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Characterization of the intestinal microorganism in patients with congenital intestinal atresia: the preliminary exploration for establishment and influence of initial intestinal flora in newborns

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Abstract

Purpose This study aimed to analyze the differences in microbial composition between the proximal and distal intestinal segments of newborns with intestinal atresia, speculating about the mechanism underlying the initial establishment of neonatal intestinal flora. Additionally, differential metabolic pathways were explored to predict their potential effects on gravidas and fetuses.

Methods The microbial characteristics of intestinal flora were assessed using 16SrRNA sequencing. The alpha and beta-diversity indices were calculated to compare the microbial composition among three groups. Principal Coordinates Analysis (PCoA) was employed to identify and quantify differences in microbial communities. Furthermore, PICRUSt software was utilized to predict the possible functional impacts of differential metabolic pathways by comparing them with public databases.

Results Samples were collected from 23 neonates with intestinal atresia (proximal and distal segments) and 25 healthy neonates (first meconium) based on predefined selection criteria. No significant differences in baseline characteristics were observed between the control and intestinal atresia groups ($P > 0.05$). Alpha-Diversity analysis revealed that the distal intestinal group exhibited greater microbial species richness. Beta-Diversity analysis indicated significant differences in bacterial composition between the control group and the distal intestinal group ($P < 0.05$), with the distal group showing a more pronounced divergence compared to the proximal group. Functional prediction analysis suggested that the differential metabolic pathways might protect the intestinal mucosal barrier. However, they could also negatively impact blood glucose regulation and lipid transport in gravidas and fetuses, potentially contributing to adverse emotional states in pregnant women.

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Conclusion The distinct microbial profiles observed among the three groups suggest that the establishment of neonatal intestinal flora may result from a combination of placental transmission and digestive tract colonization. Functional pathway analysis suggested these microbial metabolic differences may exert pleiotropic effects, demonstrating both protective roles in intestinal barrier function and potentially detrimental impacts on emotional modulation and glucose/lipid dysregulation.

Keywords Intestinal flora, Intestinal atresia, Neonate, 16SrRNA sequencing

Introduction

Congenital intestinal atresia is a developmental malformation of the digestive tract, characterized by the disruption of intestinal continuity, with a prevalence of approximately 1–3/10,000 live births [1]. Because of complete mechanical intestinal obstruction, the main clinical manifestation in newborns are progressive abdominal distension and biliary vomiting, which requires emergency surgery immediately [2, 3].

Neonates presenting with complete intestinal obstruction require urgent surgical intervention. While the spatial distribution and microbial colonization patterns within the atretic intestinal segments provide critical insights into gut microbiota development under pathological conditions. There are no studies on the intestinal microecology of children with intestinal atresia [4]. The congenital intestinal discontinuity in neonates with intestinal atresia establishes a unique experimental model: proximal segments exposed to the external environment via digestive tract colonization, while distal segments remain isolated from environmental contaminants. This anatomical dichotomy thus enables comparative investigation of digestive tract-mediated colonization versus hematogenous microbial transfer in shaping primordial gut microbiota assembly [5]. Under ethical approval, human intestinal microbiota studies have predominantly utilized neonatal meconium or animal models. Our approach capitalized on the clinical necessity of emergency intestinal resection in neonates with atresia, enabling direct acquisition of intestinal tissues and luminal contents under surgical asepsis. This methodology minimizes contamination risks while providing unique access to authentic intestinal microbial ecosystems.

This study aimed to validate and expand current understanding of neonatal gut microbiota establishment by characterizing microbial communities in congenital intestinal atresia patients through 16 S rRNA sequencing, with comparative analysis against normal neonatal meconium. Bioinformatics approaches were employed to elucidate the biological significance of differential microbial taxa.

Methods

Samples collection

This study ensured no additional harm during standard treatment. Informed consent was obtained from legal guardians before specimen collection. Conduct of research were in accordance with the relevant guidelines and regulations, and the study was approved by the Research Ethics Boards of Shenzhen Children's Hospital (Ethical approval:202104202). The samples collected in this study were proximal and distal intestinal bowels of children with intestinal atresia as well as normal neonatal meconium. All children were diagnosed intraoperatively with intestinal atresia within 24 h of birth. However, subjects were excluded if children were accompanied by intestinal perforation, pregnant women suffered from metabolic diseases or received antibiotics during pregnancy. Samples were taken from the distal and proximal bowels separately before anastomosis to avoid cross contamination. Moreover, surgical asepsis protocols in the operating theater further minimized environmental microbial contamination during sampling, preserving authentic microbial community profiles. Direct acquisition of intestinal mucosal biopsies from healthy neonates is ethically impermissible. Consequently, non-invasive meconium sampling serves as the standard control methodology for investigating primordial gut microbiota establishment. Healthy controls were established using the initial meconium of neonates collected within 24 h post-birth. These infants exhibited no congenital abnormalities, with gestational age and birth weight matched to the intestinal atresia cohort. All specimens were immediately sealed after collection, flash-frozen within 4 h, and stored at − 80℃ until analysis.

16SrDNA sequencing

Microbial DNA was extracted by the Stool DNA Kit (D4015-01; Omega) according to the manufacturer's instructions. The extracted DNA was confirmed good quality by 0.75% agarose gel electrophoresis based on comparison with standard samples. Designed primers (338 F:5'-ACTCCTACGGGAGGCAGCAG-3'; 806R:5'-GGACTACHVGGGTWTCTAAT-3') was used to amplified the V3-V4 regions of the bacterial 16SrRNA. A total of 25ul of the mixture was prepared, containing approximately 50 ng of template DNA, 12.5μL of Phusion Hot Start Flex 2X Master Mix (M0536L; NEB), 2.5μL of each primer and adequate water treated by diethylpyrocarbonate. PCR reactions were performed under the following conditions: initial denaturation at 98 °C for 30 s, 35 cycles of denaturation at 98 °C for 10 s, annealing at 54 °C for

30 s and extension at 72 °C for 45 s; and final extension at 72 °C for 10 min. The PCR products were certified to standard with 2% agarose gel electrophoresis. Amplified fragments of DNA were normalized by AxyPrep Mag PCR Normalizer (Axygen Biosciences, Union City, CA, USA), which allowed for the skipping of the quantification step regardless of the PCR volume submitted for sequencing. Furthermore, the amplicon pools were prepared for sequencing with AMPure XT beads (Beckman Coulter Genomics, Danvers, MA, USA), and the size and quantity of the amplicon library were assessed on the LabChip GX (PerkinElmer, Waltham, MA, USA) and with the Library Quantification Kit for Illumina (Kapa Biosciences, Woburn, MA, USA), respectively. PhiX Control library (Illumina) was combined with the amplicon library (expected at 30%) and sequenced on 300PE MiSeq by the standard Illumina sequencing primers.

Data processing

Raw data obtained after sequencing included dirty reads containing adapters or low-quality bases, which would affect the following assembly and analysis. Therefore, raw reads were further filtered by Fastp software (0.12.4) according to the following rules that reads containing more than 5% of unknown nucleotides or phred score less than 20 accounted for 40%. Then, the paired end clean reads were assembled as raw tags using QIIME (1.9.1) with a minimum overlap of 10 bp and mismatch error rates of 2%. Filtered data was removed chimeras using Usearch (v6.1.5). The software QIIME (1.9.1) was used to cluster the tags with more than 97% similarity into OTUs (operational taxonomic units) by open reference method. Whereafter, the Uclust software (1.2.22q) will select the tag sequence with the highest abundance in OTUs for species annotation through comparison with the database. The absolute abundance of each OTU in each sample was also calculated simultaneously.

Statistical analysis

R software (version 4.1.1) was used to analyze and plot the data obtained for the categorical OTUs. The alpha diversity, visualizing the richness and evenness of each group, was first described. The Observed feature number was calculated to indicate the number of species. Then, the Pielou index to indicate the evenness of species distribution, and the Shannon index to synthesize the level of biodiversity were also portrayed sequentially. The degree of difference in the overall abundance distribution of species can be quantified by applying the statistical algorithm Bray-Curtis to calculate the distance matrix between two samples. The closer the distance between samples, the more similar the species composition is. Then we can use Principal Coordinate Analysis (PCoA) and Permutation Multivariate Analysis of Variance (Nonparametric Multivariate Analysis of Variance, PERMANOVA) to analyze the degree of explanation of the differences by different grouping factors to indicate the differences in the compositional structure of the flora among different samples. Then the cumulative histograms of the strains were plotted according to the biological groups (phylum, order, family, genus and species) to manifest the relative abundance of the strains at different levels of classification. In the next moment, the Linear Discriminant Analysis (LDA) was performed to compare the specific differences of the flora in different samples under the same biological classification by LEfSe software. LDA distribution histograms were plotted with $P<0.05$ and LDA value >2 as significant differential groups. Finally, the feature gene data were compared with the reference genome database in the tool PICRUSt2.0 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) for the prediction of genome function of differential bacteria, and statistical tests and multiple corrections in the STAMP software were performed to map the functional prediction of the differential colonies.

Result

Basic information

A total of 23 neonatal patients who underwent emergency surgery and were diagnosed with congenital intestinal atresia in intraoperative and postoperative pathology from January 1, 2021, to October 1, 2023, were selected for this study. Meanwhile, the first meconium of 25 healthy newborns were selected as control group. Given the restricted sample size, stratification based on potential confounding variables was precluded. Case-control matching was prioritized during study design to ensure comparable baseline characteristics between groups. Specific baseline information of the children in the normal control and intestinal atresia groups were shown in Table 1. There were no significant differences in gestational age, birth weight, sex, mode of delivery, and whether they had been fed in the two groups ($P>0.05$).

Alpha diversity

All the collected samples were divided into three groups. M represents the fecal group of normal control newborns (Matched group), PB represents the proximal bowels group of children with intestinal atresia (Proximal bowels group), and DB represents the distal bowels group of children with intestinal atresia (Distal bowels group). The observed feature number was calculated separately to represent the total number of species, the Pielou index was used to represent the species distribution uniformity, and the Shannon index was used to synthesize the biodiversity. As shown in Table 2; Fig. 1, it can be seen that in terms of the total number of species, the normal

Table 1 Baseline information for the intestinal Atresia and control groups

Items	Control group ($n=25$)	Intestinal atresia group ($n=23$)	Statistic χ2/t	*P*
Gestational age/week	35.64 ± 3.87	35.8 ± 2.20	2.02	0.841
Birth weight/g	2535.4 ± 828.8	2771.7 ± 585.2	1.148	0.257
Sex(female)	14 (56.0%)	13 (48.1%)	0.001	0.971
Caesarean	19 (76.0%)	12 (52.2%)	2.973	0.085
Already fed	7 (28.0%)	7 (30.4%)	0.034	0.853

Table 2 Comparison of alpha diversity metrics among the three sample groups

Index	Matched group (M)	Proximal bowels group (PB)	Distal bowels group (DB)
Observed	91.28 ± 108.14	45.83 ± 54.47	86.28 ± 88.25
Pielou	0.43 ± 0.15	0.43 ± 0.15	0.43 ± 0.14
Shannon	2.54 ± 1.70	2.01 ± 0.85	2.62 ± 1.32

group > distal atresia > proximal atresia; the evenness of species distribution was basically the same among the three groups; while Shannon's index, which describing species richness and evenness, suggested no significant difference between the three groups.

Beta diversity

The Bray-Curtis distance between the samples were calculated to perform principal coordinate analysis (PCoA) and PERMANOVA test of variance, and the results were plotted as shown in Fig. 2. The analysis showed that there was no significant difference in the flora structure between the proximal bowel (PB) group and the normal control (M) group (R2 = 0.04, $P = 0.164$), and it was the same between the proximal bowel group and the distal bowel group (R2 = 0.03, $P = 0.419$), whereas there was significant difference between the normal control group and the distal bowel (DB) group (R2 = 0.10, $P = 0.001$). The mean Bray-Curtis distance between the different samples of was also calculated, suggesting that the difference between the proximal bowels group and the control group (0.837 ± 0.117) was significantly lower than the difference between the distal bowels group and the control group (0.868 ± 0.081), $P < 0.0001$, as shown in Fig. 3.

Differences in abundance of biologically classified flora

The relative abundance distribution of the three groups was plotted at the level of phylum and genus bioclassification as shown in Fig. 4. As far as the picture goes, the major phyla in the three groups were Proteobacteria, Firmicutes and Actinobacteria in order; The main genera in order of prevalence are Pseudomonas, Rhodococcus, Stenotrophomonas, Ochrobactrum, Staphylococcus, Lactobacillus, Bifidobacterium, Corynebacterium, Enterococcus, Streptococcus, Achromobacter and Acinetobacter.

Comparative analysis of differential bacterial genera between the two groups were also performed using the software LEfSe. The results showed that the abundance of al differential bacteria in the normal control (M) group was higher than that in the proximal bowel (PB) group. At the level of classification of bacterial families, the normal control group had more bacteria than the proximal bowels group, such as Micrococcaceae (LDA = 3.0, $p = 0.035$), Hyphomicrobiaceae (LDA = 3.04, $p = 0.03$), Methylobacteriaceae (LDA = 3.16, $p = 0.003$), Nitrospiraceae (LDA = 3.19, $p = 0.004$), Cryomorphaceae (LDA = 3.23, $p = 0.037$), Bradyrhizobiaceae (LDA = 3.26, $p = 0.014$), Oxalobacteraceae (LDA = 3.61, $p = 0.035$), Thermoactinomycetaceae (LDA = 3.76, $P = 0.014$), $p = 0.035$), Comamonadaceae (LDA = 4.34, $p = 0.013$) and Pseudomonadaceae LDA = 4.88, $p = 0.022$); while on the genera with more bacterial content in comparison were Comamonas (LDA = 3.07, $p = 0.002$), Methylobacterium (LDA = 3.12, $p = 0.003$), Delftia (LDA = 3.46, $p = 0.044$), Ralstonia (LDA = 3.60, $p = 0.009$), Nitrospira (LDA = 3.80, $p = 0.018$) and Pseudomonas (LDA = 4.88, $p = 0.022$). The linear discriminant analysis graph (Fig. 5) allowed for the more intuitive description of the species and distinctions in differential flora.

Then the results of significant difference flora analysis between normal control group and distal bowels group were performed as shown in Fig. 6. The results showed that compared to the normal control group, at the level of bacterial families, Brucelaceae (LDA = 4.56, $p = 0.005$) and Nocardiaceae (LDA = 4.54, $p = 0.007$) were significantly higher in the distal bowels group, while Methylobacteriaceae (LDA = 3.16, $p = 0.048$), Rikenellaceae (LDA = 3.34, $p = 0.008$) and Enterobacteriaceae (LDA = 4.47, $p = 0.029$) flora were significantly lower. Furthermore, At the genus level, Ochrobactrum(LDA = 4.62, $p = 0.0004$), Rhodococcus (LDA = 4.54, $p = 0.007$), Geobacillus (LDA = 3.75, $p = 0.037$), and Paenibacillus (LDA = 3.11, $p = 0.037$) were significantly more abundant in the distal intestines, while Herbaspirillum (LDA = 3.0, $p = 0.035$) and Methylobacterium (LDA = 3.16, $p = 0.028$) had significantly decreased abundance.

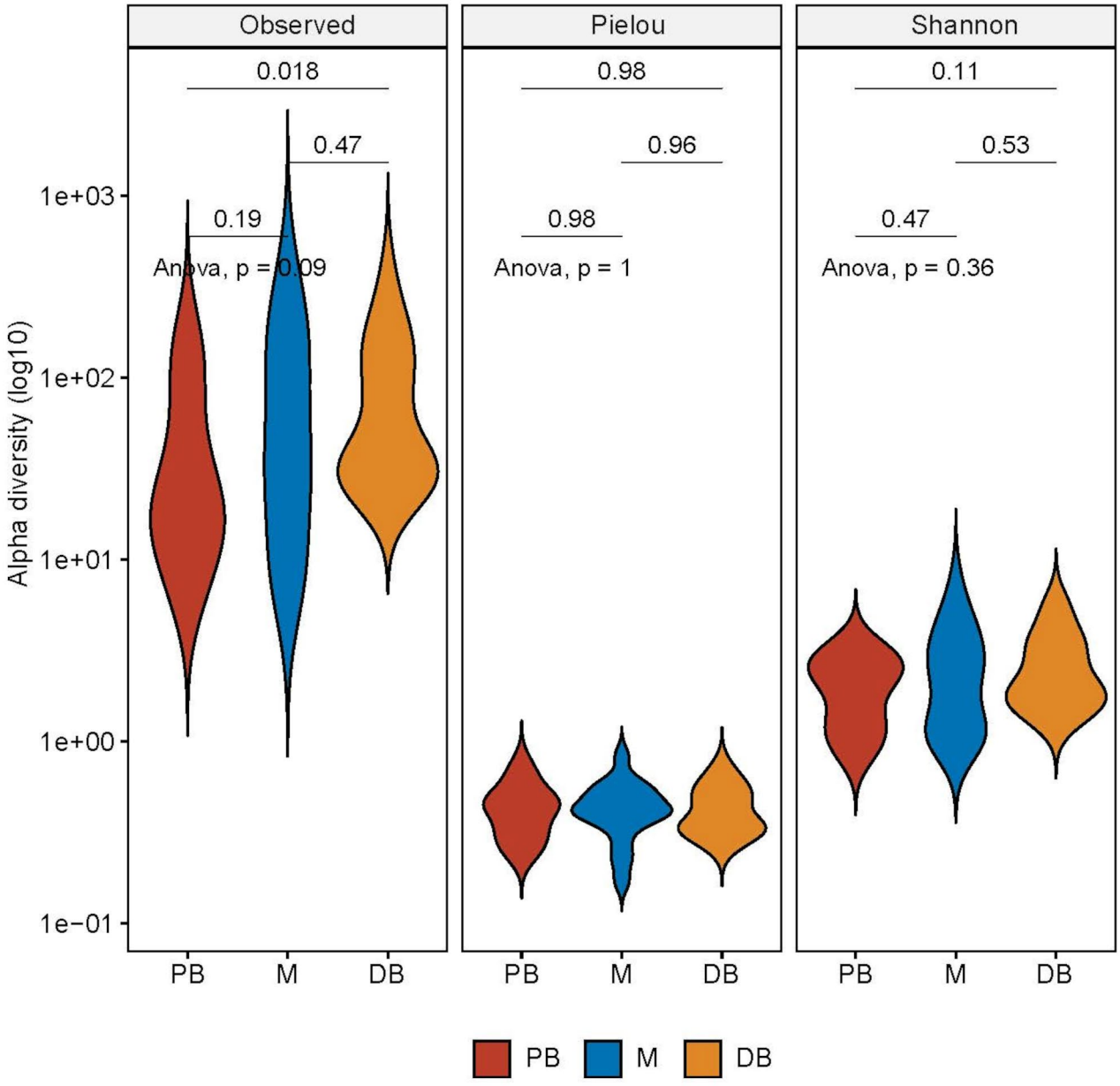

Fig. 1 Violin plot of alpha diversity among the three sample groups

Finally, the significant difference flora between the distal and proximal bowels group was compared. The results indicated that the significant differential flora of the distal intestines all presented elevated performance compared to the proximal intestines, as shown in Fig. 7. The specification at the level of mycorrhizal families were Pseudomonadaceae (LDA = 5.17, $p = 0.0007$), Nocardiaceae (LDA = 4.73, $p = 0.0001$), Brucelaceae (LDA = 4.59, $p = 0.0002$), Acetobacteraceae (LDA = 3.89, $p = 0.037$), Comamonadaceae (LDA = 3.70, $p = 0.00008$), Oxalobacteraceae (LDA = 3.53, $p = 0.005$), and Sphingomonadaceae (LDA = 3.13, $p = 0.04$); while at the genus level were Pseudomonas (LDA = 5.16, $p = 0.002$), Rhodococcus (LDA = 4.73, $p = 0.0001$), Ochrobactrum (LDA = 4.69, $p = 0.00002$), Lysobacter (LDA = 3.89, $p = 0.018$), Delftia (LDA = 3.55, $p = 0.0004$, Pelomonas (LDA = 3.55, $p = 0.005$), Ralstonia (LDA = 3.50, $p = 0.0003$) and Sphimomonas (LDA = 3.26, $p = 0.037$).

On this basis, the evolutionary branching diagram of species with different flora in the normal and distal bowels groups was drawn (Fig. 8), in which the circles radiating from inside to outside represent the taxonomic level from phylum to genus, and the size of the diameter of the solid circles were proportional to the relative

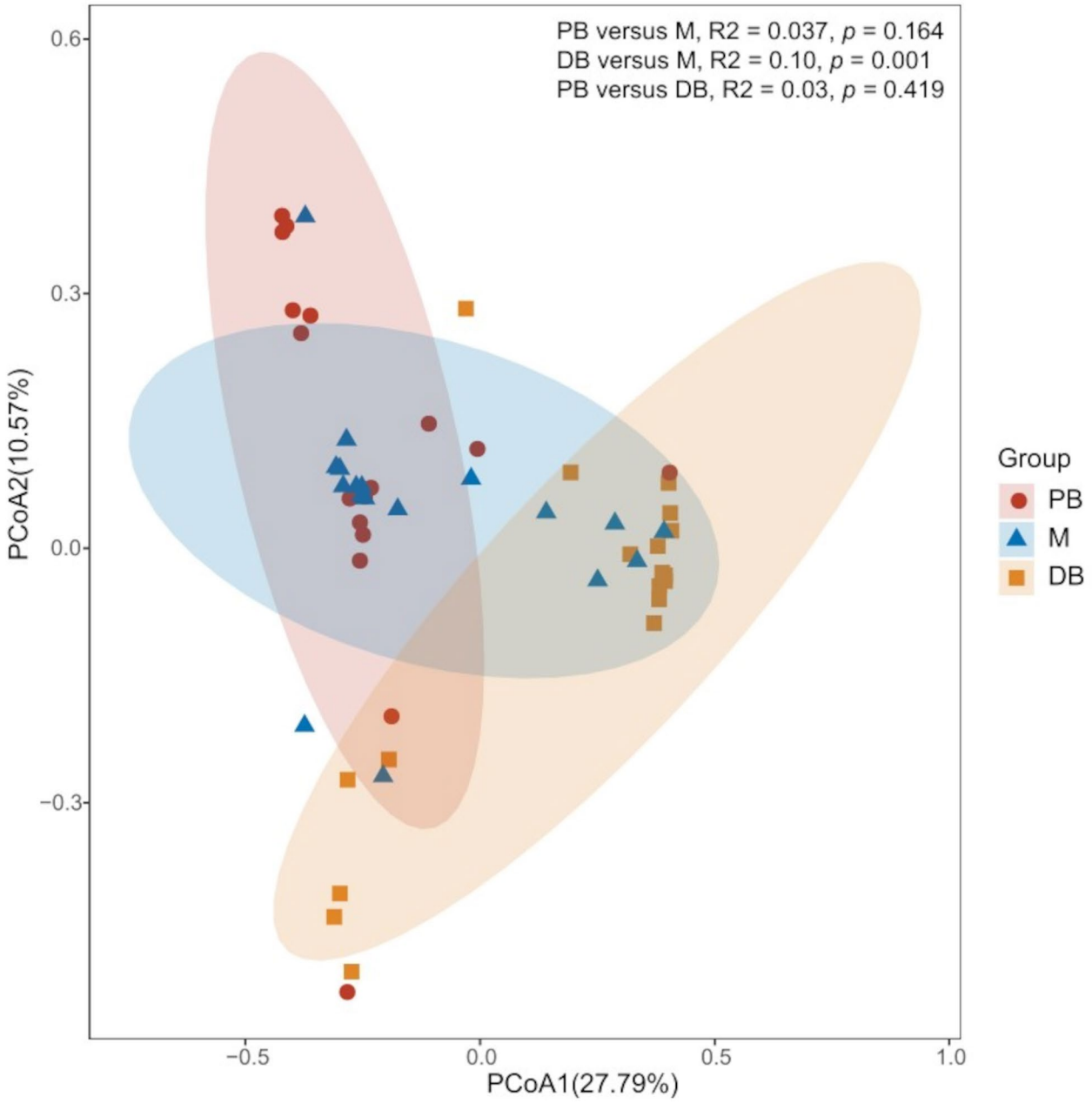

Fig. 2 Comparison of differences in bacterial structure (beta-diversity) of the flora between the three sample groups

abundance. Species with no significant differences are uniformly colored in yellow, the color of differential species indicated higher abundance in the group, and nodes denoted microbial taxa that play an important role in the corresponding group. In summary, at the level of the taxonomic classification of the phylum, the flora of the three groups of samples were predominantly Proteobacteria, Firmicutes and Actinobacteria. Compared with the normal control group, the levels of Comamonas, Methylobacterium, Delftia, Ralstonia, Nitrospira and Pseudomonas were lower in the proximal intestines; whereas the distal intestines showed significantly higher levels of Ochrobactrum, Rhodococcus, Geobacillus, Paenibacillus, and a significant reduction in the abundance of Herbaspirillum and Methylobacterium.

Functional projections

On the basis of metabolic function prediction by the tool PICRUSt2.0, the software STAMP was used to perform statistical analysis and plotted to show possible functional or phenotypic differences between the different sample flora. The final results demonstrated that

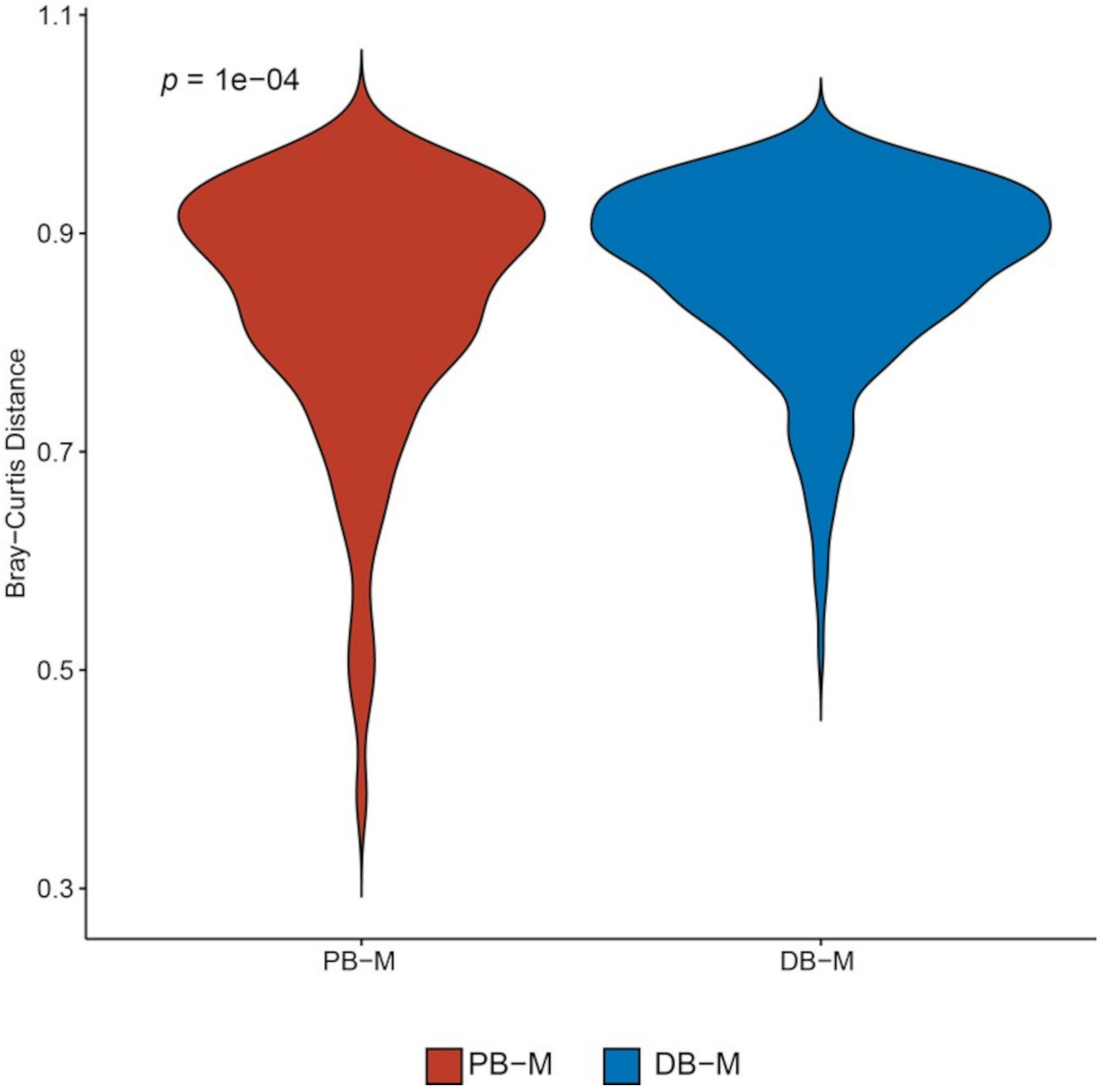

Fig. 3 Comparison of Bray-Curtis distance values between proximal and distal bowels groups with respect to control group

no metabolic pathways with significant differences were found between the normal control (M) group and the proximal bowel (PB) group. Whereas, the analysis of differential metabolic pathways between normal control and distal bowel (DB) groups suggested the metabolic pathways PWY-6282, P562-PWY, 3-HYDROXYPHENYLACETATE-DEGRADATION-PWY, PWY-722, PWY-5989, PWY-7664, KETOGLUCONMET-PWY, and PWYG-321 were significantly elevated in the distal bowels group compared to the normal control group, as detailed in Fig. 9.

Then comparing the proximal and distal bowels groups, the results revealed that metabolic pathways P101-PWY, PWY-722, PWY-7431, 3- HYDROXYPHENYLACETATE-DEGRADATION-PWY, TRYFUMCAT-PWY,

LEU-DEG2-PWY, P562-PWY, PWY – 5989, and PWY-7376 were significantly elevated in the distal intestines, and the degree of difference and corresponding *P* values were indicated in Fig. 10.

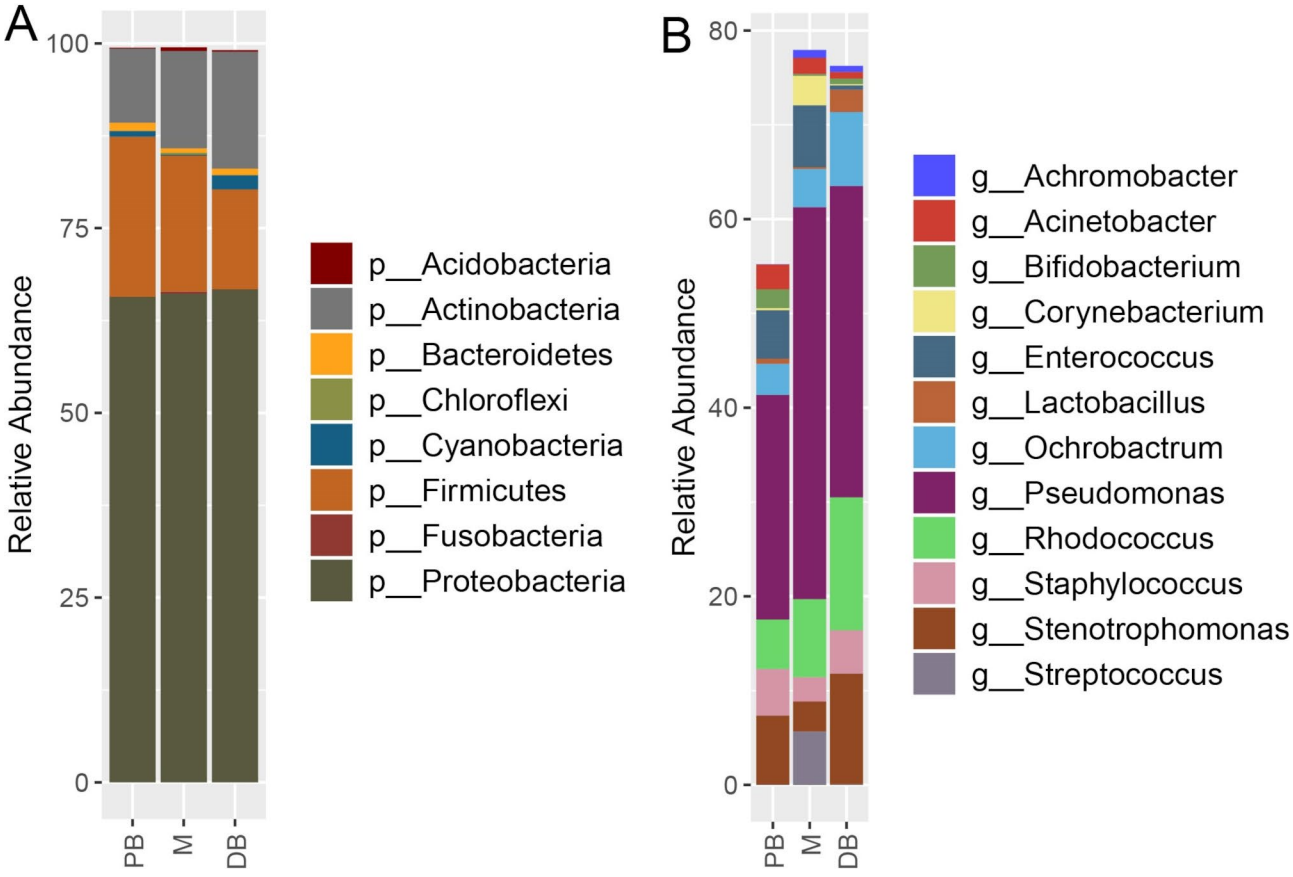

Fig. 4 Relative abundance distribution of phyla and genera for the three groups

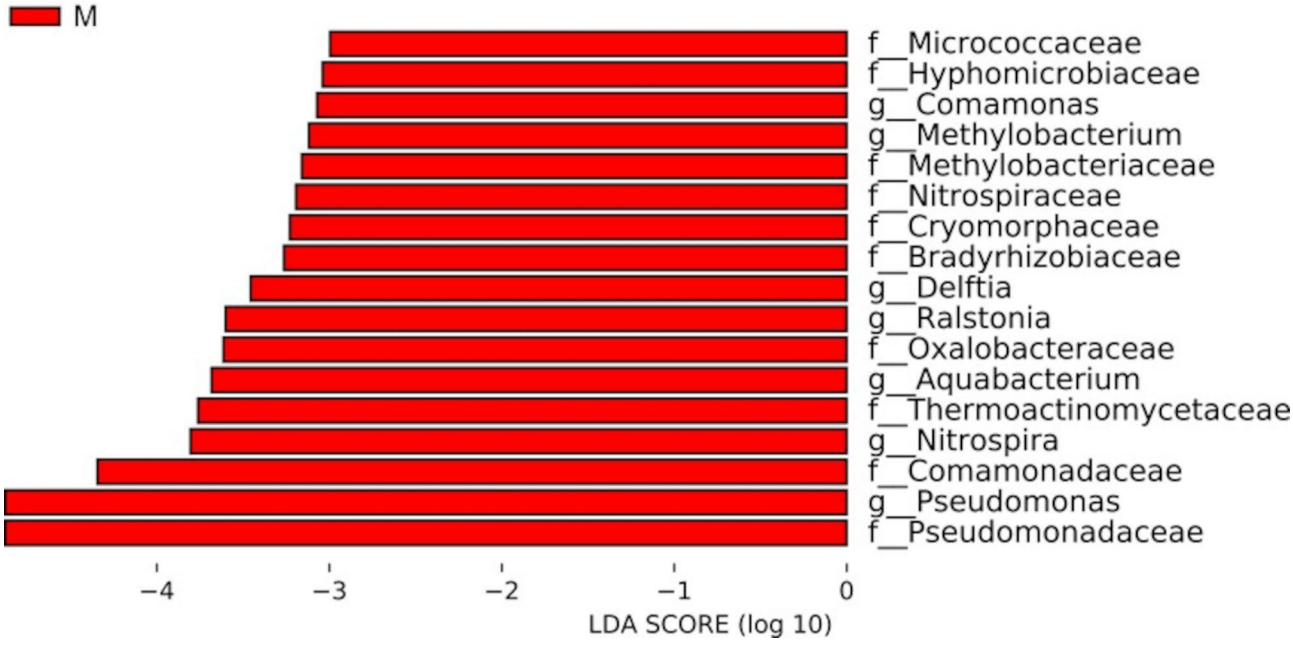

Fig. 5 Comparison of significantly different bacterial populations between normal control and proximal bowels group

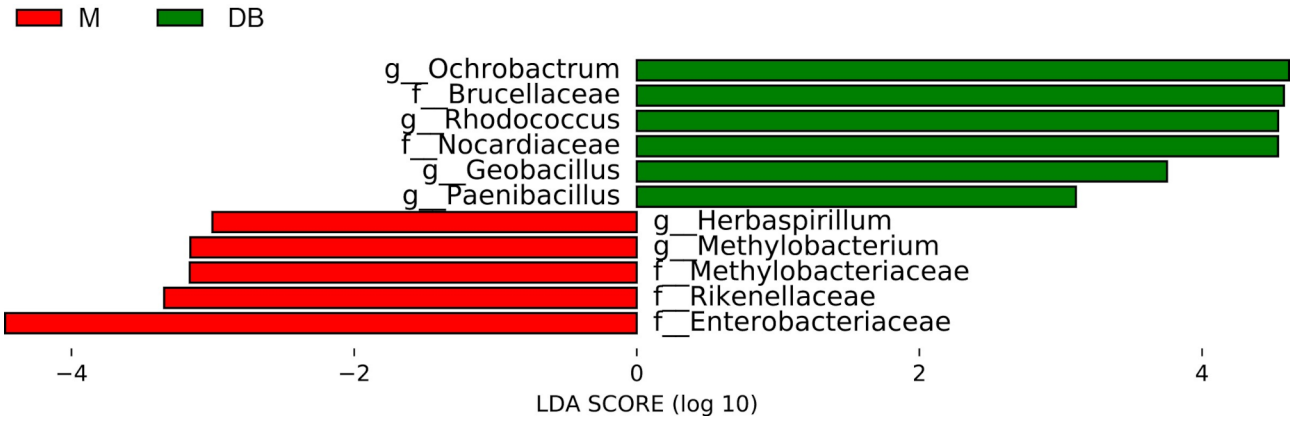

Fig. 6 Comparison of significantly different flora between normal control group and distal bowels group

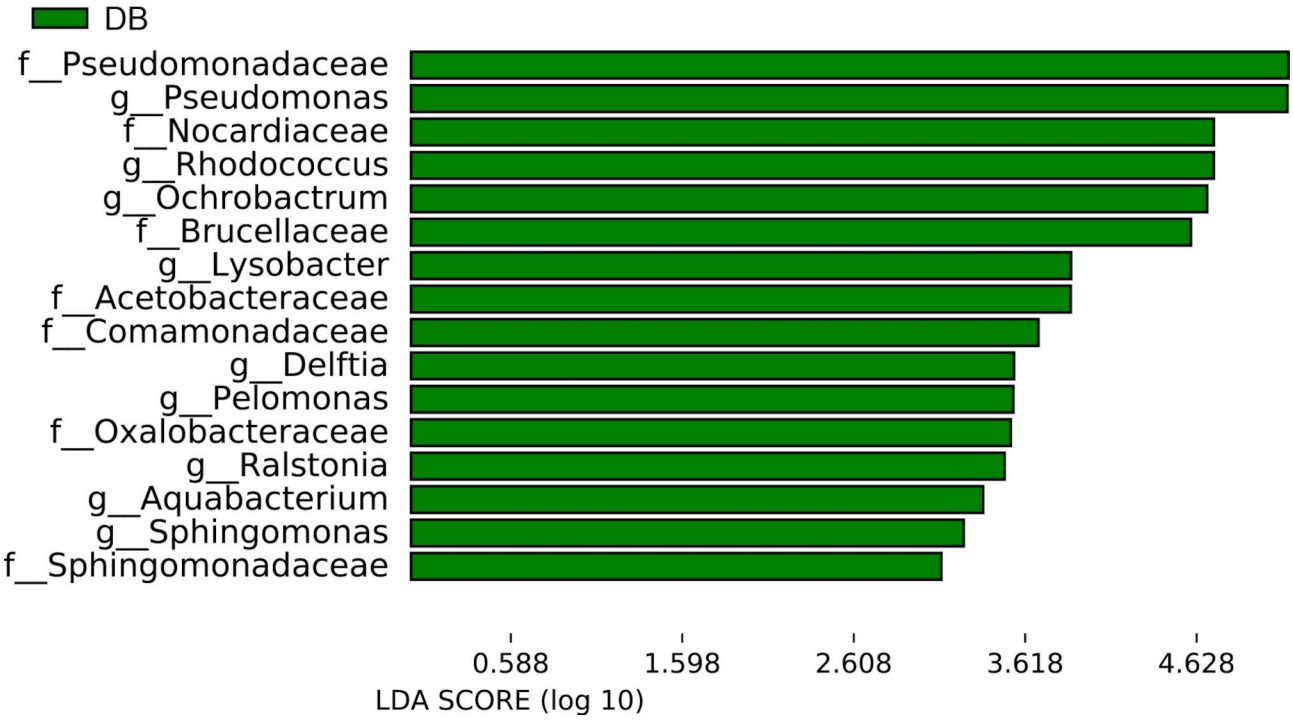

Fig. 7 Comparison of significantly different flora between proximal and distal bowels groups

Discussion

Traditional bacterial culture methods suffer from time-consuming, inefficient and costly problems, which makes high-throughput sequencing technology keep contributing to the field of microbiology. Especially in the field of less flora content or special growth conditions, the advantages of sequencing technology appear to be more unique [6, 7]. Through the sequencing analysis of bacterial conserved DNA fragments, many researchers have found the presence of a small amount of bacterial flora in fetal amniotic fluid, placenta and neonatal fetal feces, which speculates that the establishment of the initial intestinal flora of the neonate may take place in two ways: the invasion from the mother's bloodstream through the placenta into the intestinal tract, or the infection from the vagina into the intestinal canal through swallowing amniotic fluid [8, 9]. However, because the flora present in the samples was already sparse, there is still a great deal of controversy about the presence of flora in fetal amniotic fluid, placenta and neonatal feces, which shakes the reliability of the theory of flora establishment [7, 10]. Furthermore, the progress of detection technology cannot overcome the limitations of the original material acquisition. The vast majority of samples of human intestinal flora were obtained by taking the feces of the subject. Fecal microbiota analysis may not accurately reflect authentic intestinal mucosal flora composition due to enzymatic degradation and fermentation processes,

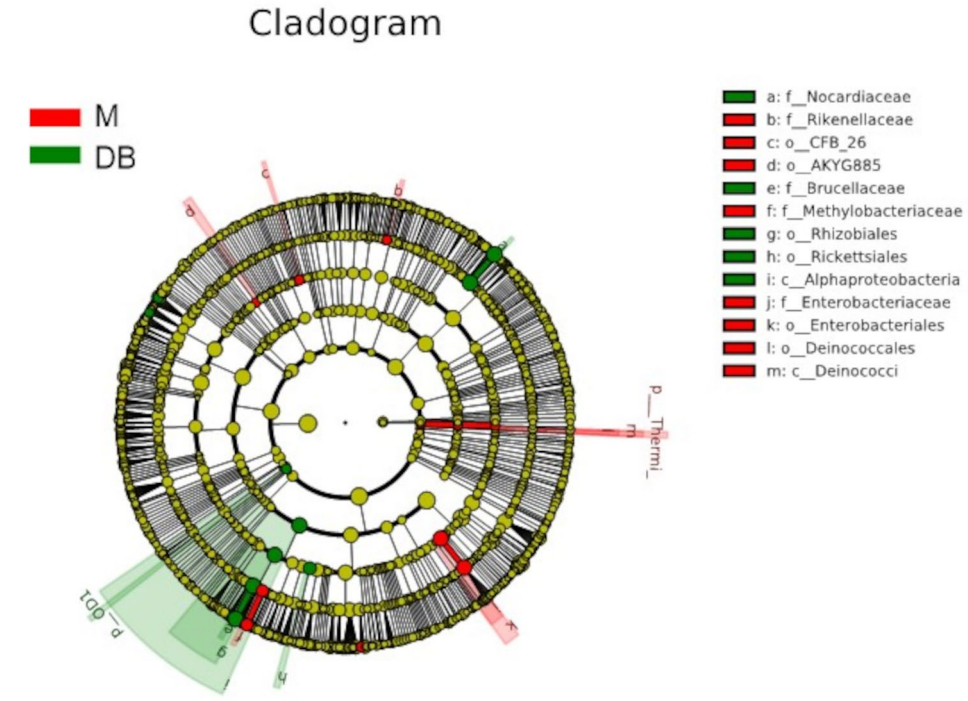

Fig. 8 Species evolutionary branching diagram of the differential flora of the normal control and distal gut tube groups

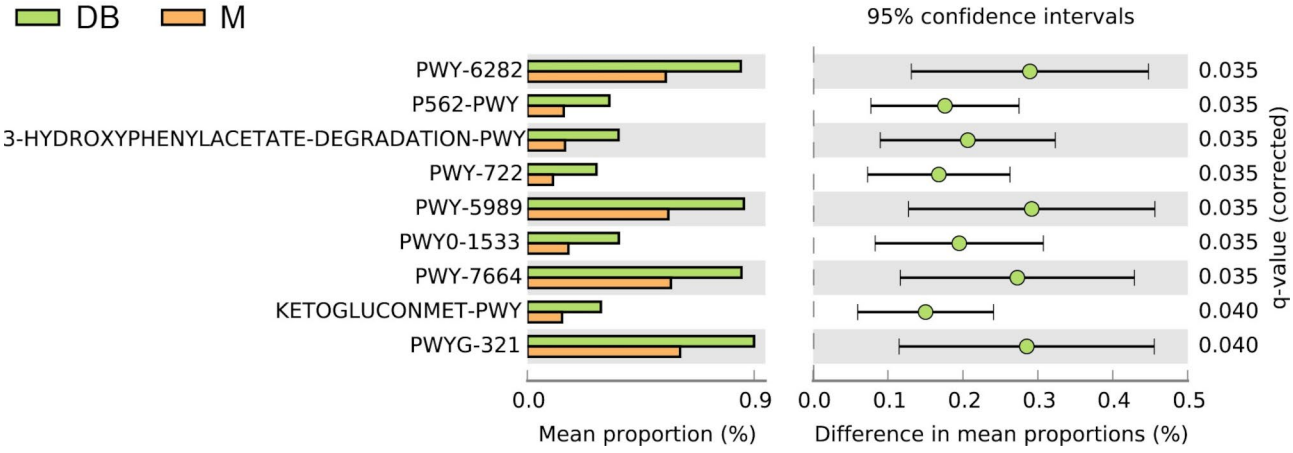

Fig. 9 Analysis of differential metabolic pathways between normal control and distal bowels groups

combined with potential environmental contamination from sampling materials (e.g., diaper components and collection containers [11, 12].

Intestinal atresia, characterized by congenital discontinuity of the intestinal lumen, may provide a unique solution to these sampling limitations. The developmental discontinuity creates a protected microenvironment proximal to the obstruction, enabling collection of uncontaminated mucosal tissues during urgent surgical intervention for neonatal intestinal obstruction. Changes of atresia was formed during pregnancy, so the distal end had a relatively isolated environment whatever during and after birth, which resulted in the composition of the microbiome in the distal intestines remained until operation. Therefore, this study not only took advantage of the normal diagnostic and treatment process of neonatal intestinal atresia, which requires emergency surgical resection of part of the intestinal tube, to avoid the ethical problems faced by direct interception of intestinal tissues, but also utilized the natural interruption of the intestines to eliminate the influence of the transdigestive tract. This approach enabled systematic investigation of the primordial establishment mechanisms governing neonatal gut microbiota development. Concurrently, further metabolic pathways were speculated for flora-host interactions.

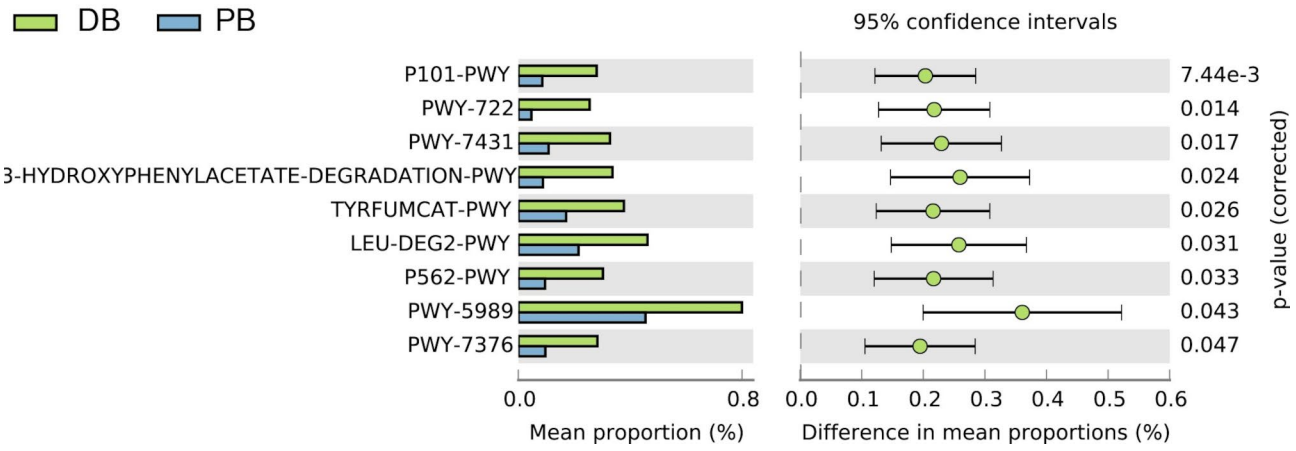

Fig. 10 Analysis of differential metabolic pathways between proximal and distal bowels groups

The results of this sequencing technique for the flora 16SrRNA between atretic intestines and normal fetal stools showed that at the alpha diversity level, which describes total number of species, demonstrating a descending gradient: normal fetal stools > distal atresia > proximal atresia. At the beta diversity level, which describes the degree of difference in colony structure, only the normal control group differed significantly from the distal intestinal tube group in terms of bacterial composition ($P < 0.05$). Further analysis revealed that the difference in flora structure between the distal bowels group and the normal control group was significantly higher than that between the proximal bowels group and the normal control group.

On this basis, the mechanisms of initial intestinal flora establishment in newborns were further explored. The source of initial flora in the fetal gut are currently theorized to be two main pathways: either bacteria infect the fetal gut via the mother's bloodstream through the placenta, or bacteria colonize the neonatal intestines from the digestive tract [9, 13]. The anatomical isolation of distal bowel segments precluded microbial translocation through the digestive tract, supporting the hypothesis of hematogenous microbial transfer. Maternal exposure to diverse environments correlates with increased blood microbiota diversity, which may facilitate microbial translocation and subsequent fetal intestinal colonization via placental circulation [1, 10]. The superior microbial number observed in normal controls likely stems from enhanced environmental exposure of fetal stool specimens, facilitating colonization of diverse bacterial taxa through external contact. Whether amniotic fluid infection during pregnancy or exposure with the surrounding environment, colonization of flora from the external environment is an important way to influence gut florae. Currently, many studies had clarified that the intestines of newborns by natural delivery were more prone to the presence of flora from their mothers' vaginas, whereas children delivered by cesarean section are more likely to exhibit flora similar to that in the air of the delivery room [14, 15]. However, the blood-borne colonization of the flora is trace, so that it was once thought that the fetal intestines should be sterile when relying solely on bacterial culture. In consequence, the invasion of a relatively large number of external flora after the birth of the children changed the structure of the intestinal flora. The heightened complexity of proximal intestinal microbiota likely arises from exogenous microbial colonization. This observed alteration may explain the more pronounced divergence observed between proximal microbiota and normal neonatal gut ecosystems compared to distal counterparts [7]. Collectively, the observed microbial characteristics suggested the establishment of initial intestinal flora in neonates may result from synergistic interactions between hematogenous colonization and exogenous exposure.

In respect of specific differential bacteria, the main intestinal flora with significant differences in intestinal atresia were Ochrobactrum, Rhodococcus, Geobacillus, Paenibacillus, Geobacillus, Herbaspirillum and Methylobacterium. Ochrobactrum is a common soil bacterium belonging to the Brucellaceae family, which is often found in heavily polluted areas, such as factories and hospitals, because of its degradation of heterologous compounds and tolerance to heavy metals. Clinical samples of this genus are often found in human tissues or blood, which was consistent with the result that its content is more abundant in distal intestinal tissue in our study [16, 17]. Similarly, Rhodococcus are widely found in human blood, feces, respiratory tracts, cerebrospinal fluid or other soft tissue abscesses. However, this genus is a group of less pathogenic opportunistic pathogens that primarily infect immunocompromised populations, such as children and AIDS patients. The usual sites of sensitization are skin mucosa, wounds and lungs, but there was no direct evidence of a strong association between this genus and

intestinal diseases [18]. Geobacillus and Paenibacillus have similar biological properties, which are reflected in the fact that the formation of spores makes them extremely tolerant to harsh environments. These bacteria are characterized by high temperature tolerance, rapid resurrection and strong secretion of enzymes, and they can survive in both aerobic and anaerobic conditions. Slightly different, Geobacillus is mainly concentrated to survive in human intestines and most of the bacteria are harmless. Not only that, the antimicrobial substances they produce have broad-spectrum bactericidal activity, which makes such bacteria play an important role in the treatment of intestinal dysbiosis signs. Increased levels of this group of bacteria in the distal intestinal tract may be conducive to the stabilization of intestinal microecology [19]. Herbaspirillum is a genus of gram-negative non-fermenting bacili, and this microorganism is most evident in humans with underlying diseases, such as cancer, cirrhosis, or applied immunosuppression [20]. Some studies have reported rare cases in which timely blood cultures in positive cases yielded positive results [20]. The environment distal to the intestinal atresia due to disruption of continuity the flora within it is mainly derived from blood dissemination, which may explain the low levels of this genus in the distal group. Whereas, the genus Methylobacterium is usually distributed in natural environment such as soil, sewage and leaf surfaces, even in hospital settings. The results of the present study suggested that their levels in the normal group were higher than those in both the proximal and distal groups. However, as an opportunistic pathogen of low pathogenicity, many of the reported cases occurred during invasive procedures [21].

The expression of differential metabolic pathways and functional genes in the differential flora allowed further exploration of the possible effects of microorganisms on the affected children and pregnant mothers. No metabolic pathways with significant differences were found between the normal control group and the proximal bowels group, which may be related to the fact that they share the same exposure to the external environment and similar flora structure. The rich flora diversity of the distal intestine resulted in more flora differences from the proximal intestine group and the normal control group, and all differential metabolic pathways in the distal intestine group were expressed at higher levels than those in the other two groups. Cross-referencing the differential metabolic pathways against the PEGG pathway database showed that distal bacteria were expressed in P562-PWY (inositol degradation), PWY-5989 (stearic acid synthesis), 3-HYDROXYPHENYLACETATE-DEGRADATION-PWY (3-Hydroxyphenylacetate degradation), PWY-7664 (oleic acid synthesis), KETOGLUCONMET-PWY (ketogluconate degradation), PWYG-321 (mycolic acid synthesis), PWY0-1533 (methylphosphate degradation), PWY-722 (nicotinate degradation), and PWY-6282 (palmitoleate degradation) metabolic pathways were higher than those of normal control group in terms of the expression level, whereas in comparison to the proximal group, P562-PWY (inositol degradation), PWY-5989 (stearic acid synthesis), 3-HYDROXYPHENYLACETATE-DEGRADATION-PWY (3-hydroxyphenylacetate degradation), P101-PWY (ectoine synthesis), PWY-7431 (aromatic amines degradation), LEU-DEG2-PWY (L-leucine degradation), PWY-7376 (vitamin B12 synthesis).

This is followed by further analysis of the possible impacts about intestines arising from metabolic pathways. The ability of inositol to reduce the diabetes had been demonstrated in many studies. Researches in animal models suggested that inositol not only inhibited intestinal glucose absorption and increased muscle glucose uptake, but also delayed gastric emptying and intestinal peristalsis in normal or diabetic animals in order to lower blood glucose levels [22]. Laboratory studies had proved that intestinal anaerobic flora could convert inositol toward propionate and acetate [23]. Besides, stearic acid, oleic acid and palmitoleic acid are saturated long-chain fatty acids that affect lipid metabolism and transport, which are widely found in various cereals. The study comparing these three fatty acids cross-sectionally suggested that stearic acid and palmitoleic acid adversely affect the transport of triacylglycerols and phospholipids in immature intestinal cells compared to oleic acid [24]. Leucine is a branched-chain amino acid associated with hemoglobin synthesis, and also studies on the gut have found it to have an important role in reducing fat deposition, improving insulin sensitivity, and increasing adipose tissue browning, as well as suppressing the ratio of Firmicutes and Bacteroidetes in the bowels [25, 26]. The effects of these metabolic pathways may indicate that the affected children and their mothers may suffer from disorders of glucose metabolism and lipid metabolism, but the exact extent of the effects and the causal relationship with intestinal atresia need to be verified by further studies. Whereas ketogluconic acid is derived from the oxidation of glucose catalyzed by Gluconobacter [27]. Regrettably, no basic studies or animal models have been found on the intestinal relevance of this metabolite, so the significance of ketogluconic acid degradation is not unclear. Mycolic acid is the specific lipid component of the Mycobacterium envelope, which is essential for the survival and virulence of this pathogen [28]. Furthermore, niacin is a water-soluble vitamin necessary for the body to form the coenzymes NAD and NADP, which has the function on pellagra treatment, hemangiectasis and lipid-lowering. The results of an animal study showed that niacin attenuated weight loss and diarrhea, increased expression of antimicrobial peptides, and enhanced the barrier function of the intestinal epithelium in weaned

piglets [29], suggesting that the increased level of niacin synthesis may have a protective effect on the closed distal intestinal canal.

Aromatic bioamines are produced from aromatic and aliphatic bioamines by decarboxylation. Many studies had shown that Escherichia coli can synthesize enzymes required for the degradation of 3-hydroxyphenylacetate, and its degradation products bioamines have important physiological functions in both eukaryotes and prokaryotes [30]. One of these, 5-hydroxytryptamine (serotonin) is an aminoalkylindole produced by the decarboxylation of L-tryptophan, which had been reported to be involved in the oxidation of tryptophan by species such as Micrococcaceae, Geobacillus and Firmicutes. A large number of studies have demonstrated that 5-hydroxytryptamine is critical for the effects of mood, hunger, sleep and pain, as well as gastrointestinal motility and secretory function, thus it is reasonable to suspect that the mood and diet of the corresponding maternities may also have been affected in some way [31, 32]. Vitamin B12, which is dependent on intestinal flora for synthesis and absorption, is involved in promoting the development and maturation of erythrocytes and maintaining the metabolism and function of nerve myelin sheaths. A meta-analysis has found that a deficiency of vitamin B12 has been associated with inflammatory bowel disease [33], but excessive amounts of vitamin B12 can also result in intestinal dysbiosis, leading to hypo-inflammation and pathogen colonization, and thus its specific impact on affected children is still uncertain [34]. Ectoine is a compatible solute that helps organisms survive extreme osmotic stress by acting as an osmotic agent, but also inhibits intestinal histologic changes and elevated inflammatory mediators to protect intestinal barrier stability [35]. Moreover, another study showed that ectoine reduced ischemia-reperfusion injury in the intestinal mucosae and muscles [36].

According to the above, the functional prediction of the flora metabolic pathway suggests that the differential metabolic pathway may have a protective effect on the intestinal mucosa of the child. Meanwhile, the metabolites produced may also bring a negative effect on the glycemic control and lipid transport of the pregnant woman and the fetus, even as well as passive emotions. Of course, this functional prediction based on the comparison of genetic information repositories were located in the theoretical level. Further investigations and experiments are needed to prove the final effects and causal links.

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Author contributions
YC and ZW together completed data collection, laboratory procedures, statistical analysis and manuscript writing; XM and ZW provided conception and design of the study, participated in the modification of the manuscript; JL and XZ were in charge of the preparation of the laboratory. FR, JY, QL, DX, LZ and LDZ contributed to data arrangement and article revision.

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Data availability
Sequence data that support the findings of this study have been deposited in the European Nucleotide Archive with the primary accession code PRJNA1160831.

Declarations

Ethics approval and consent to participate
This study involving human participants data was conducted in accordance with the ethical principles of the Declaration of Helsinki (2013 revision) and approved by the Ethics Committee of Shenzhen Children's Hospital (No.202104202). Written informed consent was obtained from all participants or their legal guardians, with detailed explanations provided regarding the study's purpose, procedures, risks, and confidentiality measures. Meanwhile, the anonymous original data has been uploaded to the corresponding website. Additional safeguards, including dual parental consent and independent ethical oversight, were implemented for neonatal participants to ensure welfare prioritization throughout the research. The experimental study obtained informed consent from all the parents of the children involved. The research process does not interfere with patient care and conflicts of interest.

Consent for publication
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Competing interests
The authors declare no competing interests.

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