Colitis*, 16(9):1397-1404. https://doi.org/10.1093/ecco-jcc/jjac043

Wang Q, Deng Z, Lan J, et al., 2022. Inhibition of gaba(a)r or application of lactobacillus casei zhang alleviates ulcerative colitis in mice: Gaba(a)r as a potential target for intestinal epithelial renewal and repair. *Int J Mol Sci*, 23(19) https://doi.org/10.3390/ijms231911210

Wang S, Ryan CA, Boyaval P, et al., 2020. Maternal vertical transmission affecting early-life microbiota development. *Trends Microbiol*, 28(1):28-45. https://doi.org/10.1016/j.tim.2019.07.010

Wang X, Xiao Y, Xu X, et al., 2021. Characteristics of fecal microbiota and machine learning strategy for fecal invasive biomarkers in pediatric inflammatory bowel disease. *Front Cell Infect Microbiol*, 11:711884. https://doi.org/10.3389/fcimb.2021.711884

Xiao L, Zhao F, 2023. Microbial transmission, colonisation and succession: From pregnancy to infancy. *Gut*, 72(4):772-786. https://doi.org/10.1136/gutjnl-2022-328970

Zhao J, Bai M, Ning X, et al., 2022. Expansion of escherichia-shigella in gut is associated with the onset and response to immunosuppressive therapy of iga nephropathy. *J Am Soc Nephrol*, 33(12):2276-2292. https://doi.org/10.1681/asn.2022020189

Supplementary information:

Figs. S1-S3; Table S1.

Unedited