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ORIGINAL ARTICLE

Abundance and Composition of the Meconium Microbiota in Preterm Infants with Infections, Feeding Intolerance, or Necrotizing Enterocolitis

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SUMMARY

Background: **The role of the microbial flora of the gut of a newborn is of scientific and practical interest. The aim of this study was to assess the abundance and composition of the meconium microbiota in preterm infants with infections, feeding intolerance, or necrotizing enterocolitis (NEC).**

Methods: **Eighty-four preterm infants born by cesarean section were prospectively enrolled in this study. Out of the 28 diseased infants, 23 developed infections, including 8 cases of sepsis, 10 cases of pneumonia, 1 case of enterocolitis, and 4 cases of NEC. Fifty-six (66.67%) preterm infants without these characteristics served as control group. General clinical information (gender, gestational age, birth weight, presence of preterm rupture of membranes, Apgar 1-minute score, and duration of hospitalization) was collected. First-pass meconium samples were collected for 16S rRNA microbiological analysis.**

Results: **Compared with the control group, the diseased infants had a lower gestational age ($p < 0.001$) and lower body weight ($p = 0.014$). In addition, the hospitalization time of the diseased infants was longer than that of the control group ($p < 0.001$). On the α-diversity measure, there was no difference in species abundance and diversity between the two groups; on the β-diversity measure, the differences in the microbial composition of the two groups were subjected to PCoA analyses, which showed that there was a difference between the disease group and the control group. At the phylum level, the dominant phylum in both groups was *p_*Proteobacteria, with higher abundance of *p_*Firmicutes in the disease group. At the genus level, the dominant genus in both groups was *g_Novosphingobium*. Microbiome phenotype prediction by BugBase revealed that microbial phenotypes 'Gram-positive' and 'Anaerobic' were abundantly increased in the disease group; microbial function prediction did not differ between the two groups in terms of significant function.**

Conclusions: **The impact of infections, feeding intolerance, and NEC on a host is complex. Preterm infants delivered by cesarean section have *p_*Proteobacteria as the dominant phylum, with a higher abundance of *p_*Firmicutes in the disease group, a difference contributed by g_*Peptoniphilus*.**

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KEYWORDS

preterm infants, meconium, gut microbiota, neonatal infections, feeding intolerance

INTRODUCTION

It was accepted early on that the fetus' gut is sterile while in its mother's womb, and with the development of high-throughput sequencing technology, there has been a growing enthusiasm for research related to the flora outcomes of fetal feces. It has been found that the construction of fetal gut microbiota may begin in the mother, and the microecological environments of the mother's gut, amniotic fluid, placenta, and birth canal may provide microorganisms for it [1]. The structure of neonatal gut microbiota is associated with development, health, and infection [2]. Neonatal infections are an important link in the development of many diseases and are one of the leading causes of neonatal mortality [3], accounting for 29% of neonatal deaths [4]. This may be due to the fact that the development of various organs and systems of neonates is immature, and the humoral and cellular immunity are in an immature state, resulting in their high susceptibility to infectious diseases [5]. Infections in preterm infants commonly include pulmonary infections, gastrointestinal infections, sepsis, and septic meningitis [6]. A review article reported that the dynamic balance of intestinal microorganisms in terms of species, number, and abundance is not only related to neonatal growth and development, but also closely associated with neonatal immunocompetence and metabolism [7]. In addition, it has been shown that the fetal flora can predict the risk of future infections in neonates. For example, increased abundance of pathogenic bacteria (Escherichia coli, Klebsiella, non-fermenting gram-negative bacilli, etc.) in the gut is closely associated with the development of neonatal sepsis [8]. Thus, dysbiosis of the gut microbiota in preterm infants may be strongly correlated with the occurrence of infectious events in neonates.

Gut microbiotas, as an integral part of the human body, live in mutualistic symbiosis with the host and act on target organs through their metabolites. When dysregulated, gut microbiotas can cause disease in newborns, as they are crucial for immune system development and maturation [9]. Following birth, the intestine serves as a crucial site for microbial colonization. The dynamic equilibrium of microbial quantity, diversity, and relative abundance is integral to material metabolism, the maturation of the immune system, the promotion of growth and development, and the establishment of intestinal barrier function [10]. The imbalance of gut microbiota in infants and young infants is closely associated with respiratory diseases and infectious diseases [11]. Since infancy is a critical period for the establishment of gut microbiota, factors such as decreased immunity and excessive use of broad-spectrum antibiotics may lead to an imbalance in the intestinal microbiome. Dysregulated gut microbiota not only affects the growth and development of newborns but also causes infectious diseases [12]. Mode of delivery, feeding mode, gestational age, etc. can have an impact on the colonization of neonatal gut microbiota [13,14]. The imbalance of gut microbiota can lead to self-infection or the transfer of colonizing bacteria to other sites and induce infection, gastrointestinal tract dysfunction, manifested as difficulty in defecation, diarrhea, etc., and the onset of certain organic intestinal lesions (e.g. necrotizing enterocolitis, NEC). Based on this, it is of diagnostic significance to analyze the microbial source, pathogenic mechanism, microbial-host interactions, and microbiota in the development of infections in preterm infants.

Therefore, an in-depth study of the dynamic changes of neonatal gut microbiota may be instrumental in modulating gut microbiota colonization within, thereby potentially reducing the incidence of neonatal infections. This study employed high-throughput DNA sequencing to analyze the bacterial communities present in fecal samples from premature infants. It focused on assessing the diversity alterations in the gut microbiota, examining the compositional characteristics of intestinal colonies in infected neonates, and provided relevant evidence of gut microbiota dysbiosis for the risk of infection in premature infants.

MATERIALS AND METHODS

Study population

This study was a prospective cohort study. We recruited preterm neonates admitted to Hangzhou Women's Hospital between January 2021 and May 2023. During the first month of the in-hospital care period, all preterm infants underwent routine blood tests, blood cultures, chest X-rays, etc. Based on the relevant diagnostic criteria and pathogenetic data, 28 cases with neonatal infections as well as feeding intolerance (4 cases with NEC, 9 cases with NEC-feeding intolerance, 5 cases with feeding intolerance) were included in the disease group, and 56 non-infected neonates served as the control group.

Inclusion criteria: 1) birth by cesarean section in Hangzhou Women's Hospital; 2) < 6 hours from birth to admission, without breastfeeding and the use of antibiotics; and 3) single fetus.

Exclusion criteria: 1) pregnant women with immune diseases, use of immunosuppressants or antibiotics; 2) infants with severe congenital diseases such as congenital heart disease, brain dysplasia, duodenal atresia, etc. requiring surgery; 3) infants with chromosomal anomalies or inherited metabolic diseases; and 4) those with incomplete clinical data.

The study was approved by the Ethics Committee of Hangzhou Women's Hospital, and the guardians of all infants signed written informed consent. Neonatal infections were defined as positive blood cultures within 48

hours after birth; positive blood cultures 48 hours or more after birth [15]. Neonatal feeding intolerance was defined as one of the following: bile retention or bilious vomiting on ≥ 2 consecutive feedings, or > 50% gastric residue prior to feeding on ≥ 2 consecutive feedings. NEC was defined as confirmed by laboratory and imaging tests [16].

Collection of first-pass meconium samples

Newborns usually pass meconium within 24 hours. Fresh meconium (3 g) was obtained using a sterile cotton swab. The swab was taken without touching the diaper or swabbing from a urine or feces collection basin to avoid contamination. Immediately after collection, it was placed in a sterile fecal collection tube, quickly frozen in liquid nitrogen, and subsequently stored at -80°C until analysis.

Collection of clinical information

General neonatal characteristics were collected, including gender, gestational age at birth, birth weight, presence of preterm rupture of membranes, Apgar 1-minute score, and length of hospitalization.

DNA extraction

Fecal samples of 200 mg were placed in 2 mL centrifuge tubes on ice. Buffer was added to the samples and mixed using a TGrinder H24 Homogenizer (OSE-TH-01) (2 cycles of oscillation at 6 M/S for 30 seconds at 30 seconds intervals). Incubation was performed at 70°C for 15 minutes, during which time the samples were shaken 2 - 3 times. Subsequently, high-speed centrifugation (12,000 rpm, 3 minutes) was performed, and the supernatant was taken; 100 μL of RNase A was added and mixed with shaking; 200 μL of buffer was added and mixed with shaking; high-speed centrifugation (13,400 × g, 3 minutes) was performed, and the supernatant was taken; an equal volume of buffer was added and mixed. The obtained solution was centrifuged (13,400 × g, 3 minutes), added with 700 μL of rinse solution, and centrifuged (13,400 × g, 3 minutes). The abovementioned rinsing step was repeated 2 times, and the residual rinsing solution was dried thoroughly. Then, 50 μL of elution buffer was added to the adsorption film and left at room temperature for 2 - 5 minutes, and the solution was collected into a centrifuge tube.

16S rRNA amplicon analysis

The 16S V3-V4 primers 341 F (5'-CCTACGGGGNGGCWGCAG-3') and 806 R (5'-GGACTACNVGGGGTATCTAAT-3') were used to amplify the 16S rRNA genes. Microbial DNA in the feces of preterm infants was qualified by using Denovix Ultra-micro UV-visible Spectrophotometer DS-11 and gel electrophoresis at appropriate concentration. The PCR products were quantified by QuantiFluor™-ST Blue Fluorescence Quantification System (Promega, WI, USA), and then mixed according to the sequencing volume required for each sample. Sequencing was performed using the Illumina MiSeq platform (major Bio-pharm, Shanghai, China).

Bioinformatics analysis

Sequencing data statistics

Raw data were quality filtered and corrected using TrimomaticV0.33 software [17], and then sequences were assembled and corrected using FLASH software [18]. High-quality reads were arranged into operational taxonomic units (OTUs) with 97% similarity using UPARSE 7.0 software [19], and each was categorized according to a classifier algorithm against the Greengenes database [20]. Based on the number of sequences in each OTU, a corresponding abundance table was obtained, and the data were analyzed based on this abundance table.

Species diversity analysis

The number of species (e.g. OTUs) shared and unique to different groups of samples was analyzed using the R language V 3.3.1 software, which allows for a more intuitive representation of similarities and overlaps in species (e.g. OTUs). α-diversity (Chao and Shannon indices) was analyzed using QIIME [21] and R software. β-diversity analysis was applied to calculate inter-sample difference based on abundance information among sample sequences to reflect whether there were significant gut microbiota differences between groups. Principal coordinate analysis (PCoA) and analysis of similarity (ANOSIM) tests were performed using the weighted UniFrac algorithm. The Wilcoxon rank sum test was used to analyze gut microbiota dysbiosis; the larger the value indicates the greater the degree of flora disturbance. According to the results of taxonomic analysis, the species composition of different subgroups at each taxonomic level could be known, and the histograms of the microbial composition of different groups were plotted by using the R language V 3.3.1 software, and the significance levels of the differences in the abundance of species among different samples were detected by the Wilcoxon rank-sum test. Meanwhile, the visualization diagram describing the correspondence between samples and species using Circos-0.67-7 software reflected the proportion of dominant species composition of each sample and also the proportion of distribution of each dominant species in different samples [22]. Using FastTreeversion V 2.1.3 software, an evolutionary tree was constructed by selecting OTUs or sequences corresponding to taxonomic information at a certain level according to the maximum likelihood method, and the results were presented in the form of a combined graph of the evolutionary tree and reads abundance [23].

Phenotype prediction by BugBase and function prediction by PICRUSt 2.0

Microbial phenotypes were predicted using BugBase software by normalizing the OTUs by the predicted 16S copy number and then using the pre-calculated files provided [24]. Among the phenotypes were ‘Gram-positive’, ‘Gram-negative, ‘Biofilm forming’, ‘Pathogenic’, ‘Mobile element containing’, ‘Oxygen demanding’, and ‘Oxygen utilizing’, including ‘Aerobic’, ‘Anaerobic’,

Table 1. General clinical baseline information of preterm infants.

Data	Disease group (n = 28)	Control group (n = 56)	p-value
Gender			**0.753**
Male	**12 (42.86)**	**22 (39.29)**	
Female	**16 (57.14)**	**34 (60.71)**	
Gestational age (weeks)	**32.30 ± 2.84**	**34.06 ± 4.38**	**< 0.001**
Extremely premature infants	**6 (21.43)**	**3 (5.36)**	**0.054**
Birth weight (g)	**1,729.1 ± 654.7**	**2,040.5 ± 407.2**	**0.014**
Premature rupture of membranes	**7 (25.00)**	**8 (14.29)**	**0.227**
Apgar 1-min score (points)	**9[8, 9]**	**9[9, 9]**	**0.823**
Duration of hospitalization (days)	**31 [12, 42]**	**13 [7, 22]**	**< 0.001**
Perinatal infections	**17**	**/**	**/**

Normally distributed measurements are shown as mean ± standard deviation and were compared between groups using Student's *t*-test, data for continuous variables with skewed distributions are shown as median [25th, 75th percentile] and were compared between groups using the Mann-Whitney U test. Count data are expressed as frequencies (n) and ratios (%), and were tested by chi-squared test or Fisher's exact test. $p < 0.05$ was considered statistically significant.
Extremely preterm: preterm infants with a gestational age of 28 to 31 weeks.
Perinatal infections: specifically, infections that occurred during the period from 28 weeks of gestation to 7 days after delivery.

'Facultatively anaerobic', and 'Oxidative stress tolerant'. Based on known microbial genes as a reference, PICRUSt analyses were conducted to predict the composition of functional genes from 16SrRNA sequencing data using PICRUSt 2 software to obtain differences in function between samples or subgroups. The function of gut microbiota was predicted from 16S rRNA amplicon data. Kyoto Encyclopedia of Genes and Genomes (KEGG) and KEGG Orthology (KO) profiles were predicted for each sample.

RESULTS

General condition of infants

A total of 86 preterm infants were included in this study. A total of 86 meconium specimens were collected. Two specimens (one each from the disease and control groups) were excluded due to quality control. Finally, 84 specimens were adopted for analysis. Out of these, 50 (59.52%) were females and 34 (40.48%) were males. In the disease group, there were 23 cases (27.38%) with infections (4 of which were NEC and 9 combined with feeding intolerance) and 5 cases (5.95%) with feeding intolerance alone. Among these infants, more than half of the infants with the disease (17 cases, 60.71%) had perinatal infections. Fifty-six (66.67%) preterm infants without these characteristics served as control group. Table 1 shows the general condition of the infants, and the results showed that the infants in the disease group had a lower gestational age ($p < 0.001$) and lower birth weight ($p = 0.014$) compared to the control group. In addition, the duration of hospitalization of the infants in the disease group was longer than in the control group ($p < 0.001$).

Comparison of α-diversity and β-diversity

The Venn diagram showed that the disease group had 400 OUTs, while the control group had 748 (Figure 1A), and the two groups shared 259 OUTs. On the α-diversity measure, analysis of the Chao index (abundance) and Shannon index (diversity) in the two groups showed that there was no difference in species abundance and diversity between the two groups (Figure 1B, C), and the dilution curves tended to be smoothed, indicating that the amount of sequencing data was asymptotically reasonable (Figure 1D). On the β-diversity measure, PCoA analysis showed a difference between the disease and control groups in microbial composition of the samples (Figure 1E). ANOSIM-based analysis showed significant differences between the two groups (Figure 1F). In addition, MDI results showed a higher level of microbial dysbiosis in the disease group compared with the control group (Figure 1G).

Analysis of species composition and abundance

At the phylum level, *p*_Proteobacteria was dominant in both groups, followed by *p_Cyanobacteria* (Figure 2A). Among them, *p*_Proteobacteria accounted for more than 90% (Figure 2B). There was a significant difference in the abundance of *p*_Firmicutes between the two groups, and the disease group had a higher abundance of *p*_Firmicutes (Figure 2C).

At the genus level, the species composition of both groups was similar, with *g_Novosphingobium* as the dominant group, followed by *g_Bradyrhizobium* (Figure 2D, E). Notably, the abundance of *g_Bradyrhizobium* was significantly reduced in the disease group, while the abundance of *g_Peptoniphilus* and *g_Peptostreptococcus* increased. In addition, the presence of *g_Alkanindiges* (microorganisms capable of digesting,

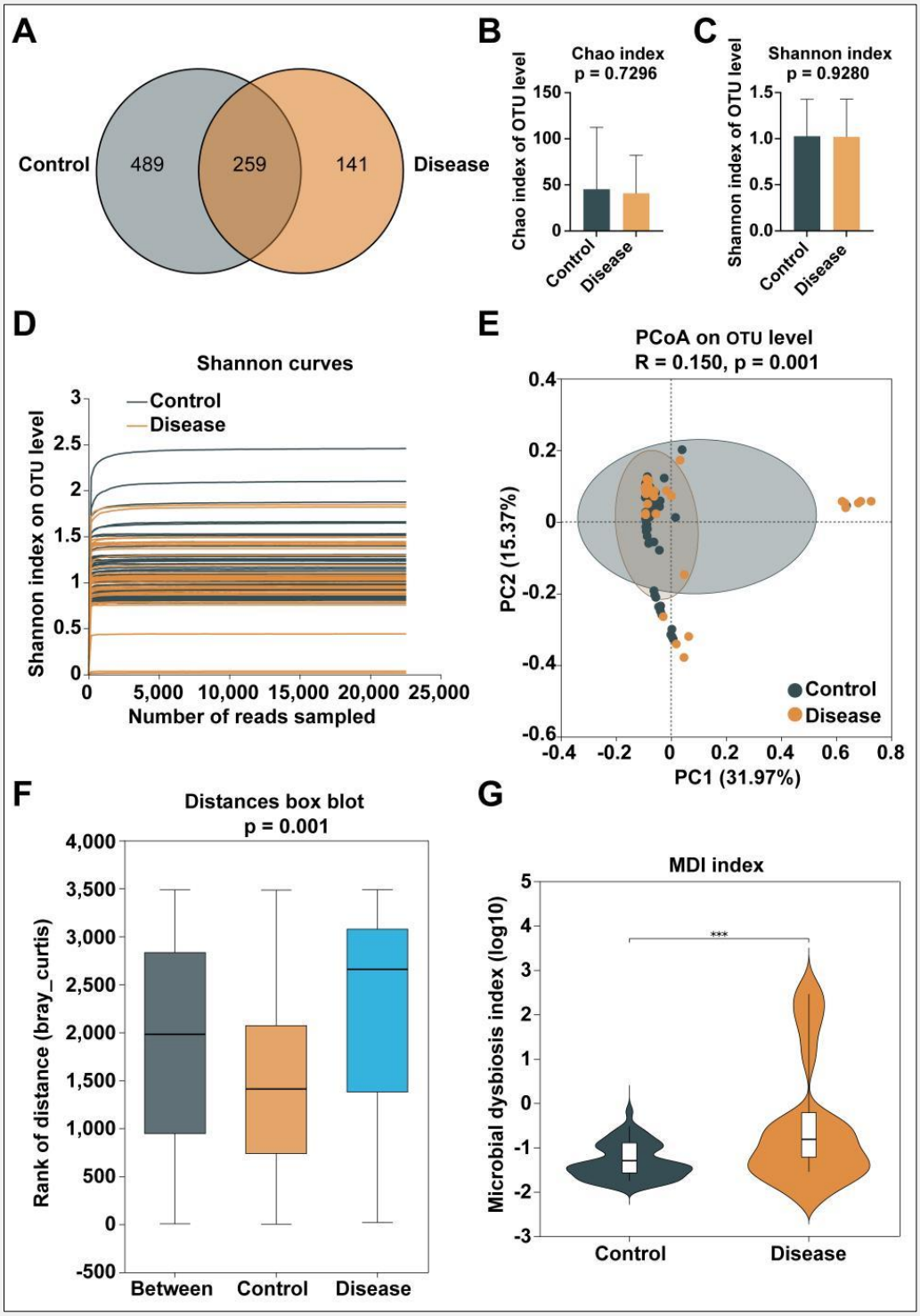

Figure 1. α-diversity and β-diversity comparison.

A) OTU-based Venn diagram. B) Chao index histogram. C) Shannon index histogram. D) Shannon index dilution curve. In the dilution curve, the horizontal coordinate is the number of randomly selected sequencing strips from a certain sample, and the vertical coordinate is the number of OTUs that can be constructed based on this number of sequencing strips. E) Weighted Unifrac distance-based PCoA analysis. Each point indicates a sample, and samples from the same group are indicated using the same color. F) Box line plot of weighted Unifrac analysis between 2 groups; "Between" describes the differences in microbial community diversity between two groups of samples. G) MDI index. $p < 0.05$ is statistically significant.

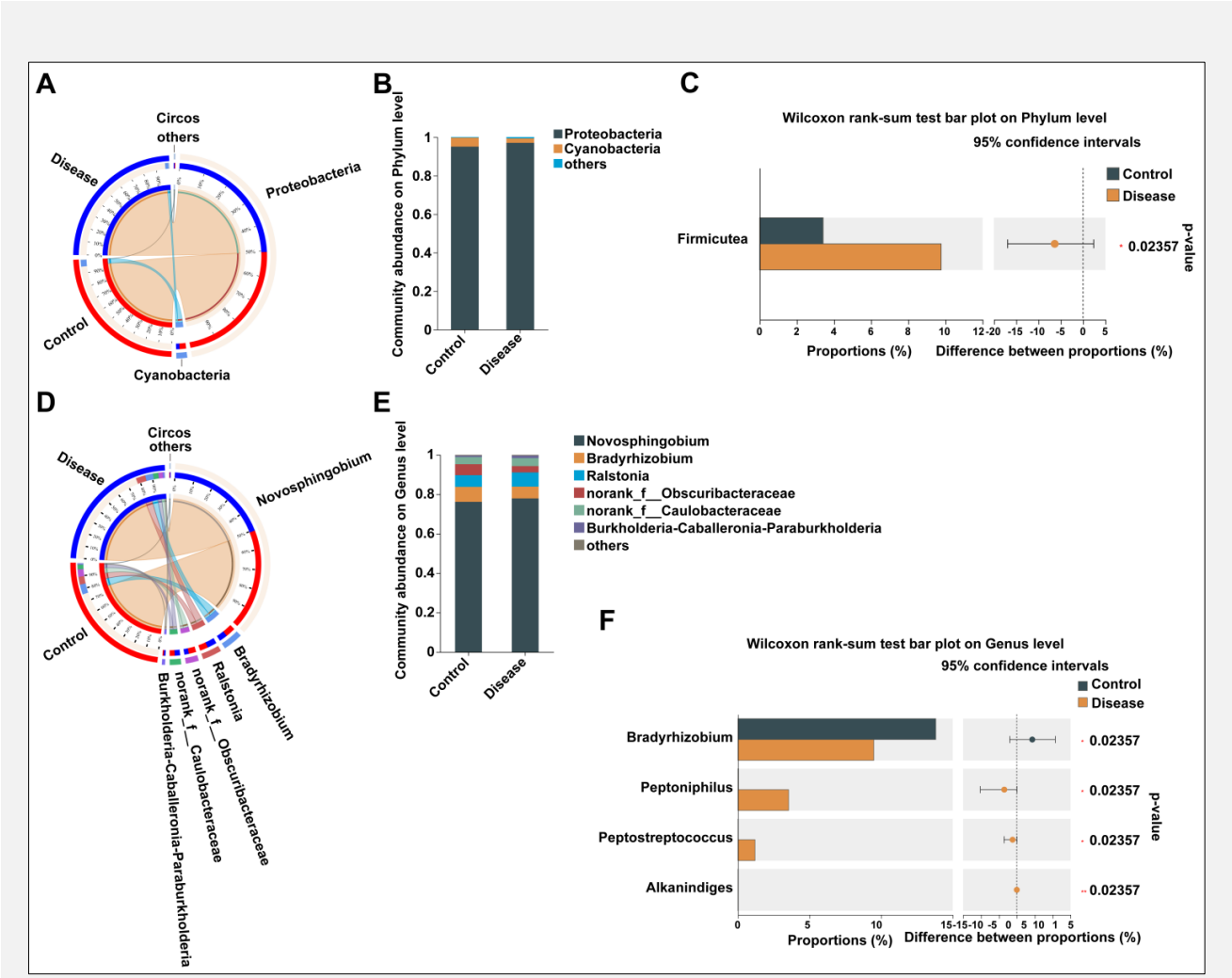

Figure 2. Species composition analysis and relative abundance.

A) Circos plot at the phylum level. The outermost layer displays information on grouping, the middle layer displays percentage information on relative abundance, and the inner layer shows bolding or thinning based on abundance to provide a more intuitive picture of the distribution and interrelationships of species or genes. B) Histogram of relative abundance of species at the phylum level. C) Histogram of species differences at the phylum level. D) Circos plot at the genus level. E) Histogram of relative abundance of species at the genus level. F) Histogram of species differences at the genus level. Microbial names preceded by p_ and g_ indicate phylum and genus, respectively. $p < 0.05$ is statistically significant.

utilizing, or metabolizing alkane compounds) was observed in the disease group (Figure 2F).

Species evolutionary tree and BugBase phenotype prediction

Representative sequences of the top 25 genera were obtained by multiple sequence alignment (Figure 3A). The results showed that the genera with higher abundance in the control group were *g_Novosphingobium*, *g_Bradyrhizobium*, *g_norank_Obscuribacteraceae*, *g_Ralstonia*, and *g_norank_Caulobacteraceae*, respectively, and the genera with higher abundance in the disease group were *g_Novosphingobium*, *g_Bradyrhizobium*, *g_Rothia*, *g_Ralstonia*, and *Burkholderia-Caballeronia-Paraburkholderia*, respectively. The abundance of *g_Bradyrhizobium* in the disease group was significantly lower than that in the control group. The composition of the seven major groups of microorganisms was analyzed by using BugBase, and the composition of the two groups is shown in Figure 3B. The microbial phenotypes 'Gram-positive' and 'Anaerobic' were increased in abundance in the disease group (Figure 3C).

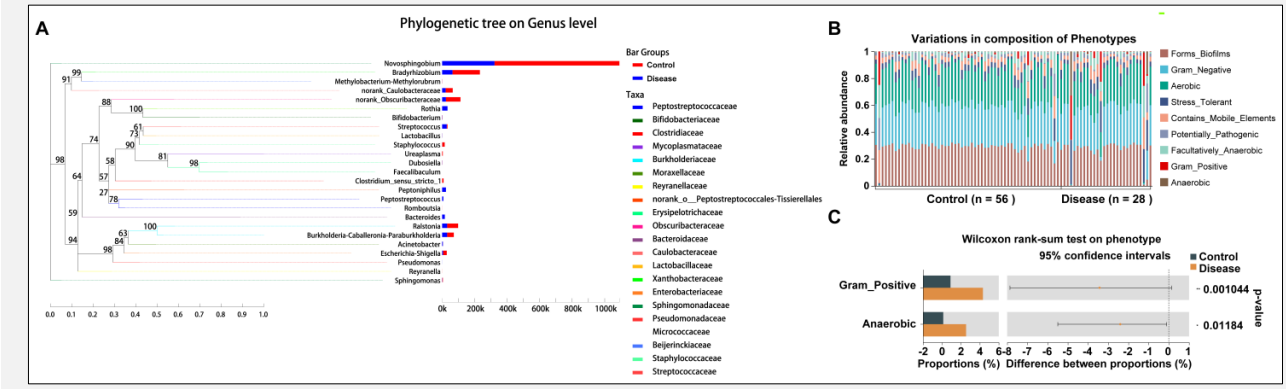

Figure 3. Genus-level species phylogenetic relationships and BugBase phenotype predictions.

A) Phylogenetic tree. On the left is the phylogenetic tree, each branch represents a class of species, the branches are colored according to the taxonomic level to which the species belongs, and the length of the branch is the evolutionary distance between two species, i.e. the difference of the species; on the right, the bar chart shows the percentage of reads of the species in different subgroups. B) BugBase phenotype prediction histogram. C) Analysis of differences between phenotype groups.

Function prediction

PICRUSt2 function prediction was utilized to predict functional information. Unfortunately, both KEGG and GOG analyses showed no significant differences between the two groups (Figure 4A, B). Weak gene expression in the pathways cellular processes (carbohydrate metabolism, amino acid metabolism, xenobiotic biodegradation and metabolism, metabolism of cofactors and vitamins, energy metabolism, signal transduction, lipid metabolism, membrane transport, cellular community - prokaryotes, etc.) and organismal systems (nervous system, immune system, transcription, circulatory system, substance dependence, immune disease, digestive system, excretory system) was only observed in few preterm infants. In addition, these few infants had weaker expression of genes related to the circulatory system and substance dependence pathways (Supplementary Figure 1).

DISCUSSION

Preterm infants are at high risk of developing infections, which may lead to sepsis, pneumonia, and other life-threatening conditions if not treated in time. Therefore, it is of great clinical significance to explore the influencing factors and related mechanisms of preterm infection, so as to screen the etiology and take symptomatic measures in clinical treatment. The microbiota of preterm infants is involved in adverse neonatal outcome events [25]. This suggests a correlation between gut microbiota and disease development in preterm infants. Eighty-four preterm infants were included in this study, out of whom 28 had significant signs of disease (23 developed infections). Although both groups of preterm infants were broadly similar in terms of gut microbial composition, subtle differences existed. This is reflected in: 1) at the phylum level, *p*_Firmicutes had a higher abundance in the disease group; at the genus level, *g_Bradyrhizobium* abundance was significantly reduced in the disease group, while *g_ Peptoniphilus* and *g_Peptostreptococcus* abundance was increased; and (2) out of the seven major microbial phenotypes, the 'Gram-positive' and 'Anaerobic' phenotypes increased abundantly in the disease group.

Preterm neonates are prone to intestinal dysbiosis because they leave the mother's body too early, and their intestinal floras are not yet fully matured in terms of colonization and development. This dysbiotic state is one of the major causes of the impaired intestinal barrier [26]. Dysbiosis of intestinal flora and impaired intestinal barrier provide favorable conditions for infections in preterm infants. Out of the 28 cases of infants with the disease in this study, 23 developed infections, including 8 cases of sepsis, 10 cases of pneumonia, 1 case of enterocolitis, and 4 cases of NEC. It is known that the multiplication of potentially pathogenic microorganisms in the intestine, such as Aspergillus phylum, Shigella spp., and Escherichia coli spp., and the metabolites produced by them can disrupt the integrity of the intestinal mucosa, leading to impaired intestinal barrier function, which can in turn lead to enterocolitis and immune responses [27]. These pathological changes affect the digestive and absorptive capacity of neonates, making it difficult for them to digest and absorb the nutrients they ingest, thus triggering feeding intolerance. In

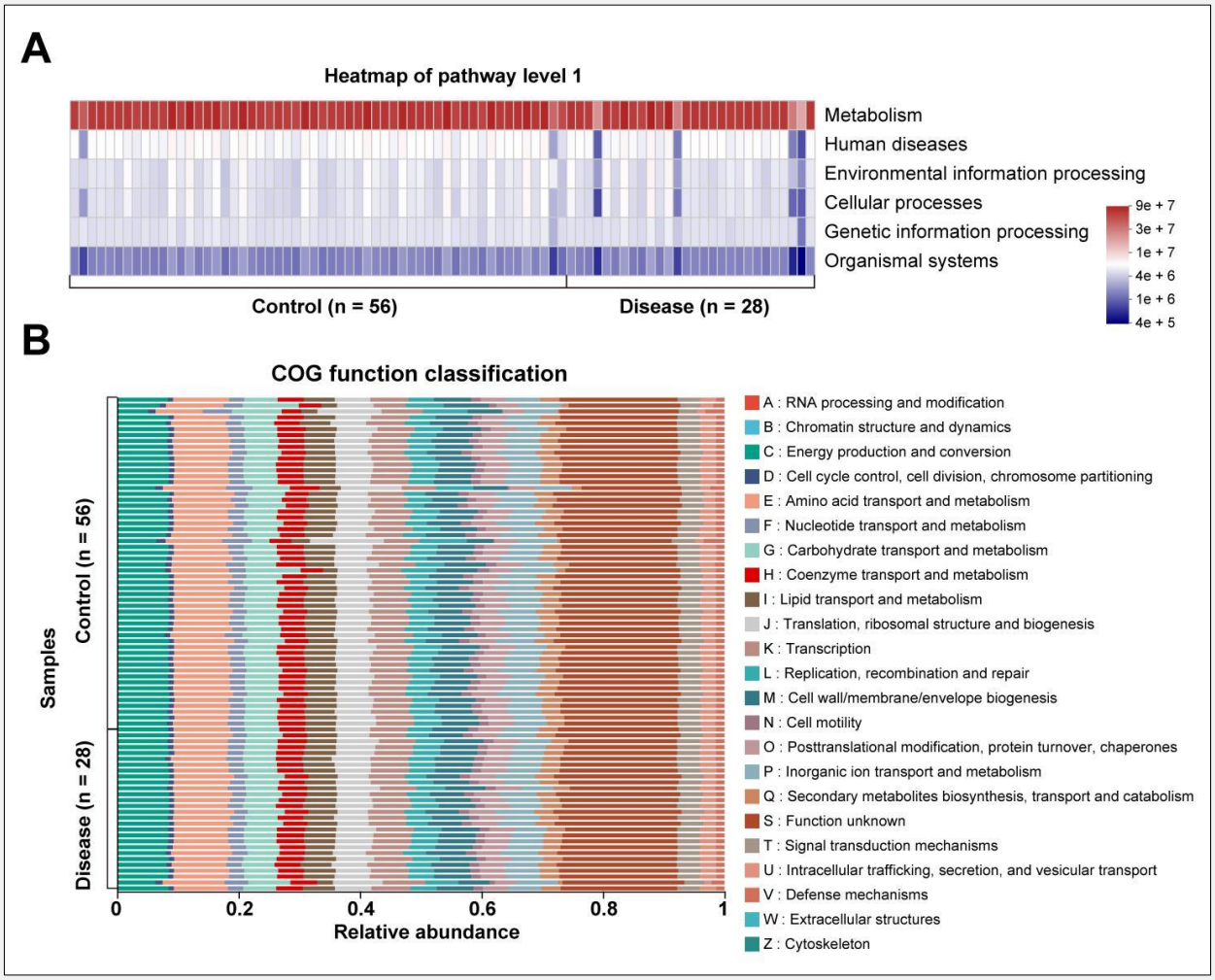

Figure 4. PICRUSt2 function prediction.

A) KEGG pathway primary classification level. B) GOG functional classification.

addition, pathogenic bacteria affect the secretion and activity of digestive enzymes, thus reducing digestion [28]. The clinical signs of feeding intolerance in preterm infants are very similar to the early manifestations of NEC, and severe feeding intolerance may also eventually progress to NEC [29]. Therefore, in the present study, it was observed that out of 23 infected patients, 9 cases had combined feeding intolerance. In addition, the remaining 5 cases in the disease group had feeding intolerance alone. The mode of delivery is associated with differences in the microbial composition of the gut in the early neonatal period. Compared to cesarean delivery, newborns delivered vaginally share 23 microbiota groups with their mothers, dominated by *Bacteroide* and *Bifidobacterium* [30]. Preterm infants delivered by cesarean section were selected for the study in order to minimize the effect of the maternal microbiota. Given that feeding is the most promising influence on the gut microbiota of newborns, meconium from preterm infants without breastfeeding was selected for microbial composition analysis. Previous studies have shown that gut microbial diversity is lower in preterm infants than in full-term infants [31-33]. There seem to be no studies reporting the diversity of microbiota in the meconium between preterm infants with infections, feeding intolerance, or NEC and preterm infants without apparent disease. Our results showed no significant difference in microbial diversity and abundance between the disease and control groups among preterm infants. However, we observed a significantly higher degree of microbial

dysbiosis in the disease group, indicating that the microbial composition of the two groups is somewhat different. β-diversity measures were confirmed by the results of PCoA analysis based on the weighted Unifrac distance algorithm and the Anosim test.

Gut microbial alterations in preterm infants are associated with diseases such as late-onset sepsis and NEC [7]. Therefore, changes in the composition and abundance of gut microbiota are related to neonatal growth and development and are also closely related to neonatal immunocompetence and metabolic capacity. In the fetal stool of both term and preterm newborns, Firmicutes, Bacteroidetes, and Proteobacteria are the phylum-level core microbiomes [34]. However, all the fetal fecal samples in the present study showed an absolute predominance of *p*_Proteobacteria. In the above study, the authors did not mention the mode of neonatal delivery. Infants delivered vaginally receive maternally transmitted microorganisms, whereas cesarean delivery may expose the fetus to microbial contamination in the surgical environment [35]. Therefore, it is possible that this may have contributed to the inconsistency of the gut microbiota in the present study with previous studies. In particular, the disease group had a higher abundance of *p*_Bradyrhizobium than the control group. At the genus level, the microbial composition of the meconium of preterm infants consisted predominantly of *Novosphingobium* and *Bradyrhizobium*, which are not typical intestinal microorganisms and have relatively limited or specific roles in the gut. It is more likely that the dominant genera in this study were derived from microbial contamination of the surgical environment from fetal exposure during cesarean delivery. Of interest, *Peptoniphilus* and *Peptostreptococcus* were in higher abundance in the disease group than in the control group. *Peptoniphilus* can cause opportunistic infections and is commonly associated with diabetic ulcers, chronic wounds, and bone and joint infections [36]. *Peptostreptococcus* is mostly reported to be beneficial in the gut, promoting nutrient absorption, modulating excessive inflammatory responses in the gut through specific metabolic pathways and metabolites, and maintaining the intestinal epithelial barrier [37]. However, their potential risks could not be ignored, as Long et al. have shown that anaerobic digesting streptococcus in the mucosal microbiota is one of the pathogens that promote the occurrence of colorectal cancer and regulate tumor immunity [38]. Among the seven microbial phenotypes predicted, 'Gram-positive' and 'Anaerobic' had increased abundance in the disease group, a feature perhaps contributed by *Peptoniphilus* at the genus level. Finally, in the function prediction, unfortunately, we did not observe significant differences in the functional information of the two groups of meconium microbiota. Overall, in preterm infants, there are differences in the composition of gut microbiota in meconium from infections, feeding intolerance, and no disease characteristics. The magnitude of these differences may be limited by exposure to microbial contamination in the surgical environment during cesarean delivery, reducing the analysis of microbial characteristics potentially affecting disease. However, it cannot be ruled out that neonatal infections, feeding intolerance, or even NEC can occur due to subsequent feeding and therapeutic interventions affecting the gut microbial composition.

CONCLUSION

Gut microbiotas are dysregulated in preterm infants. The impact of infections, feeding intolerance, and NEC on a host is complex. Preterm infants delivered by cesarean section have Proteobacteria as the dominant phylum, with a higher abundance of Firmicutes in the disease group, a difference contributed by *Peptoniphilus*.

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The effect of donor breast milk on gut microbiology and metabolites in preterm infants (A20210366).

Availability of Data and Materials:
The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Ethical Approval Statement:
The present study was approved by the Ethics Committee of Hangzhou Women's Hospital [2020]Ethical Approval No.(09)-09, and written informed consent was provided by all patients prior to the study start. All procedures were performed in accordance with the ethical standards of the Institutional Review Board and the Declaration of Helsinki and its later amendments or comparable ethical standards.

Declaration of Interest:
The authors have no conflicts of interest to declare.

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