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Does preeclampsia impact the gut microbiota of preterm offspring during early infancy?

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Abstract

Preeclampsia (PE) is a pregnancy complication characterized by high blood pressure and organ damage. This study investigates the differences in the gut microbiota between preterm neonates born to mothers with PE and those born to mothers without PE (PR), aiming to understand how maternal health conditions like PE influence neonatal gut microbiota. The early gut microbiota plays a crucial role in neonatal health, and disturbances in its development can have long-term consequences. Fecal samples were collected from preterm neonates of PE and PR mothers at 2 and 6 weeks postpartum and analyzed using shotgun metagenomic sequencing. We found that PE significantly affected the gut microbial composition of preterm neonates, particularly at 2 weeks postpartum. The gut microbial diversity in the PE_2 group was notably lower compared to the PR_2 group, with no significant difference observed between the PR_6 and PE_6 groups. At the phylum level, Firmicutes and Proteobacteria were predominant, with significant differences observed, particularly a lower abundance of Actinobacteria in the PE_2 group. At the genus level, *Escherichia, Enterococcus,* and *Klebsiella* were more prevalent in the PE_2 group, whereas *Bifidobacterium* and *Cutibacterium* dominated the PR_2 group. The gut virome analysis showed no significant differences among the groups. Functional analysis revealed distinct metabolic pathway activities across the groups, suggesting that early disturbances due to PE impact the establishment of healthy gut microbiota. These findings underscore the substantial influence of maternal health on the early development of the neonatal gut microbiota and highlight the potential long-term health consequences. Additionally, the differences in metabolic pathways further emphasize the impact of preeclampsia on gut microbiota functionality.

Keywords Preeclampsia, Preterm neonates, Gut microbiome, Gut virome, Microbial diversity, Shotgun metagenomic sequencing

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Introduction

Preeclampsia (PE) is a complex hypertensive disorder that occurs during pregnancy, impacting approximately 5–8% of pregnancies worldwide [1–3]. It is characterized by high blood pressure and proteinuria after the 20th week of gestation. PE remains a leading cause of maternal and neonatal morbidity and mortality [4–6]. It has been established that children born from pregnancies complicated by PE are more prone to a range of long-term health issues, including cardiovascular diseases, metabolic disorders, and even neurodevelopmental problems [7–10].

Despite extensive research, the exact cause of PE remains unknown. Recent studies have turned attention to the gut microbiota, the community of microorganisms residing in the gastrointestinal tract. Evidence suggests that the composition and diversity of gut microbiota during early life are crucial in shaping an individual's immune system and metabolic functions, potentially influencing their health trajectory throughout life [11]. Furthermore, maternal health conditions during pregnancy, including PE, can affect the development of newborn's gut microbiota, as well as their immune system and overall health [12, 13]. Research has shown that the gut microbiota of the mother and fetus share some degree of overlap. Maqsood et al. [14] reported that the maternal gut microbiota significantly influences infant's microbial acquisition, with approximately 63% of the infant's bacterial microbiota and 15% of viral communities traceable to the mother's gut microbiota. Earlier studies conducted by our research team have identified distinct patterns in the gut microbiota and virome of PE pregnant women, which may be linked to the mechanisms underlying the onset of this condition [15–17]. However, the question of whether or how PE impacts the gut microbiota of neonates remains largely unanswered. This knowledge gap is critical, as understanding the relationship between maternal health conditions like PE and the subsequent development of newborn's gut microbiota could provide insights into long-term health outcomes and open avenues for preventive strategies.

The development and health of infants are intertwined with the protective and regulatory functions of microorganisms in gut. Preterm infants born with an imbalanced gut microbiota are at substantial risk of diseases such as inflammatory intestinal diseases, necrotizing enterocolitis, late-onset sepsis, neurodevelopmental disorders, and allergies, which may persist into adulthood [11, 12, 18, 19]. The offspring of mothers with PE have a high incidence of preterm birth, particularly in cases of early-onset PE. Therefore, we selected preterm infants with similar gestational ages and comparable preterm complications as controls to assess whether PE has a specific impact on the gut microbiota of neonates born preterm. Current research has predominantly focused on the bacterial component of the gut microbiome, but the gut ecosystem also includes viruses, archaea, and eukaryotes. The neonate's gut virome is highly dynamic during the first year of life, gradually transitioning to a composition resembling adults [20, 21]. Viruses can influence the host indirectly by modulating bacterial composition and adaptation or directly by interacting with human cells, potentially triggering immune responses [22, 23]. Thus, further research is essential to elucidate these connections, exploring not only the microbiological changes but also the associated physiological and health implications for the newborn, which could have enduring effects into adulthood.

This study aims to investigate the characteristics of the gut microbiota in preterm neonates born to mothers with PE compared to those born to mothers without preeclampsia. By analyzing fecal samples collected at 2nd and 6th week postnatally, the study aims to uncover the impact of maternal PE on the early development of the neonatal gut microbiome. Understanding these microbial differences may providing critical insights into the pathophysiology of PE and help inform strategies to improve neonatal health outcomes in this vulnerable population.

Methods

Study cohort

A longitudinal observational study was conducted in the NICU of Guangdong Women and Children Hospital from March 2022 to March 2023. The neonates were all born spontaneously and categorized into two groups: the PE group, consisting of 15 preterm neonates born to mothers with preeclampsia, and the normal preterm (PR) group, comprising 15 preterm neonates born to mothers without preeclampsia. Clinical history data for both the mothers and their offspring were meticulously documented, and fecal samples were collected at two weeks (2W) and six weeks (6W) postpartum during their hospital stay. These two time points represent key stages in the early development of the neonatal gut microbiota [24, 25]. Following this period, regular follow-ups will be conducted for the newborns until discharge. A total of 43 fecal samples were collected at 2 weeks and 6 weeks of age, Notably, only 13 participants (6 PE and 7 PR) were available for the second sampling in the 6th week. A major reason for this is that some participants were discharged from the neonatal intensive care unit (NICU) before the 6th week or were excluded due to failure to collect the required samples on time. The collected samples were transported to the laboratory in secure containers at 4°C, then stored at −80°C until DNA extraction.

The diagnosis of PE in this study followed the guidelines of the American College of Obstetricians and Gynecologists (ACOG) [26]. The diagnostic criteria for PE include: after the 20th week of gestation, the patient's blood pressure reaches at least 140/90 mmHg on two separate occasions; proteinuria reaches 300 mg. In the absence of proteinuria, PE can also be diagnosed if any of the following conditions are present: platelet count below $100{,}000 \times 10^{\wedge}9$/L; serum creatinine concentration exceeding 1.1 mg/dL or doubling in the absence of other renal diseases; elevated liver transaminase concentrations twice the normal level; pulmonary edema; or cerebral or visual symptoms. Preterm birth is defined as birth between 20 weeks of gestation and 36 weeks of gestation.

Preterm neonates were included based on the following criteria: (1) mothers diagnosed with PE or those without serious pregnancy complications (Gestational hypertension, placental abruption, gestational Diabetes, et.al) who delivered before 37 weeks' gestation; (2) admission to our hospital's NICU after birth. Exclusion criteria included: (1) maternal factors such as twin or multiple pregnancies; (2) newborn factors including (a) chromosomal abnormalities or single-gene disorders; (b) inability to cooperate due to severe physical conditions; (c) congenital malformations; (d) inherited metabolic disorders; (e) endocrine disorders; (f) digestive tract malformations; (g) incomplete data or cases lost to follow-up.

No statistically significant differences were found in the mother's age, body mass index (BMI), gestational age, or diet. None of the mothers of the neonates had diabetes, intrahepatic cholestasis of pregnancy, anemia, smoked, consumed alcohol, autoimmune diseases, or a history of gastrointestinal surgery. Additionally, none of the mothers in the study cohort were on antihypertensive medications during pregnancy. The systolic blood pressures of PE and PR mothers before hospital delivery were 156.0 ± 7.9 mmHg and 95.5 ± 7.5 mmHg, respectively ($p < 0.001$ for PE vs. PR), while the diastolic blood pressures were 119.0 ± 8.5 mmHg and 69.0 ± 8.4 mmHg, respectively ($p < 0.001$ for PE vs. PR). Written informed consent was obtained from the legal guardians of all participants.

Shotgun metagenomic sequencing

Fecal samples were processed with the E.Z.N.A.® DNA Kit for DNA extraction, followed by evaluation of concentration, purity, and integrity. DNA that met quality standards was fragmented into approximately 300bp pieces using the Covaris M220 for library preparation. The samples underwent shotgun metagenomic sequencing with Illumina NovaSeq platform.

Microbial taxonomic profiling

Microbial taxonomic composition of all samples was analyzed using MetaPhlAn4 [27], which integrates information from metagenome assemblies and microbial isolate genomes for more comprehensive metagenomic taxonomic profiling. The estimation of taxonomic clade abundance within metagenomic samples was based on reads homology and coverage of species-specific marker genes. Bowtie2 [28] was used for mapping with the default settings ' -sam-no-sq -no-unal -fast'. Based on the quality-controlled mapping results, MetaPhlAn4 (a tool for metagenomic profiling) [27] calculated the coverage of each marker and determines the clade's coverage.

To compare taxon abundance between different taxa, the Linear Discriminant Analysis Effect Size (LEfSe) [29] was applied, a tool for high-dimensional biomarker mining that identifies genomic features (such as genes, pathways, and taxonomies) that significantly characterize two or more groups in microbiome data. The analysis was conducted utilizing a Kruskal–Wallis sum-rank test with an alpha value of < 0.5, followed by the Wilcoxon-rank sum test with an alpha score < 0.05. A one-against-all approach was used for multi-class analysis. In this study, an effect size greater than 2 (on a logarithmic scale) was deemed significant.

Functional analysis

The HUMAnN3 algorithm [30] for annotating genes with high-quality reads across all samples, show exceptional accuracy in detecting and quantifying species' roles in community functional profiles when compared to other software. HUMAnN3 uses native UniRef50 [31] annotations from the ChocoPhlAn species pan-genome to analyze genes, pathways, and modules from metagenomes. Briefly, reads were mapped to the UniRef50 database using DIAMOND [32], with the relative abundance of each gene family quantified. The UniRef50-based gene family abundances were regrouped into Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologous groups [33], which were further used to infer metabolic pathways.

Analyses of the gut virome

To analyze the composition of the gut viral community in metagenomic samples, we used a gut virus catalog comprising over 67,000 nonredundant viral operational taxonomic units (vOTUs) as the reference. This Chinese gut virus catalog (cnGVC) was constructed from over 10,000 publicly available fecal metagenomes from the Chinese population [34]. To create taxonomic profiles of gut viral communities, clean reads from each metagenomic sample were aligned to the cnGVC

database using Bowtie2 with parameters '–end-to-end –fast –no-unal'. Subsequently, the total mapped reads across all samples were randomly down-sampled to a uniform sequencing depth of 1.2 million reads. The relative abundance of each vOTU was determined by the proportion of reads mapped to the respective vOTU compared to the total number of reads mapped to any vOTU within each metagenome. Additionally, the relative abundance of each viral family was calculated by summing the relative abundances of vOTUs associated with that specific family.

Statistical analyses

Statistical analysis and visualization were implemented in R v 4.2.2 platform. The Shannon, Richness, Observed, and Simpson diversity indices were computed using the diversity function within the *vegan* package [35]. Bray–Curtis dissimilarities among samples were assessed using the *vegdist* function, also from the vegan package. Principal coordinate analysis (PCoA) of these dissimilarities was conducted using the PCoA function available in the *ape* package. Permutational multivariate analysis of variance (PERMANOVA) was performed utilizing the *adonis* function within the vegan package.

Results

Study cohort and sequencing summary

This study investigated gut microbiota differences between preterm neonates born to mothers with PE and those born to mothers without preeclampsia (PR). Fecal samples were collected from neonates of PE and PR mothers at 2 weeks (PE_2, n = 15; PR_2, n = 15) and 6 weeks (PE_6, n = 6; PR_6, n = 7) postpartum for analysis. Participants had similar dietary habits and lifestyles, and the cohort included both cesarean and vaginal deliveries. Comprehensive clinical and phenotypic characteristics of the participants are presented in Table 1. There was no statistically significant difference between the PE and PR groups with respect to maternal age, gestational age, advanced maternal age, primiparity, history of abortion, mode of delivery, fetal lung maturation status, presence of cardiac dysfunction during pregnancy, liver or kidney dysfunctions, or placental abruption ($P > 0.05$). However, a statistically significant difference in birth weight was noted between the two groups($p < 0.05$; Table 2).

Regarding complications, there were no statistically significant differences between the PE and PR groups in terms of respiratory distress syndrome (RDS), bronchopulmonary dysplasia (BPD), vitreous opacity, retinopathy of prematurity (ROP), neonatal jaundice, necrotizing enterocolitis (NEC), dysphagia, patent ductus arteriosus, sepsis, septic shock, cerebral hemorrhage, and pneumonia ($P > 0.05$) (Table 3).

Table 1 Basic characteristics of the pregnant women with preeclampsia and healthy controls included in this study

		Pregnant women with preeclampsia (N, %)	Healthy pregnant women (N, %)	*P*-value
No. of individuals		15	15	–
Age of pregnancy (year)		31.6 ± 3.7	31.5 ± 4.7	0.928
Advanced gestational age (year > 35)		2 (13.3)	4 (26.7)	0.651
Gestational age (week)	28–1 + 6	6 (40.0)	6 (40.0)	1.000
	32–33 + 6	4 (26.7)	3 (20.0)	–
	34–6 + 6	5 (33.3)	6 (40.0)	–
Primipara		4 (26.7)	3 (20.0)	1.000
History of miscarriage		6 (40.0)	4 (26.7)	0.700
History of cesarean section		4 (26.7)	2 (13.3)	0.651
Promote fetal lung maturity		7 (46.7)	12 (80.0)	0.128
Vaginal delivery		1 (6.7)	6 (40.0)	0.080
Cesarean section		12 (80.0)	11 (73.3)	1.000
HELLP syndrome		0	0	–
Pulmonary edema		0	0	–
Cardiac insufficiency		2 (13.3)	0 (0)	0.483
Abruption of the placenta		1 (6.7)	1 (6.7)	1.000
Liver and kidney insufficiency		3 (20.0)	0 (0)	0.224
Hydrothorax and abdomen		4 (26.7)	0 (0)	0.100

P-values were calculated by Student's t-test and Fisher's exact test for continuous and discrete variables, respectively. HELLP, hemolysis, elevated liver enzymes and low platelets count syndrome

Table 2 The perinatal characteristics of the PE and PR groups in this study

		PE group (N, %)	PR group (N, %)	*P*-value
No. of individuals		15	15	–
Infant gender	Male/female	7/8	10/5	0.462
Duration of hospitalization (day)		34.1 ± 21.4	29.3 ± 22.3	0.588
Antibiotic treatment		8 (53.5)	10 (66.7)	0.710
Duration of antibiotic treatment (day)		11 ± 7.6	10 ± 5.7	1.000
NIV		6 (40.0)	9 (60.0)	0.466
VLBWI		8 (53.3)	5 (33.3)	0.462
Birth weight (kg)		1.45 ± 0.48	1.93 ± 0.52	0.014
SGA		2 (13.3)	0 (0)	0.483
Apgar score (< 8)		8 (53.3)	9 (60.0)	1.000
Fetal distress		2 (13.3)	4 (26.7)	0.651
Neonatal asphyxia		1 (6.7)	1 (6.7)	1.000

P-values were calculated by Student's t-test and Fisher's exact test for continuous and discrete variables, respectively. NIV, non-invasive ventilation. VLBWI, very low birth weight infant (birth weight < 1500 g). SGA, small for gestational age

Table 3 The clinical complications of the PE and PR groups in this study

	PE group (N, %)	PR group (N, %)	*P*-value
No. of individuals	15	15	–
RDS	7 (46.7)	12 (80.0)	0.128
BPD	2 (13.3)	1 (6.7)	1.000
NEC	3 (20.0)	4 (26.7)	1.000
ROP	2 (13.3)	8 (53.3)	0.050
Vitreous opacity	5 (33.3)	5 (33.3)	1.000
Neonatal jaundice	11 (73.3)	14 (93.3)	0.330
Dysphagia	6 (40.0)	8 (53.3)	0.715
PDA	5 (33.3)	8 (53.3)	0.462
Neonatal sepsis	3 (20.0)	1 (6.7)	0.578
Infectious shock	1 (6.7)	1 (6.7)	1.000
Cerebral hemorrhage	1 (6.7)	4 (26.7)	0.330

P-values were calculated by 'Fisher's exact test. PE groups, preterm neonates of mothers with preeclampsia. PR groups, preterm neonates of mothers without preeclampsia. RDS, Respiratory distress syndrome. BPD, Bronchopulmonary dysplasia. NEC, Necrotizing enterocolitis. ROP, Respiratory distress syndrome. PDA, patent ductus arteriosus

Diversity and structure of gut microbiota associated with PE

To explore the gut microbiota of preterm neonates born to mothers with PE and PR between 2 and 6 weeks of age, relationships within and between samples across the four groups were analyzed using alpha and beta diversity indexes. The PCoA based on Bray–Curtis distance revealed a clear distinction between the gut microbiota of neonates with PE and those with PR (Fig. 1A). This finding was further supported by PERMANOVA analysis ($R^2 = 0.173$, $P < 0.001$) (Fig. 1B), indicating that approximately 17% of the variation in gut microbiota composition can be attributed to differences between the groups. In comparison, the delivery model explained only approximately 7% of the variation in gut microbial composition (PERMANOVA $P = 0.081$). The Richness diversity index, Shannon index, Simpson index and Observed index displayed significant differences between the PE_2 group and the other three groups ($P < 0.05$) (Fig. 1C, D). Interestingly, the gut microbiota diversity in the PE_2 group was notably lower compared to the PR_2 group, although no significant difference was observed between the PR_6 and PE_6 groups. This indicates that the gut microbial diversity of preterm neonates born to mothers with PE may be initially affected by maternal factors at 2 weeks but normalize by 6 weeks.

The microbial composition of gut microbiota in neonates of mothers with PE

The gut microbial composition of preterm neonates from PE and PR was compared at 2 and 6 weeks postpartum to investigate potential microbial signature of PE. At the phylum level, we found that Firmicutes (average relative abundance 44.2 ± 4.1% in all groups) and Proteobacteria (39.8 ± 12.7%) were the predominant components, followed by Actinobacteria (14.6 ± 18%), Bacteroidetes (1.3 ± 3.77%) and Ascomycota (0.005 ± 0.015%) (Fig. 2A). Among these phyla, we found that PE_2 and PR_2 groups were significantly different. Actinobacteria were significantly lower in the PE group compared with the PR group (Wilcoxon rank-sum test $P < 0.05$). It is worth mentioning that the Proteobacteria in the PE_6 and PR_6 groups were significantly higher than those in the PR_2 group ($P < 0.05$), however, Firmicutes did not show differences among the four groups (Fig. 2B–E).

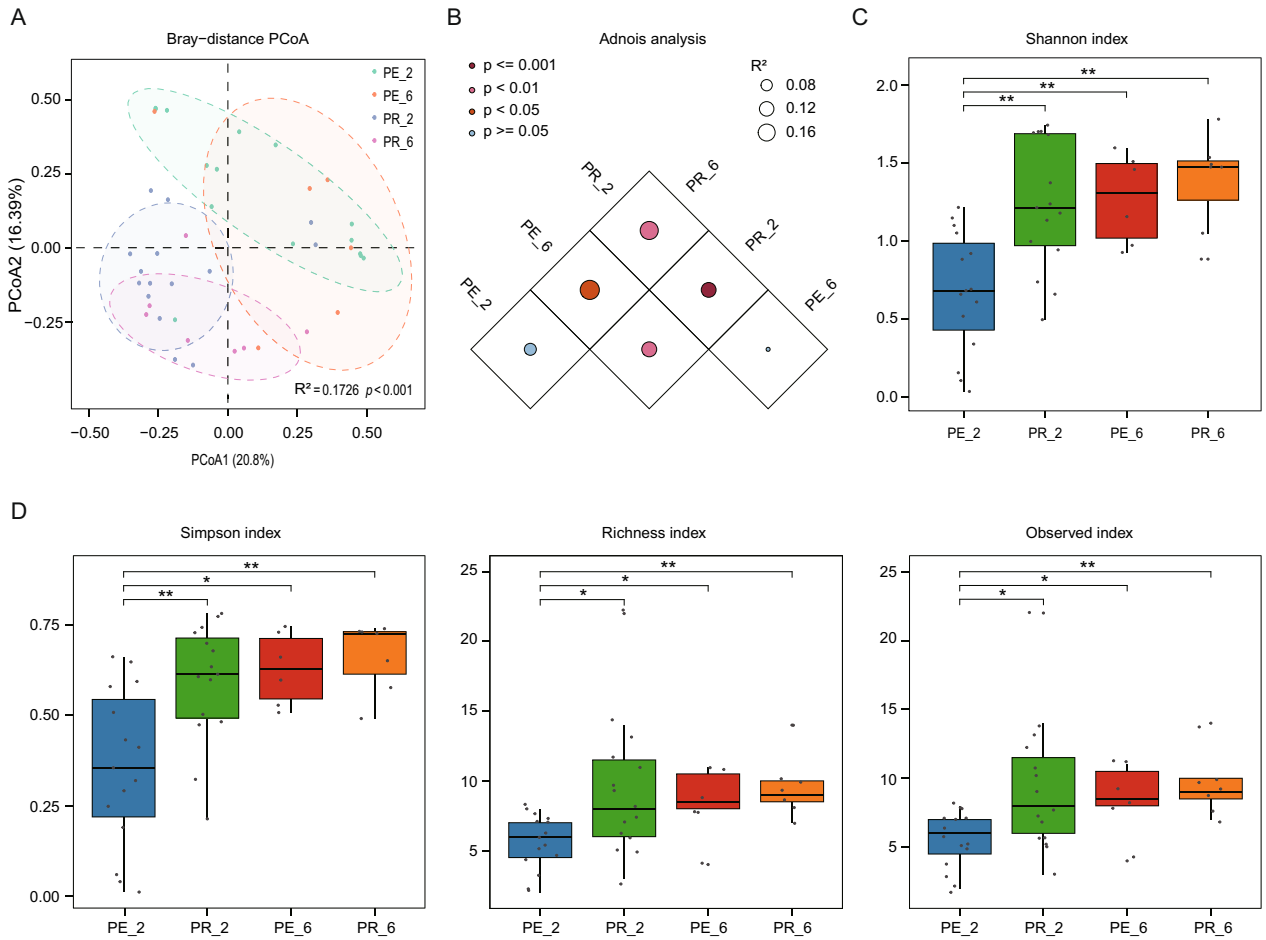

Fig. 1 **A** Scatter plots showing beta diversity, representing the compositional variation of the gut microbiome across all groups. Samples were plotted along the first and second principal coordinates (PCoA1 and PCoA2) with the associated explained variance ratios of these coordinates. Ellipsoids represent 75% confidence intervals around each group. **B** PERMANOVA results revealed the overall effect sizes of different groupings. *P* values were calculated using the *adonis* test. **C, D** Boxplots depict species Shannon, Simpson, richness and Shannon index for all groups, respectively. Significance levels were determined using the Wilcoxon rank-sum test

The analysis at the genus level showed that *Escherichia* (36.1%), *Enterococcus* (28.8%), and *Klebsiella* (14.4%) were the most prevalent genera in the PE_2 group, while *Bifidobacterium* (19.6%), *Cutibacterium* (12.0%), and *Klebsiella* (11.0%) dominated in the PR_2 group. At the 6 weeks, *Escherichia* (39.3%), *Enterococcus* (22.3%) and *Veillonella* (9.9%) were the predominant genus in the PE_6 group, with *Klebsiella* (27.0%), *Veillonella* (21.2%) and *Enterococcus* (18.4%) dominating in the PR_6 group (Fig. 2F). Particularly, *Bifidobacterium* and *Veillonella* in the PR_2 group were significantly more abundant than in the PE_2 group ($P < 0.05$) (Fig. 2H). To further elucidate

(See figure on next page.)

Fig. 2 **A** Bar graph showing community composition of gut microbiota at the phylum level. **B-E** Box plots show the relative abundance of the three phyla in all groups. Wilcoxon rank sum test: *, $P < 0.05$; **, $P < 0.01$, ***, $P < 0.001$. PERMANOVA analysis of the gut microbial results of each group, darker points indicate larger p values, while larger circles correspond to larger R2 values. **F** Bar graph showing community composition of gut microbiota at the genus level. (H) Box plots show the relative abundance of the four genera in all groups. Wilcoxon rank sum test: *, $P < 0.05$; **, $P < 0.01$, ***, $P < 0.001$. (I) A bar chart representing the Linear Discriminant Analysis (LDA) scores for taxa with significant differences in abundance (LDA score > 2) across the four groups: PE_2 (red), PE_6 (green), PR_2 (blue), and PR_6 (purple). A cladogram representing the phylogenetic distribution of significant taxa across the four groups. The highlighted areas indicate the presence of specific taxa in each group, with colors corresponding to the group in which they are significantly more abundant

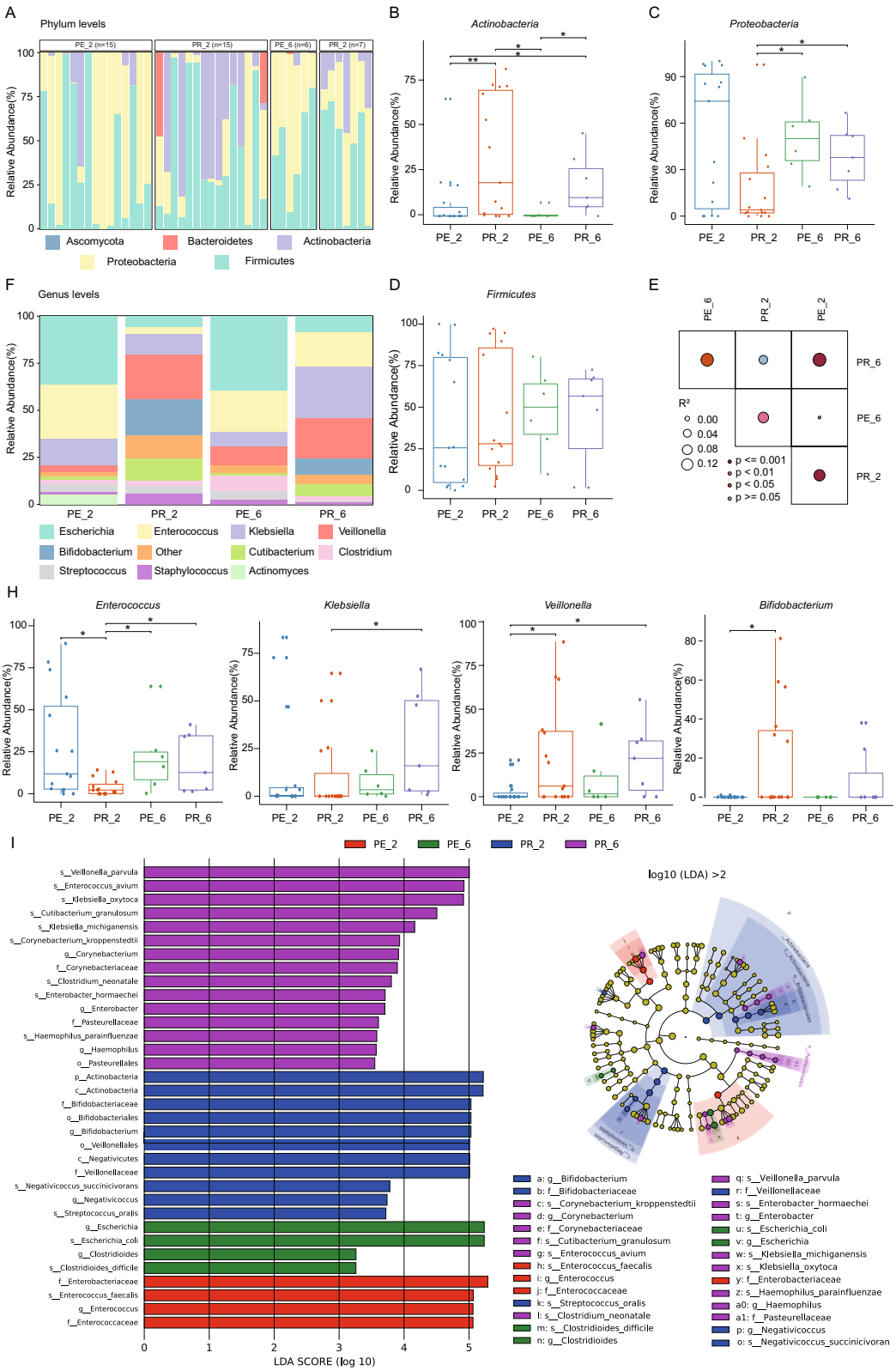

Fig. 2 (See legend on previous page.)

bacterial community differences at other taxonomic levels, a cladogram representation of significantly different taxa at various taxonomic levels was generated using the LEfSe tool. The results indicated that at the family level, *Corynebacteriaceae, Bifidobacteriaceae*, and *Enterococcaceae* exhibited significantly higher abundance in PE_2, PE_6, and PR_6 (LDA score > 2). At the species level, *Veillonella parvula, Negativicoccus succinicivorans, Escherichia coli,* and *Enterococcaceae spp.* displayed significantly higher abundance in PE_2, PE_6, PR_2, and PR_6, respectively (LDA score > 2) (Fig. 2I).

The results suggest that preeclampsia has a distinct impact on the gut microbial composition of preterm neonates, particularly evident at 2 weeks after birth. The lower levels of Actinobacteria and higher levels of Proteobacteria in neonates born to mothers with PE may indicate early disruptions in the development of a healthy gut microbiota. Differences at the genus and species levels underscore the significant influence of maternal health on the gut microbiota of neonates.

Gut viral structure and diversity

We performed PCoA based on the Bray–Curtis distance of vOTU level profiles to assess differences in the gut virome composition of preterm neonates from PE and PR mothers and visualized the first two principal axes, explaining 91.6% of the total variation. The PCoA result showed that the beta diversity between different groups did not fifer significantly ($P > 0.05$ for all comparisons) (Fig. 3A), indicating no major shifts in gut virome composition across the four groups. To estimate the gut viral richness and evenness of four groups, alpha diversities of each sample were calculated using two indices (Shannon diversity index, and Simpson index). Comparison analysis was conducted based on Fisher's method of combining *P* values from Wilcoxon rank-sum tests. Similarly, the alpha diversity between different groups did not differ significantly ($P > 0.05$) (Fig. 3B, C). These results highlighted no alteration in the gut virome of neonates from PE patients.

At the family level, it was found that the known viral accounted for only 70% of the total abundance of the gut virome across all samples. Among these, *Siphoviridae* (average relative abundance 41.0 ± 3.9% in all samples) and *Myoviridae* (24.34 ± 3.78%) were the main components, followed by *Podoviridae* (4.09 ± 1.57%) (Fig. 3D). only *Podoviridae* showed a significant difference with an increasing trend in the virome of PE_2 compared to PR_2 (Fig. 3E).

Analyses of microbial functions and correlation with complications

We analyzed the gut microbiota functionality in preterm neonates born to PE and PR mothers at two different time points: the second week (PE_2 and PR_2) and the sixth week (PE_6 and PR_6) postpartum. The analysis identified the top 50 most abundant functions annotated in the fecal samples of these neonates. Hierarchical clustering represented in the heatmap reveals distinct patterns of functional annotations across the different groups. Notably, metabolic pathways such as the Calvin-Benson-Bassham cycle and superpathways of threonine and lysine biosynthesis showed higher activity in the PE_2 and PR_2 groups compared to their respective 6-week groups. Nucleotide and amino acid metabolism pathways, including those involved in folate transformations and ribonucleotide biosynthesis, displayed differential expression, with significant differences observed in the superpathway of guanosine nucleotides de novo biosynthesis between the PE and PR groups. Lipid and carbohydrate metabolism pathways, such as the superpathway of fatty acid biosynthesis and glycolysis, were more active in the PE groups. Additionally, the biosynthesis of secondary metabolites, vitamin and cofactor metabolism, and pathways related to stress response and DNA repair were prominently featured, with notable variations across the groups (Fig. 4A). These findings suggest that the gut microbiota of preterm neonates from PE mothers exhibits distinct functional profiles compared to those from PR mothers, reflecting altered metabolic demands and environmental exposures. The observed temporal changes from the second to the sixth week postpartum indicate an adaptive progression in gut microbiota functionality, potentially influenced by the neonates' development and external factors. Understanding these functional capabilities is crucial, as it may have significant implications for the growth, development, and overall health outcomes of preterm neonates.

To ascertain the potential correlation between neonatal gut microbiota and complications, we employed PERMANOVA to assess the association between neonatal complications (such as cardiac abnormalities, dysphagia, retinopathy) and gut microbiota. The results revealed that these complications have no significant impact on the gut microbiota (Fig. 4B), suggesting that, at least in this cohort, the gut microbiota is not strongly influenced by the presence of these neonatal complications.

Discussion

This study provided a comprehensive analysis of the gut microbiota in preterm neonates born to mothers with PE compared to those born to mothers with PR. Our findings revealed significant differences in microbial diversity and composition between these groups, particularly evident at 2 weeks postpartum. These differences have potential implications for the health and development of

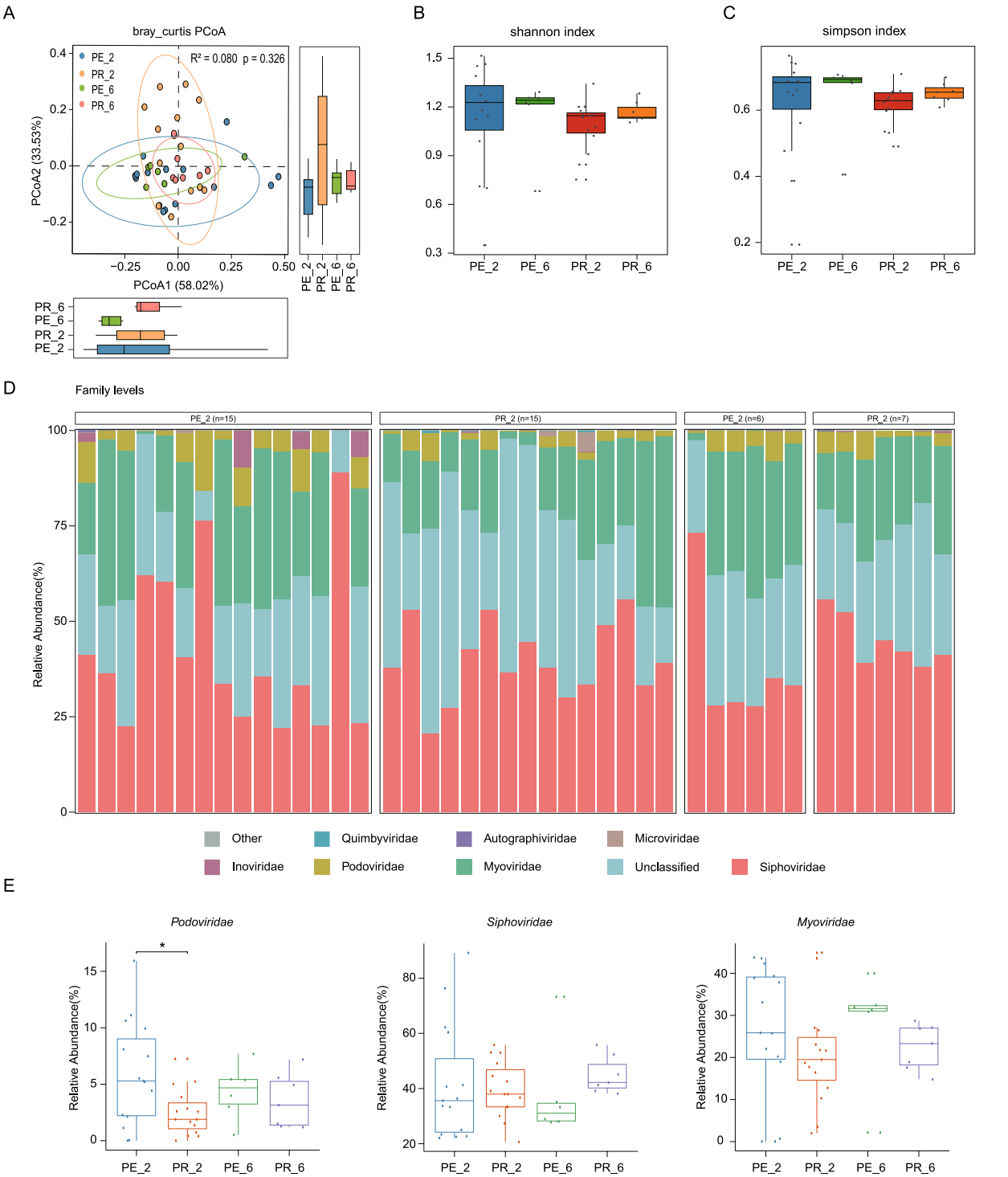

Fig. 3 **A** Scatter plots showing beta diversity, representing the compositional variation of the gut virome across all groups. Samples were plotted along the first and second principal coordinates (PCoA1 and PCoA2) with the associated explained variance ratios of these coordinates. **B, C** Boxplots depict species Shannon and Simpson index for all groups, respectively. Significance levels were determined using the Wilcoxon rank-sum test. **D** Bar chart showing the family-level community composition of the gut virome. **E** Box plot showing the relative abundance of the three families in all groups. Wilcoxon rank sum test: *, $P < 0.05$; **, $P < 0.01$, ***, $P < 0.001$

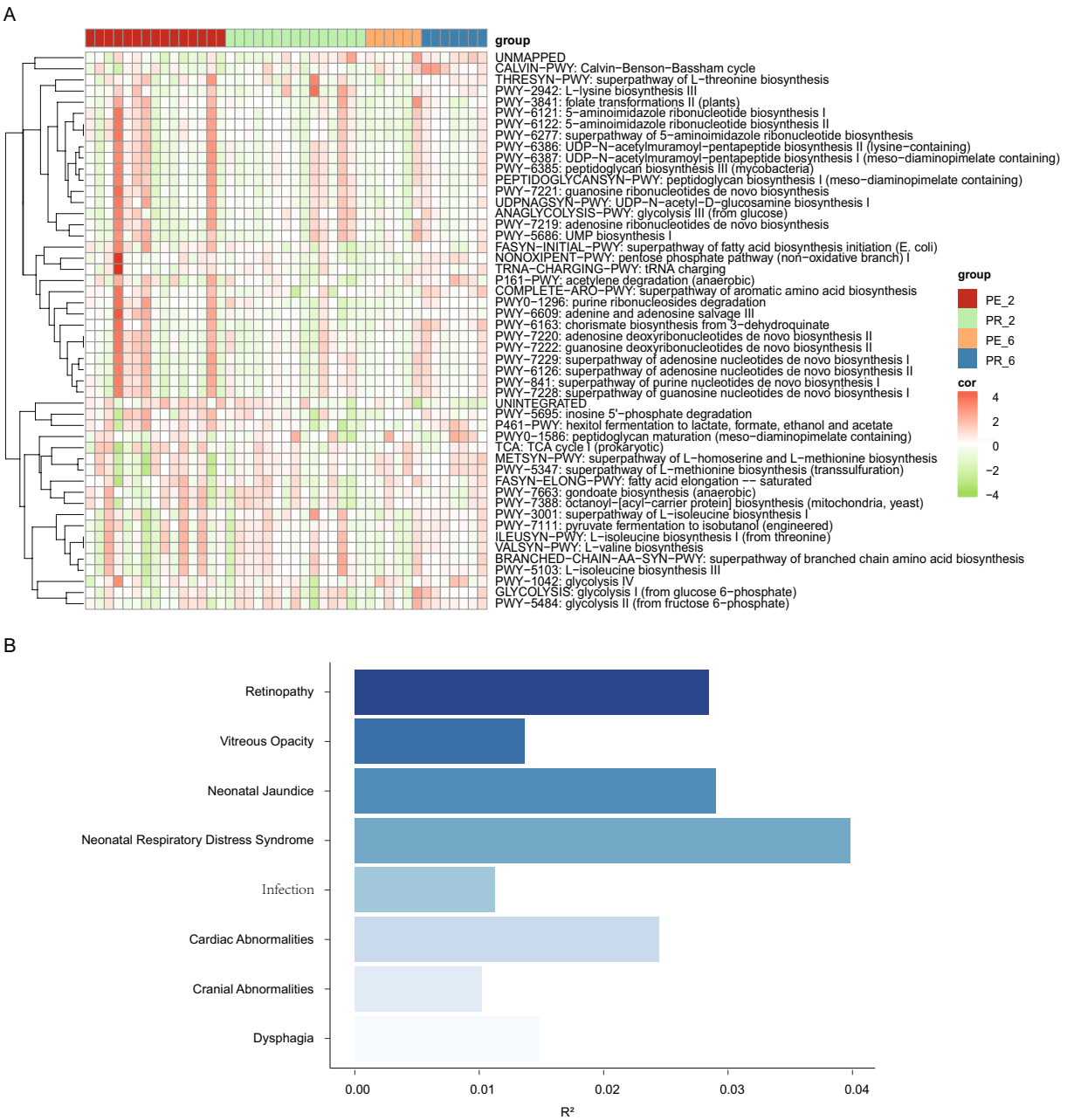

Fig. 4 **A** The heatmap reveals the functional annotations of the gut microbiota in different groups, represented as follows: PE_2 (red), PE_6 (green), PR_2 (blue), and PR_6 (purple). Each row represents a specific functional pathway identified in the gut microbiota, annotated with pathway descriptions. Each column represents an individual sample from one of the four groups. The color intensity reflects the activity level of each pathway, with a gradient from red (high activity) to green (low activity), and white indicating intermediate levels. **B** The bar graph illustrates the R^2 values for various neonatal health conditions, indicating the proportion of variance explained by the independent variables in each case. *P* values were calculated using the *adonis* test

preterm neonates, highlighting the impact of maternal health on early microbial colonization.

Our study identified a clear distinction in the gut microbiota between PE and PR neonates. Notably, both the microbial richness and diversity were significantly lower in the PE_2 group compared to others three groups. This reduced diversity is consistent with previous research indicating that maternal health conditions, such as PE [15], can negatively impact neonatal

gut microbiota diversity, potentially affecting immune development and disease susceptibility [18, 36].

At the phylum level, we observed that Firmicutes and Proteobacteria were the main components across all groups, with significant differences in Actinobacteria levels, which were notably lower in the PE_2 group. Previous studies have linked lower levels of beneficial bacteria such as Actinobacteria with adverse health outcomes, including impaired immune function and developmental delays [24, 37]. Although these changes recover by 6 weeks, early microbiota adjustments can affect the newborn's immune system, metabolic functions, and other physiological processes, potentially leading to long-term effects on health [38, 39]. Interestingly, while previous research by others have found that Fusobacteria is significantly enriched in the gut microbiota of PE mothers, we did not observe this enrichment in the gut microbiota of PE neonates. This suggests that the microbes significantly enriched in the gut microbiota of PE offspring neonates are not the same as those enriched in the gut microbiota of PE mothers [15, 16]. Conversely, the increased abundance of Proteobacteria in the PE_2 group may indicate a disrupted microbial environment, as Proteobacteria are commonly associated with inflammation, dysbiosis, and increased susceptibility to infections[40–43].

At the genus level, differences were also prominent. The PE_2 group was dominated by *Escherichia, Enterococcus,* and *Klebsiella*, whereas *Bifidobacterium* and *Cutibacterium* were more prevalent in the PR_2 group. *Bifidobacterium*, in particular, is known for its beneficial effects on gut health and immune function [44], and its lower abundance in PE neonates may reflect a compromised microbial environment. On the other hand, *Escherichia* and *Klebsiella* are generally considered pathogenic, with associations to gastrointestinal infections and inflammation [45]. *Enterococcus*, although sometimes considered part of the beneficial gut microbiota, has been linked to infections in immunocompromised individuals [46]. The increased presence of these potentially harmful genera in the PE_2 group may suggest a microbial imbalance, potentially contributing to the observed health risks in these neonates.

Interestingly, our analysis of the gut virome revealed no significant differences in beta or alpha diversity between the groups, which contrasts with the findings in pregnant women with PE [17]. This suggests that while the bacterial component of the microbiota is affected by maternal preeclampsia, the viral component remains relatively stable. This finding suggests that newborns' gut virome may be less susceptible to maternal influence than their bacterial microbes. The composition of gut virome in neonates may be shaped more by other early-life factors, such as breastfeeding, rather than by maternal preeclampsia [47].

Functional analysis of the gut microbiota indicated distinct metabolic pathway activities between the PE and PR groups. Notably, pathways such as the Calvin-Benson-Bassham cycle and superpathways of threonine and lysine biosynthesis were more active in the PE_2 and PR_2 groups compared to their respective 6-week counterparts. These differences in metabolic pathways reflect altered metabolic demands and environmental exposures due to maternal preeclampsia. Specifically, the Calvin-Benson-Bassham cycle, which plays a key role in carbon fixation and energy production, was notably more active in the PE_2 group, indicating potential shifts in energy metabolism due to maternal preeclampsia. Similarly, pathways involved in amino acid biosynthesis, such as those for threonine and lysine, were upregulated, possibly reflecting a response to altered nutrient availability in the neonates. These findings suggest that maternal preeclampsia may lead to metabolic shifts that impact the functionality of the neonatal gut microbiota, potentially influencing overall health outcomes.

The findings of this study underscore the significant impact of maternal preeclampsia on the early gut microbiota development of preterm neonates. While our study provides valuable insights into the neonatal gut microbiota but one important limitation is the absence of analysis on microbial metabolites and their potential associations with neonatal complications. Understanding the metabolites of the gut microbiota could offer further insights into the functional implications of the observed microbial compositions, as well as their potential impact on neonatal health outcomes, allowing for the exploration of targeted interventions (such as probiotics or prebiotics) to support healthy gut microbiota in neonates born to mothers with PE, helping establish a healthy gut microbiota in neonates at risk due to maternal health conditions [48]. Additionally, the conclusions of this study were constrained by the relatively small number of preterm neonates, particularly in the PE group, and the strict inclusion criteria, which limited the number of eligible participants. Despite the small sample size, we observed significant differences in both the diversity and composition of the gut microbiota between the PE and normal preterm groups, suggesting that the current sample size was adequate to detect meaningful differences between the two groups. However, future studies based on larger datasets are still needed to explore more precise and comprehensive PE-associated gut microbiota characteristics.

In conclusion, our study highlights the distinct differences in gut microbiota composition and function in preterm neonates born to mothers with preeclampsia. These findings emphasize the need for heightened awareness and potential therapeutic strategies to mitigate the

adverse effects of maternal preeclampsia on neonatal health. Understanding the intricate relationship between maternal health and neonatal gut microbiota is crucial for developing interventions aimed at promoting optimal health outcomes for preterm neonates.

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Author contributions
LJL, JYW, SHL, and QLY contributed to the conception, planning, and carrying out of the study. ZTY, XYW,JPW and CN collected the samples and information. JYW, YC, HL, MCC and XYY were responsible for sample storage and follow-up of the cohort. SHL,HLY and QLY had a critical role in data analysis and inter pretation. LJL, YC, SHL and HLY drafted and revised the manuscript. All authors read the manuscript, contributed to the article, and approved the submitted version.

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Data availability
The authors declare that all other data supporting the findings of the study are available in the main text, or from the corresponding author upon request.

Declarations

Competing interests
All authors have no competing interests to disclose.

Informed consent
An informed consent form was signed by all volunteers and all experiments were performed in accordance with the Declaration of Helsinki. The study was approved by the Ethics Committee of Guangdong Women and Children Hospital (approval ID: 202301226).

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