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Effect of Gut Microbiotas Diversity During 32-39 Weeks of Gestation on Postpartum Depression

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Research article

Keywords: Postpartum depression, gut microbiota, third trimester

Posted Date: October 20th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-91665/v1

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Abstract

Background: Patients with major depression are accompanied by intestinal flora flocculation; however, the relationship between the composition of gut microbiota in pregnancy and postpartum depression (PPD) has not been established. In this study we determined the effect of the gut microbiota in pregnant women during 32-39 weeks of gestation on PPD.

Methods: Participants (n = 74) were enrolled between 2016–2017 from the Guangzhou Women and Children's Medical Centre (GWCMC). Stool samples were collected during 32-39 weeks of gestation, and the relative abundance of fecal microbiota was characterized by 16S rRNA sequencing. The parturients completed the mainland Chinese version of the Edinburgh Postnatal Depression Scale (EPDS) 42 days postpartum to detect PPD. The linear discriminant analysis (LDA) effect size (LEfSe) method was used to identify bacterial population differences between the PPD and control groups.

Results: The top three bacteria phyla in the PPD and control groups were Firmicutes, Bacteroidetes, and Actinobacteria. Compared with healthy pregnant women, the alpha diversity index of the PPD group was lower. Beta diversity analysis was performed by PCoA showing that no significant differences in bacterial community structures between the two groups (R^2 = 0.013, P = 0.549). The composition of gut microbiota during 32-39 weeks of gestation of the two groups was different. At the genera level, *Acinetobacter, Plesiomonas, Enterococcus, Olsenella, Alloscardovia,* and *Anaerotruncus* were increased in the PPD group, while *Lactococcus, Adlercreutzia, Clostridium, Coprococcus*, and unclassified-*Clostridiales* were decreased. At the species level, *hypermegale, uli, casseliflavus,* and *hathewayi* were increased in the PPD group, and *celatum* was increased in the control group.

Conclusions: During 32-39 weeks of gestation, a reduction in diversity of gut microbiota and anti-inflammatory bacteria, and an increase in opportunistic pathogenic bacteria are more likely to cause PPD.

Introduction

Postpartum depression (PPD) refers to a non-psychotic depressive episode lasting > 2 weeks after delivery. Women with PPD were more likely to cry, more irritable, more emotionally unstable, attention decreased, sleep disturbances, loss of appetite, and lack of interest in recreational activities than usual. Suicidal thoughts are extremely common, affecting about 20% of women with PPD symptoms [1–5]. PPD is one of the most common complications of childbirth and a major public health problem. The high prevalence rate is ranging from 6.9–12.9% in high-income countries to more than 20% in some low- or middle-income countries [6]. When untreated, it has the potential for a profound negative impact on mothers, children, and families. Case identification and accurate diagnosis are important which needs global attention.

There are 10^{13}–10^{14} microbes in the human gut, which is thought to be 10 times the number of human cells and 150 times the size of the human genome [7]. Indeed, the gut microbiota is considered to be another "organ" in human beings [7]. There is a two-way communication network between the gut

microbiota and the brain, which is referred to as the “brain-enteric-axis.” Changes in the composition and number of gut microbiotas can disturb the “brain-enteric-axis,” which has an impact on the central nervous system and is closely related to neuropsychiatric diseases, including anxiety, cognitive decline, autism spectrum disorder, schizophrenia, bipolar disorder, and depression [8]. Previous studies have revealed different bacterial populations, including *Blautia, Clostridium, Faecalibacterium, Ruminococcus Prevotella*, and *Roseburia*, between people with depression and the general population [9]. Of note, the research subjects with depression have mostly involved patients with major depression, and little attention has been paid to patients with PPD. Because the course and physiological state of PPD and severe depression are different, we cannot directly infer the relationship between PPD and gut microbiota from the study results involving major depression. The interrelationships between PPD, intestinal flora, and stress during pregnancy, hormone levels, and immune regulation indicate an association between PPD and gut microbiota [10]. Supplementation of probiotics during pregnancy can reduce the PPD score, further confirming the association between intestinal bacteria and PPD [11]. Indeed, the association between gut microbiota composition and PPD has not been established. In this study we demonstrated that the gut microbiota is associated with postpartum depression which will be helpful to the case identification and accurate diagnosis.

Methods

Study design and participants

Between July 2016 and October 2017, a prospective cohort study including 14 women with PPD and 60 gestational healthy control women was performed in Guangzhou Women and Children’s Medical Centre (GWCMC), a large modern city located in Guangdong Province in South China. The pregnant women were recruited into the study at the third trimester, and followed-up to 42 days postpartum. The exclusion criteria included participants who had taken antibiotic treatment or probiotic supplements in the four weeks prior to sample collection, strict vegetarians, individuals with alcoholism or with other unusual dietary habits or with diseases, such as gestational diabetes mellitus, gestational hypertension, thyroid disease, gastroenteritis, and major mental illnesses.

The information regarding the regular prenatal examinations, basic personal information, past medical history, psychiatric history, pregnancy history, family history, co-existing conditions during pregnancy, and other clinical data were entered by trained healthcare workers in the medical records. The protocol for this study was approved by the Ethics Review Committees of Guangzhou Medical University, and all participants provided voluntary signed informed consent. All procedures performed in studies involving human participants were in accordance with the requirements of the ethics committee and with the 1964 Helsinki Declaration.

Stool sample collection

Stool samples were collected from all participants during the third trimester. The collection time of fecal samples from the 74 subjects included in this study was 32–39 weeks gestation. Stool samples were

self-collected by the participants. Briefly, the subjects were instructed on how to self-collect the samples, and all materials were provided in a convenient specimen collection kit. Participants used polypropylene spoons (three scoops of approximately 10 g) to collect stool samples at home or in the hospital, transfer the samples to sterile sampling containers, and immediately stored at 4 °C. The specimens were transported to the laboratory within 12 h of collection at a refrigerated temperature. Containers were immediately stored at − 80 °C.

DNA extraction and sequencing

DNA extraction was carried out according to the MOBIO PowerSoil DNA Isolation Kit 12888-100 protocol, and DNA was stored at − 80 °C in Tris-EDTA buffer solution before use. To enable amplification of the V4 region of the 16S rRNA gene and add barcode sequences, unique fusion primers were designed based on the following universal primer set with barcode sequences: 515F (5′-GTGYCAGCMGCCGCGGTAA-3′); and 806R (5′-GGACTACNVGGGTWTCTAAT-3′). PCR mixtures contained 1 μL of each forward and reverse primer (10 μM), 1 μL of template DNA, 4 μL of dNTPs (2.5 mM), 5 μL of 10 × EasyPfu buffer, 1 μL of Easy Pfu DNA polymerase (2.5 U/μL), and 1 μL of double-distilled water in a 50-μL reaction volume. Thermal cycling consisted of an initial denaturation step at 95 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, extension at 72 °C for 40 s, and a final extension step at 72 °C for 4 min. The amplicons from each sample were run on agarose gels. The expected band size for 515f-806r was ~ 300–350 bp. The amplicons were quantified with a Quant-iT PicoGreen dsDNA Assay Kit (ThermoFisher/Invitrogen catalog no. P11496) according to the manufacturer's instructions. The amplicon library for high-throughput sequencing on the Illumina MiSeq platform was combined in equal amounts and subsequently quantified (KAPA Library Quantification Kit KK4824) according to the manufacturer's instructions.

The 16S rRNA sequencing analysis

Quantitative Insights into Microbial Ecology (QIIME2) was used to process 16S rRNA amplicon sequences. All reads were truncated at the 150th base with a median Q score > 20 to avoid sequencing errors at the end of the reads. Noisy sequences, chimeric sequences, and singletons in the sequence data were removed using DATA. Denoised paired-end reads were joined, setting a maximum mismatch parameter of two bases. The representative sequences (i.e., the features) were defined as 100% similar-merged sequences. We have used the term "operational taxonomic unit" (OTU) instead of "feature" in the current study for convenience. Then, the taxonomy of the features was identified using the classify-sklearn classification method based on the Greengenes 13.8 database (https://data.qiime2.org/2018.11/common/gg-13-8-99-515-806-nb-classifier.qza) via the q2-feature-classifier plugin. The phylogenetic analysis was performed in QIIME2 with "qiime alignment mafft," "qiime alignment mask," and "qiime phylogeny fasttree" commands, based on the tutorials at https://docs.qiime2.org/2019.1/tutorials/moving-pictures/. The phylogenetic tree of the core OTUs was visualized using iTOL v4. To measure the gut microbiota diversity and quantify the taxonomic composition of the samples, all samples were rarefied to an even sampling depth of 20,000 sequences.

Edinburgh Postnatal Depression Scale (EPDS)

The participants completed the mainland Chinese version of the EPDS on postpartum day 42, which has been validated for Chinese women in other studies [11]. The EPDS is a 10-item self-reporting instrument for evaluating depressive symptoms over the past 7 days. Participants rated the severity or frequency of each item based on four levels, where 0 indicates the most favorable condition and 3 indicates the least favorable condition, for each item. The total score ranged from 0–30. A score ≥ 10 was diagnosed as postpartum depression [12].

Statistical methods

Descriptive statistics are presented as the mean ± standard deviation (SD) for continuous variables and as a frequency (percentage) for categorical variables. Anthropometric parameters between the two groups were compared using a *t*-test/chi square test. The stacked graph was drawn using the ggplot2 package in the R statistical program. The alpha diversity index (a measure of richness and evenness) was calculated using the vegan package in the R statistical program. The Wilcoxon test was used to compare the alpha diversity index between the two groups. Beta diversity analysis was performed by principal coordinates analysis (PCoA) based on Bray-Curtis distances at the OTU level. The analysis of similarities (Adonis) based on Bray-Curtis distances was conducted to compare different groups. Microorganism features distinguishing fecal microbiota between the PPD and non-PPD groups were identified using the linear discriminant analysis (LDA) effect size (LEfSe) method (http://huttenhower.sph.harvard.edu/lefse/). LEfSe used the Kruskal-Wallis rank-sum test with a normalized relative abundance matrix to detect features with significantly different abundances between assigned taxa and performs LDA to estimate the effect size of each feature. An alpha significance level of 0.05 and an effect-size threshold of 2 were used for all biomarkers. All analyses were conducted in R statistical package (version 3.5.1) and figures were produced using RStudio. All tests for significance were two-sided, and a $P < 0.05$ was considered to indicate statistical significance.

Results

Demographic characteristics

This study involved 74 participants, including 14 participants with PPD and 60 healthy participants. The mean ages of the PPD and control groups were 29.29 ± 4.30 and 30.78 ± 4.60 years, respectively. The average height and weight before pregnancy of the PPD group was 159.79 ± 7.20 cm and 51.89 ± 8.33 kg, respectively. The height of the control group was less than the PPD group (158.57 ± 5.42 cm), but the weight before pregnancy was greater (52.19 ± 7.03 kg). The pre-pregnancy body mass index (BMIs) of the PPD and the control group were 20.30 ± 2.78 and 20.79 ± 2.89 kg/m^2, respectively. The cesarean section rates in the PPD and control groups were 35.71% and 35.00%, respectively. Additional general demographic information is shown in Table 1.

Table 1
Basic characteristics of the PPD and control groups.

Characteristics		Non-PPD (n = 60, %)	PPD (n = 14, %)	P
Age (year)	Mean ± SD	30.78 ± 4.60	29.29 ± 4.30	0.261
Age groups (year)	≤ 29	26 (43.33)	8 (57.14)	0.740
	30 ~ 35	21(35.00)	4 (28.57)	
	≥ 35	13 (21.67)	2 (14.29)	
Height (cm)	Mean ± SD	158.57 ± 5.42	159.79 ± 7.20	0.477
Weight before pregnancy (kg)	Mean ± SD	52.19 ± 7.03	51.89 ± 8.33	0.892
Weight at delivery (kg)	Mean ± SD	66.38 ± 7.46	64.56 ± 10.38	0.451
BMI before pregnancy (weight/height^2)	Mean ± SD	20.79 ± 2.89	20.30 ± 2.78	0.569
BMI before pregnancy groups (weight/height^2)	BMI < 18.5	11 (18.33)	4 (28.57)	0.359
	18.5 ≤ BMI ≤ 24	44 (73.33)	8 (57.14)	
	BMI > 24	5 (8.33)	2 (14.29)	
Gravidity (time)	1	26 (43.33)	8 (57.14)	0.525
	≥ 2	34 (56.67)	6 (42.86)	
Parity (time)	1	31 (51.67)	9 (64.29)	0.579
	≥ 2	29 (48.33)	5 (35.71)	
History of abortion	Yes	15 (25.00)	2 (14.29)	0.500
	No	45 (75.00)	12 (85.71)	
Delivery mode	Vaginal	39 (65.00)	9 (64.29)	1.000
	Cesarean	21 (35.00)	5 (35.71)	
Duration of gestation (week)	Mean ± SD	39.70 ± 1.05	39.29 ± 0.73	0.095
Season of conception	Summer	19 (31.67)	7 (40.00)	0.299
	Autumn	38 (63.33)	6 (42.86)	
	Winter	3 (5.00)	1 (7.14)	

Composition of gut microbiota in gravidas (who subsequently developed PPD) during 32–39 weeks of

gestation

At the level of the phylum, the top three bacteria in the PPD group were Firmicutes (72.16%), Bacteroidetes (18.67%), and Actinobacteria (5.36%). Similarly, the top three bacteria in the control group were Firmicutes, Bacteroidetes, and Actinobacteria (78.69%, 12.76%, and 5.31%, respectively). The results are shown in Fig. 1.

Alpha diversity analysis of the gut microbiota during 32–39 weeks of gestation between the PPD and control groups

Alpha (α) diversity was used to evaluate the variety of species in the samples, which reflects OTU richness and evenness using several different indices. The alpha diversity index of the PPD group was lower than the control group (Supplementa Table 1). The Wilcoxon test showed a statistical difference in the Richness, Inverse Simpson, Chao1, ACE, Shannon, and Simpson indices between the two groups ($P < 0.05$), while there was no statistical difference in the Pielou and Good's indices [$P > 0.05$; Figs. 2(a) to 1(i)].

Beta diversity analysis of the gut microbiota during 32–39 weeks of gestation between the PPD and control groups

The similarity of the bacterial community structures among the two groups was evaluated by PCoA (Fig. 3). NO clear segregation of gut microbiota was observed between samples of PPD group and control group. Adonis analysis also demonstrated no significant distinction between PPD and control groups ($R^2 = 0.013$, $P = 0.549$). This finding indicated that PPD patients and healthy controls have similar gut bacterial community structures during 32–39 weeks of gestation. PCoA1 explained 9.835% of the variation observed, and PCoA2 explained 9.576% of the variation.

Altered gut microbiota composition during 32–39 weeks of gestation between the PPD and control groups

To determine different taxa between the non-PPD and PPD groups, the LEfSe algorithm on the Galaxy browser was used. LEfSe detected 31 bacterial taxonomic clades showing statistically significant differences (16 increased and 15 decreased) in the PPD group compared to the control group (Fig. 4a and b). At the class level, Bacilli were more abundant in the control group. At the order level, Pseudomonadales were more abundant in the PPD group, while Lactobacillales were more abundant in the control group. At the family level, Moraxellaceae were increased in the PPD group, whereas Clostridiaceae and unclassified-Clostridiales were decreased. Among the different predominant genera, *Acinetobacter, Plesiomonas, Enterococcus, Olsenella, Alloscardovia*, and *Anaerotruncus* were more abundant in the PPD group compared to the non-PPD group. Lactococcus, *Adlercreutzia, Clostridium, Coprococcus*, and unclassified-*Clostridiales* were more abundant in the non-PPD group compared to the PPD group. Among the identified species, compared with the non-PPD group, the abundance of more species (*hypermegale, uli, casseliflavus*, and *hathewayi*) were increased in the PPD group, while *celatum* were decreased.

Discussion

In this study we determined the composition of intestinal microflora in gravidas (who subsequently developed PPD) during 32–39 weeks of gestation. The results showed that the alpha diversity index and the anti-inflammatory bacteria of gut microbiota in gravidas (who subsequently developed PPD) were decreased during 32–39 weeks of gestation compared with healthy pregnant women.

Based on the literature involving human clinical microbiota [9], we identified very few studies with consistent results for depression. In our study patients with PPD had a lower alpha diversity compared with the normal population. Similar to our results, Huang [13] reported that the alpha diversity indices of major depressive disorder were lower than healthy controls. In other studies [14, 15], different results have been presented. Jiang [14] found that patients with active depression had a higher alpha diversity index compared with the normal population. Naseribafrouei [15] did not detect any significant differences in the alpha diversity index.

Acinetobacter is a genus of Moraxellaceae, both of which belong to Pseudomonadales. We observed that all three were increased in PPD. *Acinetobacter* is a conditionally pathogenic bacteria that is usually associated with respiratory tract infections, accounting for approximately 33.7% of the pathogens causing respiratory tract infections [16]. The researchers also found that the abundance of *Acinetobacter* is elevated in the gut of patients with neuroinflammatory diseases, such as multiple sclerosis [17]. This association is closely related to the fact that *Acinetobacter* can induce a proinflammatory response in human peripheral blood mononuclear cells [17]. In a murine study the abundance of *Enterococcus* was increased in a depression model induced by chronic unpredicted mild stress [18]. This suggests that stress may induce depression by increasing the abundance of *Enterococcus*. *Alloscardovia* is a newly discovered genus of Bifidobacterium. The link between *Alloscardovia* and disease has not been established, but high concentrations of *Alloscardovia* have been detected in the feces of patients with intrahepatic cholangiocarcinoma, and *Alloscardovia* may be involved in the metabolism of bile acids [19]. Previous studies have shown that patients with Parkinson disease have a higher abundance of *Anaerotruncus* in their intestines than healthy individuals [20]. In addition, *Anaerotruncus* is enriched in the feces of the patients with gestational diabetes mellitus, and negatively correlated with insulin sensitivity [21]. This finding suggests that *Anaerotruncus* may play a negative role in disease.

Like our results, a reduction in the anti-inflammatory gut microbiota was observed in depressed patients, including *Clostridium* [22]. Two other studies arrived at an opposite conclusion, with higher levels of clostridia in patients with major depression [14, 23]. The difference in observations may be due to diet. *Clostridium* can metabolize carbohydrates to produce short-chain fatty acids (SCFAs), and when the intestinal protein is rich, *Clostridium* will metabolize proteins to produce harmful substances [9]. Patients with major depression may have relatively rich protein remaining in the intestine due to poor appetite, causing *Clostridium* to grow [9]. We did not include diet in the current study, which was a limitation. In a murine study it has been reported that *Clostridium butyricum* can modulate inflammatory factors and microglial activation to prevent depression-like behavior [24], which also supports our findings. Butyrate-

producing *Coprococcus* bacteria are consistently associated with higher quality of life indicators. *Coprococcus* spp. are also depleted in depression, even after correcting for the confounding effects of anti-depressants [25]. *Adlercreutzia* is an equol-producing bacterium isolated from human feces that contains a single species (*Adlercreutzia equolifaciens*) [26]. Equol attenuates microglial activation and potentiates neuroprotection *in vitro* [27]. Animal studies have also shown that equol is beneficial in mitigating depression and anxiety disorders [28]. *Lactobacillus* and *Lactococcus* are two common genera of Lactobacillus. *Lactobacillus* can reduce oxidative stress markers and inflammatory cytokines in the brain and serum to prevent depression [11, 29]. Certain species of *Lactococcus* can improve depression and anxiety through antioxidant effects [30]. Among the identified species, *Clostridium hathewayi* can be used as a biomarker for colorectal adenoma and cancer [31], and *Enterococcus casseliflavus* is a relatively rare enterococcus that causes human infectious diseases, including bacteremia, endocarditis, and meningitis [32]. No studies have found a direct link between *Clostridium hathewayi, casseliflavu* and depression. Among patients with Behcet's disease, *Megamonas hypermegale* may synthesize SCFAs in the intestine and reduce the concentration of SCFAs. These changes will affect the function of the immune system, and thus affect the nervous system [33].

The mechanism underlying the brain-gut axis consists of the interaction of the nervous, endocrine, and immune systems [34]. The inflammation caused by the disorder of intestinal flora and the neuroendocrine hormones produced work together on the intestinal wall, changing the intestinal permeability, and interact with the central system through the vagus nerve. Metabolites produced by intestinal flora (such as SCFAs and tryptophan) can interact with the immune system, changing the body's immune state and thus changing the brain's behavior and mood. In addition, an imbalance of neurotransmitters and neuropeptides produced by gut bacteria, such as para-aminobutyrate (GABA), serotonin, and brain-derived neurotrophic factor (BDNF), which act as nerve signaling messengers, can also affect the central nervous system [34].

Conclusions

During 32–39 weeks of gestation, the reduction of the diversity of gut microbiota and anti-inflammatory bacteria and the increase in opportunistic pathogenic bacteria were more likely to cause PPD. This finding provides the basis for further exploring the relationship between intestinal flora and PPD.

Abbreviations

PPD Postpartum depression

GWCMC Guangzhou Women and Children's Medical Centre

EPDS Edinburgh Postnatal Depression Scale

LDA Linear discriminant analysis

LEfSe Linear discriminant analysis effect size

OTU Operational taxonomic unit

PCoA Principal coordinates analysis

Adonis Analysis of similarities

SD Standard deviation

SCFAs Short-chain fatty acids

BMI Body mass index

Declarations

Ethics approval and consent to participate

The protocol for this study was approved by the Ethics Review Committees of Guangzhou Medical University, and all participants provided voluntary signed informed consent. All procedures performed in studies involving human participants were in accordance with the requirements of the ethics committee and with the 1964 Helsinki Declaration.

Consent for publication

Not applicable.

Conflict of Interest

The authors declare that they have no conflicts of interest with the content of this article.

Funding

This study was funded by the Science and Technology Fund of Guangzhou (201704020109), the National Natural Science Foundation of China (81871716), the Guangzhou Municipal Health Bureau Project (20141A011030) and the Natural Science Foundation of Guangdong Province (2016A030313497).

Authors' contributions

Chunyan Zhu and Yanwei Hu directed the study implementation, including quality assurance and control, and drafting the article. Qiangsheng Gan and Xiaoyan Zhang analyzed and interpreted the data. Qingshan Geng designed the study and reviewed the article. Hongling Yang, Xueqin Zhao, Yan Long and Shanshan Mei designed the study's analytic strategy and helped conduct the literature review. Weitao Ye,

Fangling Zeng, Jun Ma, and Rehemayi·Rehemutula carried out acquisition of data, helped conduct the literature review.

Acknowledgements

First and foremost, we would like to express our gratitude to Guangzhou Municipal Science and Technology Project who gave financial support to this study. Besides, we are grateful to the Guangzhou Medical University, Guangzhou Women and Children's Medical Centre and all women that participated in this study. Last but not least, we want to thank all the staff in our research team who gave helping hands throughout the process.

Availability of data and material

The datasets generated during the current study and analysis are available from the corresponding author upon reasonable request.

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Figures

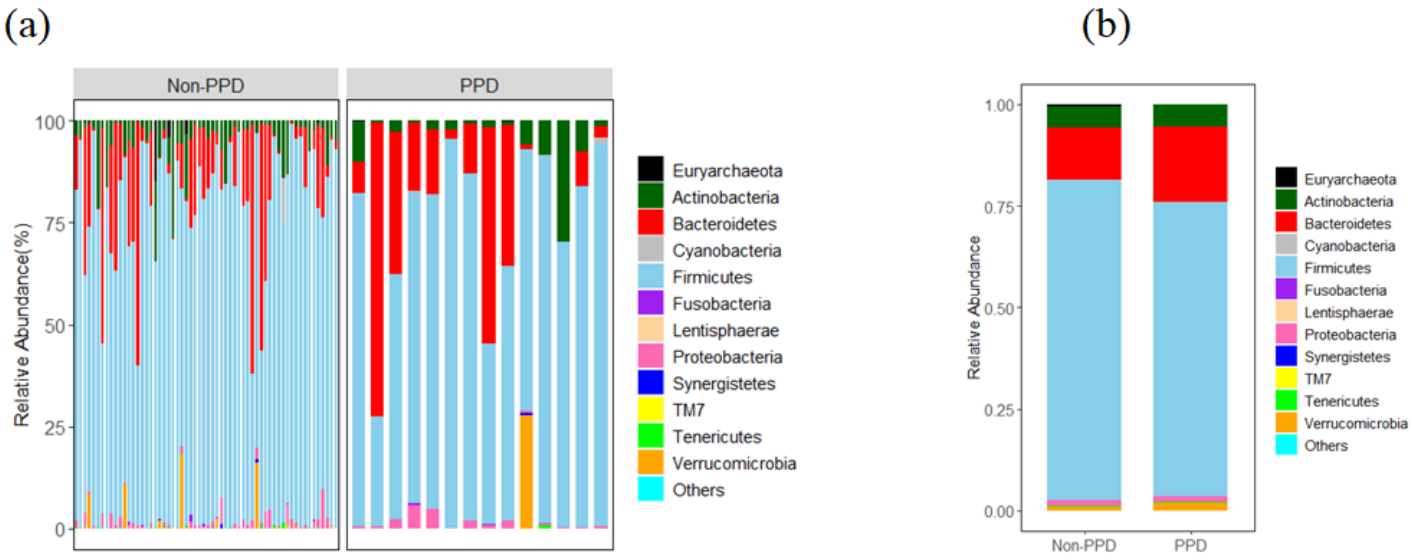

Figure 1

Composition of bacterial phyla in gravidas during 32-39 weeks of gestation (PPD and control groups). (a) The composition of the phylum abundance for each sample. (b) The composition of the phylum abundance for the two groups.

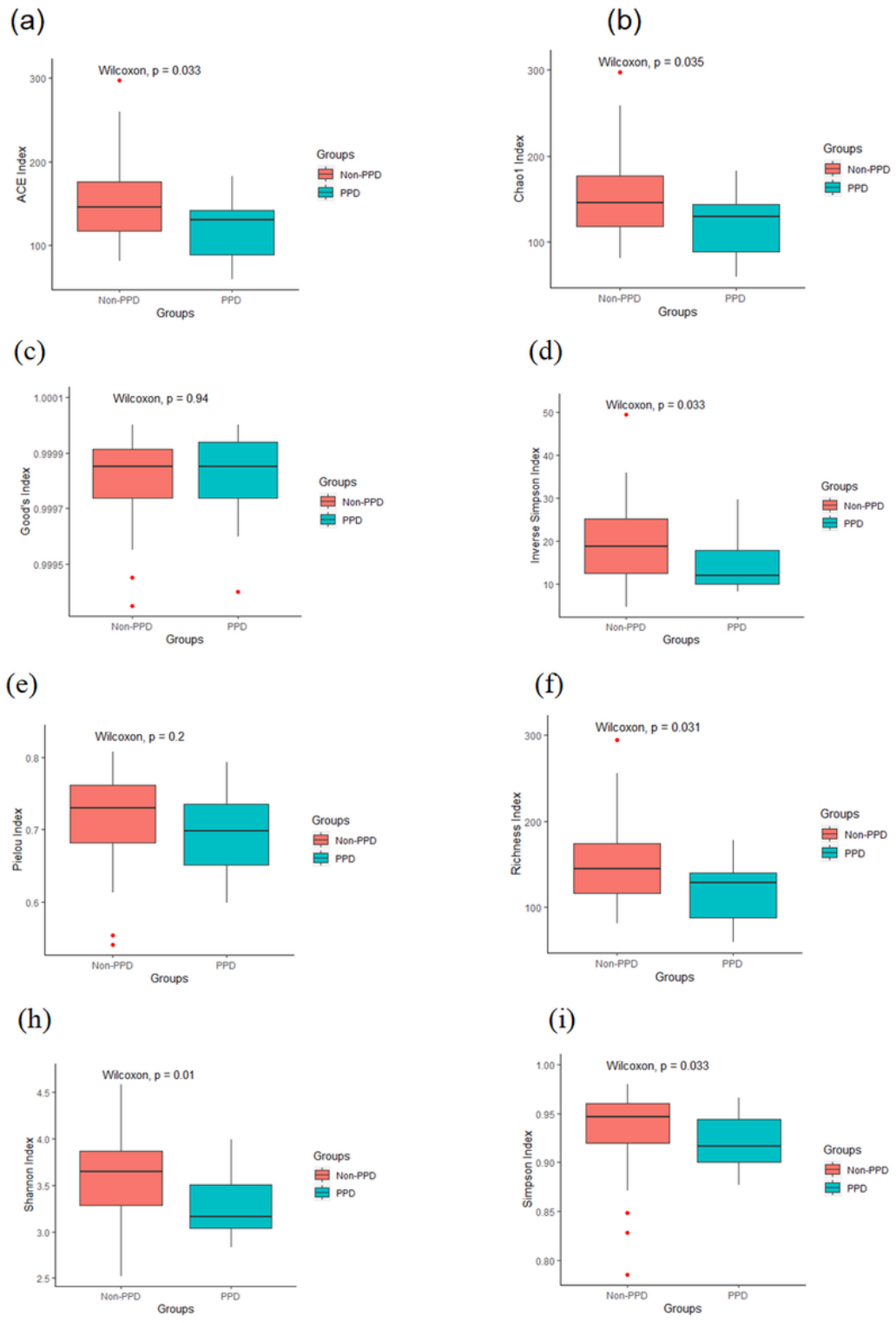

Figure 2

Comparison of the alpha diversity index between the PPD and control groups. The Wilcoxon test was used to compare the alpha diversity index between the PPD and control groups. (a): Comparison of the ACE index between the PPD and control groups; (b): comparison of the Chao1 index between the PPD and control groups; (c): comparison of the Good's index between the PPD and control groups; (d): comparison of the Inverse Simpson index between the PPD and control groups; (e): comparison of the

Pielou index between the PPD and control groups; (f): comparison of the Richness index between the PPD and control groups; (h): comparison of the Shannon index between the PPD and control groups; (i): comparison of the Simpson index between the PPD and control groups.

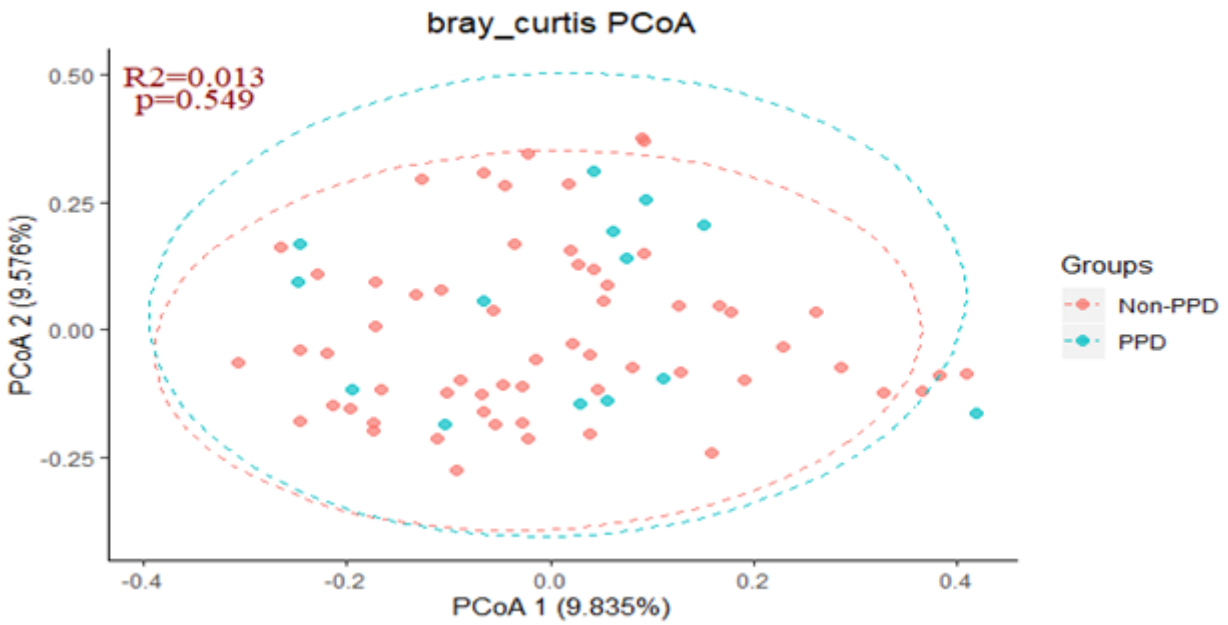

Figure 3

PCoA based on Bray-Curtis distances at the OTU level. Each sample is represented by a dot. Circles in different colors represent different groups.

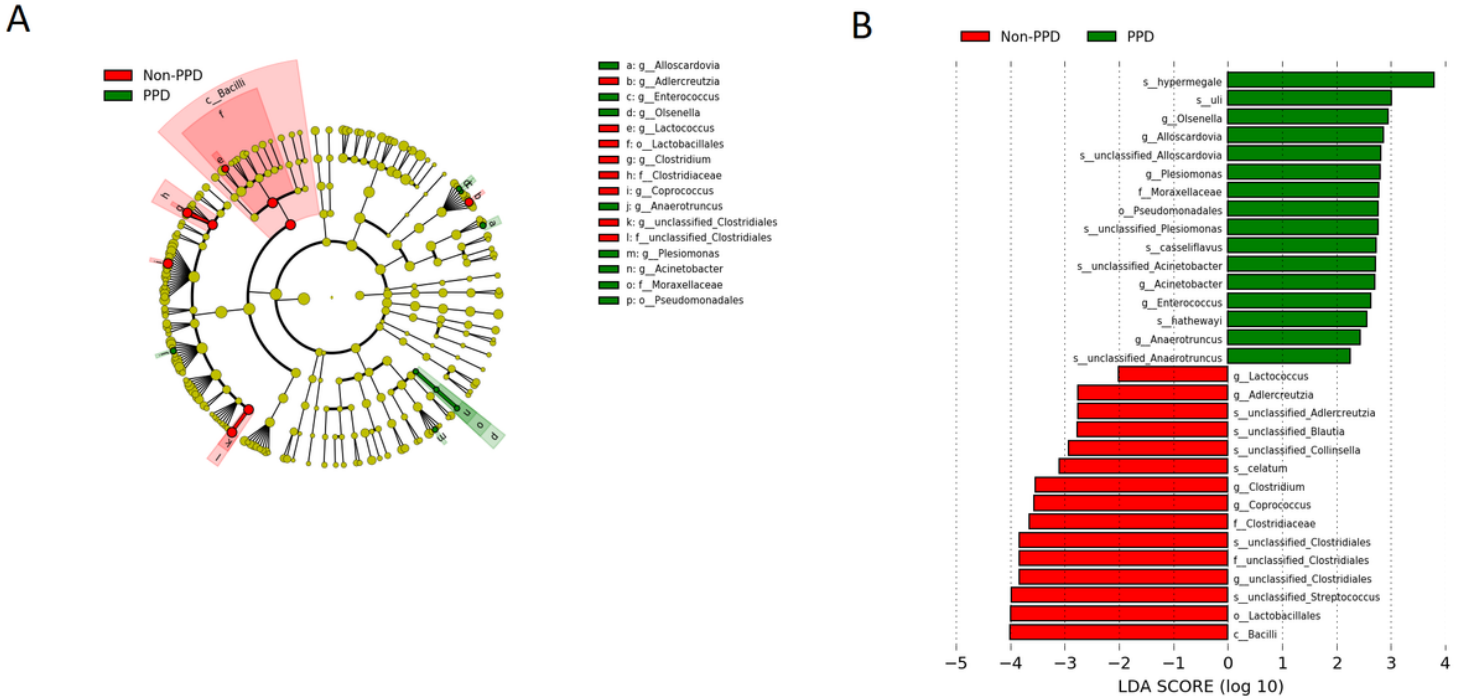

Figure 4

LEfSe identified the most differentially abundant taxa between the PPD and non-PPD groups. The taxonomic cladogram obtained 16S sequences (relative abundance ≥0.5%) from LEfSe analysis. (a) Red indicates non-PPD group-rich taxa. Green indicates PPD group-rich taxa. The brightness of each dot is proportional to the effect size. (b) PPD-enriched taxa are indicated with a positive LDA score (green), and taxa enriched in the non-PPD group have a negative score (red). Only taxa meeting a LDA significant threshold >2 are shown.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalTable1.docx