Gut microbiota changes in pre-eclampsia, abnormal placental growth and healthy pregnant women

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Research

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Abstract

Pre-eclampsia (PE) is a condition of high blood pressure which usually concurrent with proteinuria in pregnancy. PE complicate the management of both maternal and fetal health and contribute the most of adverse pregnancy outcome, but the mechanism underlying the development of PE remains unclear. In this study, we performed a case-control study to compare the gut microbiota of PE, abnormal placental growth (APG) and health pregnant women and analyzed the potential pathogenic role of gut microbiota in PE progression. Although the clinical pathophysiological state did not affect the bacterial diversity, the compositions of the gut microbiota were significant altered in both PE and APG groups when compared with health pregnant woman. At phylum level, *TM7* was significantly increased in woman with APG and, although with no significance, the percentage of *Proteobacteria* were extended especially in patient with PE. Heterogeneity was observed at genus level, especially in the genus with positive LDA scores suggested the stage-dependent manner effort of gut microbiota to the development of PE. The beneficial bacteria *lactobacillus* was markedly depleted in PE and APG group but only correlated with blood pressure (BP) and proteinuria levels in PE group and, different *lactobacillus* species shown different contributions (otu255 and otu784 were significant related to PE and APG separately). Our results indicated that shifts in the gut microbiota might occur from the early stages of the development of PE, which is of possible etiological and therapeutic importance.

Background

PE is a disorder of pregnancy characterized by one set of hypertension and usually concurrent with proteinuria in pregnancy (1). Most of PE occurs third trimester pregnancy and worse outcomes were commonly prevailed when it occurs earlier (2, 3). Since 2015, PE became a leading cause of maternal and infant morbidity and mortality and affect 2 ~ 8% of all pregnancies worldwide annually (4, 5).

Depressingly, although many studies indicated that PE should be considered as a subtype of metabolic disease (6, 7), the pathogenesis of PE has not been clarified and, there was no clear and effective clinic therapy. Part of studies suggested that placental obstruction, abnormal placentation, diffuse inflammatory response, vascular endothelial damage and environmental factors were considered as possible etiologies to PE (1). The most accepted pathogenesis of PE is placental dysplasia caused anoxia, ischemia and hypoperfusion. In animal models, the increased heterodimer formed by angiotensin II and bradykinin B2, two G protein-coupled receptors with opposing, can cause abnormal vasoconstriction which triggering PE-like symptoms in pregnant mice (8). But the causes of the above-mentioned abnormal remains obscure and thus pathogenetic heterogeneity complicate the management of both maternal and fetal health. For this reason, no reliable biomarkers or clinic test can predict the emergence of PE in the earlier stages of pregnancy, and the only way to cure PE is termination (1).

A very encouraging and innovative hypothesis had recently been proposed whereby the mutual relationships between microbes and host could be an important factor affecting health (9). Tens of trillions of microbes composed a complex ecosystem inhabited mainly in our intestine also known as gut

microbiota (10), and own distinctive characteristics during pregnancy (11). Numerous microbes in our gut help us to digested complex and indigestible carbohydrates to produce beneficial metabolic output (12, 13). Highly diverse bacterial related proteins maintained our intestinal and immune homeostasis (14). Dysbiosis in our gut was associated with metabolism disorder (15), immune system disfunction (16), and even the development of obesity (17, 18) and diabetes (19, 20) of the host. But the relationship between the gut microbiota and PE remains largely unclarified till now. Some studies glimpsed the disturbance of both gut and placenta microbiota in PE cohorts (21–23), but there is lack of stage-dependent or chronological analysis. In this study, healthy and pre-eclampsia pregnant women were recruited. Because increasing evidences indicated the placentation abnormal was associated with PE (24) and such abnormal can often be predicted by clinical test of placental growth factor (PIGF) (25) early of pregnancy, we also recruited 25 pregnant women with significant decreased PIGF levels. Using 16s rRNA gene sequencing on stool samples we revealed the gut microbiota profile of women with PE.

Results

Subject clinical features description

A total of 100 women were recruited, which included 21 healthy women (NW group), 28 healthy pregnant women (NP group), 25 pregnant women with abnormal placental growth (APG group, decreased PIGF MoMs) and 26 pregnant women with pre-eclampsia (PE group). There were no significant differences on age and body mass index (BMI) between the four groups. The gestational age of APG is significantly lower than that of NP and PE group. Setting of APG group is more conducive for us to estimate the gut microbiota changes at earlier gestational weeks and, the diastolic blood pressure (DBP), systolic blood pressure (SBP) and urinary protein concentration (UP) of PE group were significantly higher than those of other three groups (Table 1).

Table 1
Summary of subject characteristics.

Groups	NW	NP	APG	PE
Subjects, n	21	28	25	26
Age range, years (SD)	31.04 ± 3.73	29.03 ± 3.80	27.50 ± 0.57	29.23 ± 4.85
gestational week (SD)	-	36.50 ± 7.43	23.71 ± 5.80*	36.73 ± 3.44
BMI (SD)	20.49 ± 1.30	21.87 ± 2.62	25.01 ± 3.55	24.27 ± 4.34
SBP, mmHg (SD)	110.95 ± 9.38	113.46 ± 8.79	115.00 ± 9.37	145.61 ± 10.44**
DBP, mmHg (SD)	71.38 ± 5.62	73.21 ± 7.28	70.27 ± 6.99	95 ± 7.14**
Urine protein, scores (SD)	0	0	0	1.57 ± 0.70**

#All values are mean ± standard deviation. An asterisk indicated a significant difference (* at $p < .05$, ** $p < .01$) between the NP and the labelled group.

Effects of PE on the Overall Structure of Gut Microbiota.

In order to better analyze the reads range from 74949 to 201741 (about 124761 clean reads per sample) obtained by fecal sample sequencing, ≥ 97% similarity of sequences were clustered into 1 operational taxonomic units (OTUs). This was an efficient and widely used strategy for the investigation of the fecal microbiota.

Principal component analysis (PCoA) based on the unweighted UniFrac index showed no significant difference in bacterial composition between NW and all pregnancies (Fig. 1A, B). As in the three pregnant woman groups, the gut microbiota composition of APG group and PE group and all the abnormal pregnancies (APG group plus PE group) were significantly shifted compared with the NP group (Adonis, P = 0.002, P = 0.015, P = 0.001, respectly) (Fig. 1B). There was no significant difference in the composition of gut microbiota between APG and PE groups even a notable gestational age gap existed (Adonis, P = 0.463). Although the pathophysiological state of pregnant women affected their gut microbiota, the alpha diversity, which included shannon and chao1 index, exhibited only marginally difference (Fig. 1C, D). The total numbers of OTUs (detected in more than 2 samples) in each group did not changed (Fig. 1E). A total of 2332 OTUs were observed as the core shared feature in all the four groups (Fig. 1F), a higher number of detectable unique OTUs were observed in the NW and APG groups (228 unique OTUs in the NW group and 495 in the APG group).

Gut microbiota compositional shifts in APG and PE at phylum level.

As many other studies indicated, the mainland of human gut microbiota were the microbial communities mainly belonged to *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria* and *Verrucomicrobia* (26). The same results were found in our data (Fig. 2A). The ratio of the phyla *Firmicutes* and *Bacteroidetes* (F/B) have been associated with obesity, type 2 diabetes and systemic inflammation, we compared the F/B ratios between the four groups. Although with no significance, an obvious increasing trend of F/B ratios among NP, APG and PE groups were observed (Fig. 2B). Two phyla, *TM7* and *Proteobacteria*, were disturbed. The percentage of *TM7* was significantly increased in APG group (Fig. 2C), and the rates of patients with higher content of *Proteobacteria* were observed in APG and, especially, PE groups (Fig. 2D). Because the APG and NW groups carried more unique OTUs, Venn diagram combined with phylogenetic relationships of OTUs at the phylum level was exhibited. Bacteria belonged to *Firmicutes* dominanted the core shared microbial community (Fig. 1F and 2E). In the NP group, bacterium belonged to *Firmicutes* were the main driver which constituted the unique microbial community (Fig. 2E). But in the APG group, bacterium belonged to *Bacteroidetes* and *Actinobacteria* were prevailed (Fig. 2E). Just as the APG group, the PE group showed same unique OTUs composition (Fig. 2E).

Disturbance of gut microbiota was associated with the clinical characteristics of PE.

The composition of the gut microbiome at the genus level revealed by OTUs showed some more detail changes on microbes. The composition of the most abundant 10 genera in all subjects was shown in Fig. 3A. Apparently, *g_Prevotella* was the most common and prevailed genus in the NP group when

compared with NW or any patient groups (Fig. 3A). For further identify the relationship between the gut microbiota changes and the development of PE, linear discriminant analysis (LDA) was used to catch the core bacterial differences and identified a total of ten genera with significant differences.

Detailly, the relative abundance of *g_Prevotella*, *g_WAL_1855D*, *g_1_68*, *g_Porphyromonas*, *g_Varibaculum* and *g_Lactobacillus* between NP and PE groups were significant decreased (Fig. 3B). Between the APG and the NP groups, *g_Prevotella*, *g_1_68*, *g_Porphyromonas*,*g_Lactobacillus*、*g_Mobiluncus*、*g_Campylobacter* and *g_Peptostreptococcus* were decreased significantly (Fig. 3B). When consider the abnormal pregnant women as a whole group, 6 genera with significant difference were detected whereby the significant decreased abundance of *g_Prevotella*, *g_1_68*, *g_Porphyromonas*, *g_Lactobacillus*, *g_Varibaculum* and the significant increased abundance of *g_Lactococcus* (Fig. 3B).

Obviously, *g_Lactobacillus* showed unanimous correlations with both blood pressure and UP (Fig. 3C). *g_1_68*, *g_Porphyromonas*, *g_Mobiluncus*, and *g_Lactococcus* were significant correlated with SBP and DBP. Although with no significant LDA difference in PE group compared with NP group (Fig. 3B), the relative abundance of *g_Staphylococus*, a potential pathogen, was inversely correlated with BMI index in the NP and the PE groups (Fig. 3C).

The loss of g_Lactobacilli **is only related to the abnormal clinical indicators of Pre-eclampsia patients.**

Over the past decades, *Lactobacillus* has been regarded as the most common probiotics in human intestine and been world-widely used in food processing, drug development and clinical treatment (27). In our cohort, *g_Lactobacillus* was the most detectable genus in the four groups with detection rates range from 68–100% (Fig. 4A). The detection rate and the relative abundance of *g_Lactobacillus* were significantly higher in NP than any other groups (Fig. 4A) implied that the *g_Lactobacillus* played an important role in maintaining the health of pregnant women. Moreover, we analyzed the correlations between the relative abundance of *g_Lactobacillus* and the blood pressure and UP in different grouping modes. The abundance of *g_Lactobacillus* was significant inversely correlated with SBP, DBP and UP in NP and PE groups (Fig. 4b). But in the NW and NP, NP and APG or all samples groups, no statistically significant correlations were found (Fig. 4B). We further found 20 OTUs (species) belonged to *g_Lactobacillus* and identified 2 OTUs, otu255 and otu784, showing significant differences (both of them with negative LDA score) (Fig. 4C). The relative abundance of otu255 was significant decreased in PE group when compared with NP group and, the otu784 was decreased with significance only between the APG and NP groups (Fig. 4C). Only otu255 was negatively related to DBP in the PE and NP groups with statistical significance (Fig. 4D). These results reveal profound changes in the intestinal microbiome structure of the APG and PE groups, indicating the importance of gut microbiota changes in the development of PE.

Discussion

Pregnancy is one of the most special and vulnerable physiological state of human beings under natural conditions. The maintenance of physical and mental health during pregnancy means great deal to both maternal and fetal safety. Since PE first described by Hippocrates (24), toxemia caused by external toxic substances, nutritional imbalance, genetic factors and placental dysfunction1 were proposed as pathogenesis of PE. The heterogeneity of etiology led to the lack of effective methods for both clinical prediction, prevention and intervention. We aimed to explore the potential role of gut microbiota in the development of PE. To our knowledge, the cohort in this study was the first one within which included pregnant women abnormal PIGF levels. Our results proposed that the gut microbiota dysbiosis could play an important role in the development of PE with a chronological manner.

Microbial community disturbances in the gut (21–23) and the planta (28) were previously detected in the PE members, and using fecal microbiota transplantation, the gut–placenta axis was proposed (23). Consistent with previous studies (23), we observed that the compositions of the gut microbiota in the PE group was clearly and firmly shifted (Fig. 1A, B). Exclusively in our data, a more robust shift of the gut microbiota was also detected in the APG group (Fig. 1A, B). If we taken all the abnormal pregnant women as a whole group, the gut microbiota was also significantly different from that of healthy women (Fig. 1B). Gestational age was one of the main factors affecting pregnant women's gut microbiota (11, 21). Interestingly, there was no difference between the PE and APG (Adonis, P = 0.463) even a notable gestational age gap existed (Table 1), indicating that the gut microbiota changes may occurred in the early stage of pregnant (considering the abnormal PIGF levels implied the abnormal pathophysiology of pregnant woman). Many diseases, such as obesity (17, 18), colorectal cancer (29) and arteriosclerosis (30), were accompanied by the decreased gut microbiota diversity. A relatively high diversity of gut microbiota was considered a sign of health (31). But only marginally differences in shannon, chao1 and observed OTUs were observed (Fig. 1C, D).

The expansion of low proportion microbial community was usually a manifestation of enteric imbalance (32) and represented the physical and chemical changes of intestinal environment (33, 34). The increased abundance of *Proteobacteria* was considered to be one of the causes of the inflammation in IBS and IBD patients and, as the result of high oxygen content in intestine (35). At late pregnancy, for better adapt to the physiologic, metabolic and immune changes in healthy pregnant women, the proportion of *Proteobacteria* was commonly increased (11). Noteworthy, the rates of patients with higher content of *Proteobacteria* were observed in APG and, especially, PE groups (Fig. 2D). The relative abundance of *TM7* was significantly increased in the APG (Fig. 2D). Restlessness of rarer communities of gut microbiota result in the reconstitution of the unique OTUs composition in the APG and PE (Fig. 2E). As the core shared OTUs, bacteria belonged to *Firmicutes*, which is believed to help us to improve the utilization of calories in the food (36), dominanted (Fig. 2E) and also composed the unique OTUs community in the healthy pregnant women group (NP). But more bacteria belonged to *Bacteroidetes*, which usually contained more gram-negative bacteria that could produce lipopolysaccharide (LPS) (37, 38), were observed in the compositions of unique OTUs community in APG and PE (Fig. 2E).

More significantly were the patterns of gut dysbiosis in the APG and PE. The relative abundance of *g_Prevotella* was significantly decreased (from ~ 20% in NP down to ~ 10% in APG and PE with significance). *g_Prevotella*, a main functional bacterial group in human intestinal tract (39). Recent studies reported that *g_Prevotella* can use fibre and polysaccharides to produce short chain fatty acids (SCFAs) (40), such as butyrate, the main energy source of intestinal epithelial cells (41) and the regulator of colonic T cell differentiation (42). At the same time, the colonization of *g_Prevotella* is also helpful to resist the infection of pathogens (43, 44). The beneficial effects of probiotics on human health has been widely studied. For pregnant women, the role of probiotics has not been fully revealed, but the homeostasis of hormone in their body can affect the content of probiotics in their gut. For example, progesterone can be used as nutrient to help the colonization and proliferation of *Bifidobacteria* in pregnant women's faeces (45). In our cohort, we found the detection rate of *g_Lactobacillus* in NP was, unexpectedly, 100% (Fig. 4A). And its relative abundance was significantly higher than any other three groups implied that *g_Lactobacillus* may play an important role in healthy pregnancy (Fig. 4A). In response, a study of 33399 primiparas showed that daily or weekly intake of *Lactobacilli* or their yoghurt products during pregnancy significantly reduced the risk of PE (46). Another study of 70149 Norwegian mothers showed a significant correlation between supplementation of probiotic milk in late pregnancy and a lower risk of PE (47). The loss of beneficial and functional genera may lead to the bred of potential pathogens both in the APG and PE, such as *g_Lactococcus*, and finally contributed to the development of PE. In the future, probiotic supplementation and gut microbiota targeted special diet may has expectable therapeutic effects in improving or blocking the occurrence and development of preeclampsia.

Taken together, we revealed the clearly gut microbiota dysbiosis in PE and APG. This shifts in the gut microbiota may occur from the early stages of the development of PE and future longitudinal multiregion large-cohort study are needed to r constitute a microecological perspective for PE managements.

Materials And Methods

Study subject recruitment

All subjects were from the outpatient or ward of Changsha Hospital for Maternal and Child Health Care, Hunan, China.

The definition of PE was according to the diagnosis standard and the subjects were screened by experienced clinician. Diagnosis standard was as followed: hypertension (systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≥ 90 mm Hg) and high urine protein (≥ 0.3g/24h urine collection or random dipstick reading urine protein positive). Additionally, PE woman with systolic blood pressure ≥ 160 mm Hg and/or diastolic blood pressure ≥ 110 mm Hg, thrombocytopenia, impaired liver /kidney function, pulmonary edema or emerging nervous system abnormalities were clinically defined as severe.

The definition of patients with abnormal PIGF value in early and middle pregnancy. The values were obtained by normalize the concentrations of PIGF with the median value in the localized database

(anthropometry-matched pregnant women's database), also known as MOM value, in early and middle pregnancy.

Patients with malignant tumors, depression, cardiovascular diseases, diabetes, chronic nephritis, liver and kidney function damage or immune system diseases were excluded.

Fecal sample collection and DNA extraction

Fecal samples of each subject were obtained at home. Fresh faeces was immediately removed into our storage kit, storage and stabilizing stool samples for microbiome study at ambient temperature for up to a month with minimal alterations when compared with deeply freezing samples, and frozen immediately until delivered to the laboratory. Fecal samples were delivered together with ice and were stored at – 20°C at the laboratory until extraction.

16S rRNA gene amplicon sequencing and analysis

Microbial genome DNA was extracted from frozen fecal pellets using the e.Z.N.A.TM stool DNA kit (OMEGA bio-tek, USA) following the manufacturer's instructions. The resultant DNA was quantified by Nanodrop and then stored at -80°C for further analysis. The V4 region of the 16S rRNA gene was PCR-amplified from microbial genome DNA using primers (forward primer, 5'-GTG CCA GCM GCC GCG GTA A-3'; reverse primer, 5'-GGA CTA CHV GGG TWT CTA AT-3'). The PCR products were detected using dual-indexing amplification and sequencing approach on the Illumina MiSeq platform and then using QIIME (vision 2.0) to reveal the gut microbiota change. Sequencing and preliminary analysis were completed in Hangzhou Guhe Info Co., Ltd, Hangzhou, China.

Statistical analysis.

Differences between two or more groups were evaluated by one-way ANOVA followed by Dunnett's test or 50 Fisher's protected least significant difference test using SPSS 24.0 (SPSS, Chicago, Illinois). Values are expressed as the mean ± SEM.

Declarations

Ethics approval and consent to participate

The study was approved by Research Ethics Committee of Changsha Hospital for Maternal and Child Health Care (NO: 2020005). Each participant provided written informed consent before any study procedure.

Consent for publication

NA

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

LH Huang, WT Yang, J He and Min Cai designed the research;

L Li, Xin Zhang, Y Xu, JY Liu, Q Huang and GJ Luo programmed the task and collected the fecal samples and the clinical data;

LH Huang, L Li, M Cai, ZZ Zeng and CY Jin analyzed the data and drafted the manuscript;

CY Jin and YX Jin revised the manuscript. All authors reviewed and approved the manuscript.

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Abbreviations

PE: Pre-eclampsia; APG: Abnormal placental growth; BP: Blood pressure; PIGF: Placental growth factor; BMI: Body mass index; DBP: Diastolic blood pressure; SBP: Systolic blood pressure; UP: Urinary protein; OTUs: Operational taxonomic units; PCoA: Principal component analysis; F/B: *Firmicutes* and *Bacteroidetes*; LDA: Linear discriminant analysis; LPS: Lipopolysaccharide; SCFAs: Short chain fatty acids.

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Figures

figure 1

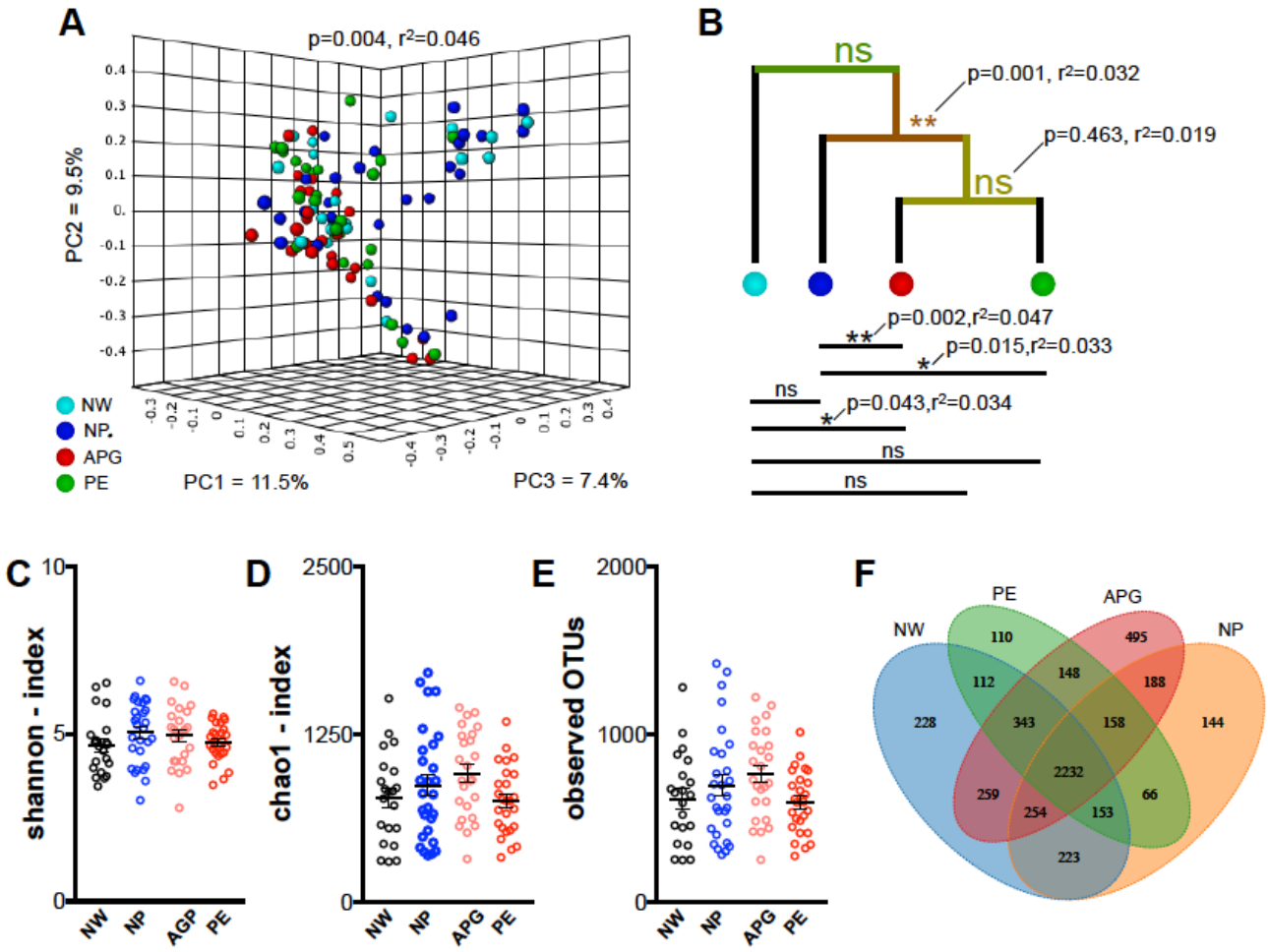

Figure 1

Effects of PE on the Overall Structure of Gut Microbiota. (A, B) Principal coordinate analysis based on unweighted UniFrac index of the bacterial communities in NW, NP, APG and PE. The differences in beta diversity between each group were tested by permutational multivariate analysis of variance (Adonis). (C, D, E) The Shannon, Chao1 and observed OTUs estimates of fecal microbiota in each group. (F) Venn diagram of the numbers of the identified OTUs. Dots in box plot show values in each individual. An asterisk indicated a significant difference (* at p < .05, ** p < .01) between the labelled groups.

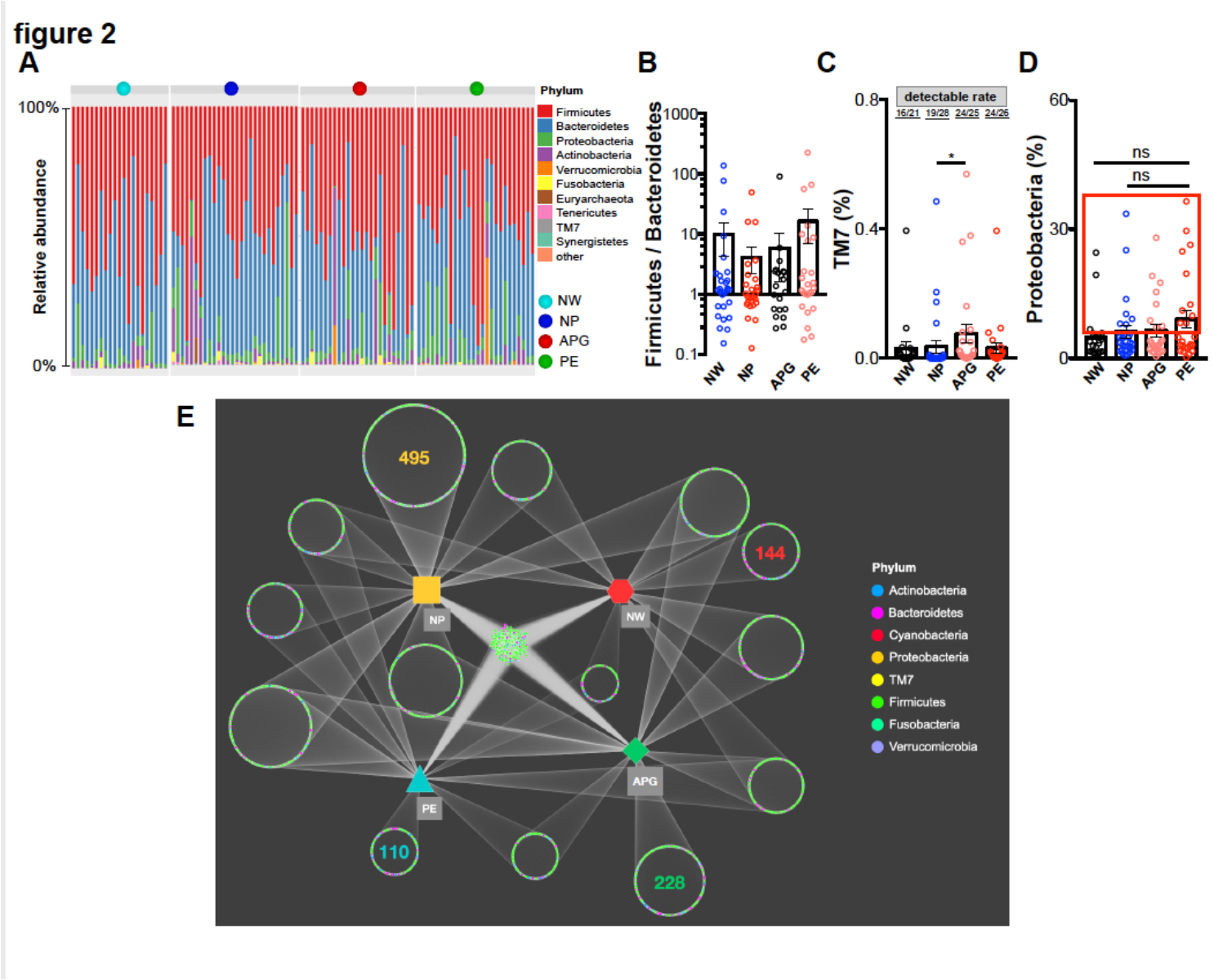

Figure 2

Gut microbiota compositional shifts in APG and PE at phylum level. (A) Relative abundances of phyla in samples. (B) The Firmicutes/Bacteroidetes ratios of gut microbiomes in each group. (C, D) Relative abundances of two phyla, TM7 and Proteobacteria, in samples. An asterisk indicated a significant difference (* at $p < .05$, ** $p < .01$) between the labelled paired groups. (E) Venn diagram combined with phylogenetic relationships of OTUs at the phylum levels.

figure 3

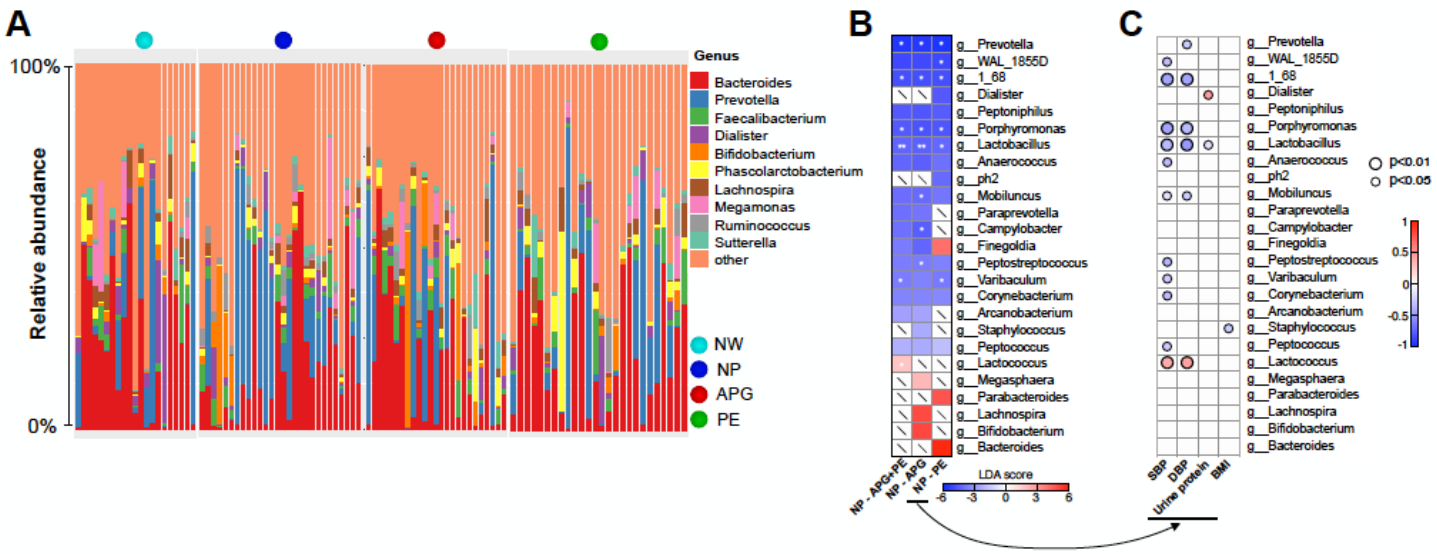

Figure 3

Disturbance of gut microbiota was associated with the development of PE. (A) Relative abundances of the most abundant ten genera in samples. (B) linear discriminant analysis effect size identified the most differentially abundant taxa between the selected two groups. The enriched taxa were indicated with a positive LDA score, and taxa enriched in NP have a negative score. Only taxa meeting an LDA significant threshold of >3 were shown. The asterisk indicated a significant relative abundance change of the taxa (* at $p < .05$, ** $p < .01$) between the selected paired groups. (C) Correlations between the relative abundance of the selected genus and the clinical parameters.

figure 4

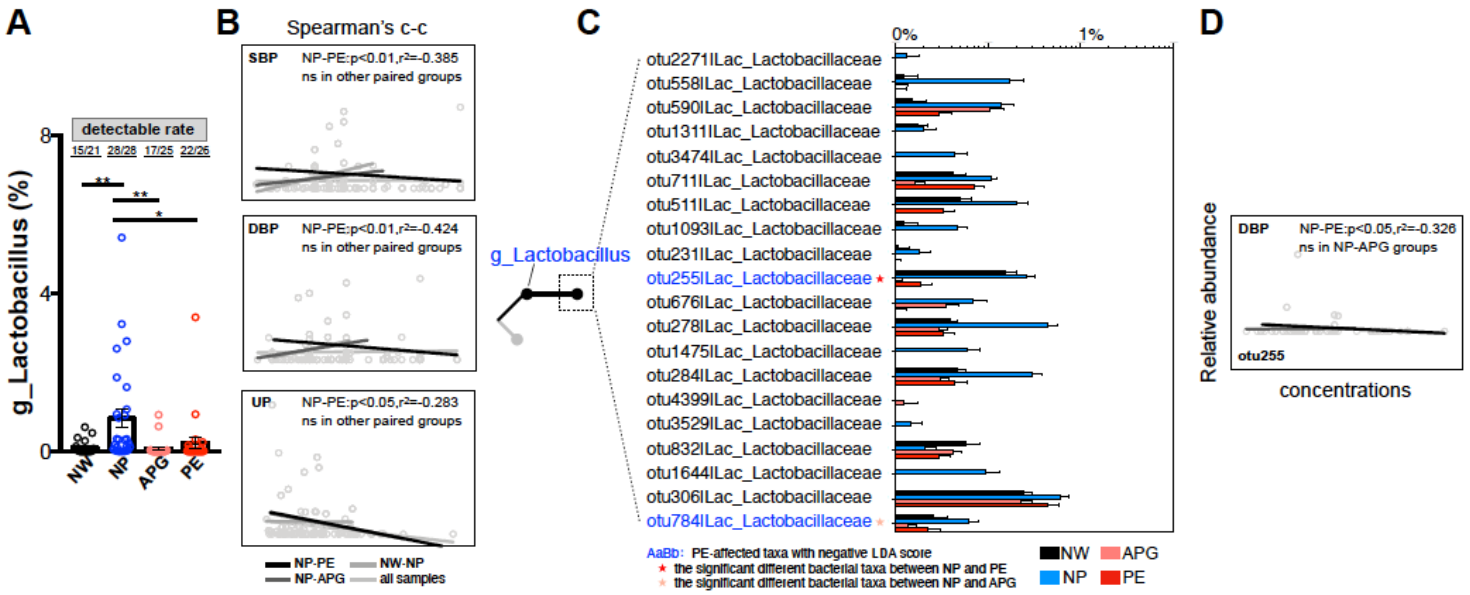

Figure 4

The loss of g_Lactobacilli is only related to the abnormal clinical indicators of Pre-eclampsia patients. (A) Relative abundances of g_Lactobacilli in samples. (B) The correlations between the relative abundance of g_Lactobacilli and the concentrations of SBP, DBP and UP. (C) The relative abundances of the OTUs belonged to the g_Lactobacilli. (D) The correlations between the relative abundance of otu255 and the concentrations of DBP.