ORIGINAL RESEARCH

Gut dysbiosis induces the development of pre-eclampsia through bacterial translocation

Xia Chen 1, Pan Li 2, Mian Liu 1, Huimin Zheng 2,3, Yan He 2, Mu-Xuan Chen 2, Wenli Tang 2, Xiaojing Yue 1, Yongxin Huang 1, Lingling Zhuang 4, Zhijian Wang 1, Mei Zhong 1, Guibao Ke 5, Haoyue Hu 1, Yinglin Feng 1, Yun Chen 1, Yanhong Yu 1, Hongwei Zhou 2, Liping Huang 1

ABSTRACT

Objective Pre-eclampsia (PE) is one of the malignant metabolic diseases that complicate pregnancy. Gut dysbiosis has been identified for causing metabolic diseases, but the role of gut microbiome in the pathogenesis of PE remains unknown.

Design We performed a case–control study to compare the faecal microbiome of PE and normotensive pregnant women by 16S ribosomal RNA (rRNA) sequencing. To address the causative relationship between gut dysbiosis and PE, we used faecal microbiota transplantation (FMT) in an antibiotic-treated mouse model. Finally, we determined the microbiome translocation and immune responses in human and mouse placental samples by 16S rRNA sequencing, quantitative PCR and in situ hybridisation.

Results Patients with PE showed reduced bacterial diversity with obvious dysbiosis. Opportunistic pathogens, particularly Fusobacterium and Veillonella, were enriched, whereas beneficial bacteria, including Faecalibacterium and Akkermansia, were markedly depleted in the PE group. The abundances of these discriminating bacteria were correlated with blood pressure (BP), proteinuria, aminotransferase and creatinine levels. On successful colonisation, the gut microbiome from patients with PE triggered a dramatic, increased pregestational BP of recipient mice, which further increased after gestation. In addition, the PE-transplanted group showed increased proteinuria, embryonic resorption and lower fetal and placental weights. Their T regulatory/helper-17 balance compared with in normotensive samples. The dysbiotic gut microbiome induced immune responses in pregnant mice, facilitating translocation of bacteria to the intrauterine cavity, ultimately eliciting inflammation in the placenta and contributing to poor placental development.

Conclusions This study suggests that the gut microbiome of patients with PE is dysbiotic and contributes to disease pathogenesis.

INTRODUCTION

Pre-eclampsia (PE) is a disorder of pregnancy associated with new-onset hypertension, which occurs most often after 20 weeks of gestation combined with proteinuria. PE affects 2%–8% of pregnancies and is associated with an increased risk of adverse pregnancy outcomes, including placental abruption, prematurity and intrauterine growth restriction.

To date, no first-trimester or second-trimester tests can reliably predict the development of all cases of PE, and thus, it is of particular interest to find effective and accessible clinical biomarkers in the field of obstetrics. The pathogenesis of this morbidity is still vague to date. Emerging studies have indicated that PE should be considered as a type of metabolic disease closely associated with metabolic disorders such as obesity, diabetes mellitus, insulin resistance, atherogenic dyslipidaemia and hyperglycaemia.
The presence of glucose intolerance and dyslipidaemia, along with hypertension, can contribute to endothelial dysfunction, poor placentation and abnormal placental development, which are common pathological abnormalities in PE.

Numerous studies have suggested that gut dysbiosis plays a causative role in the development of metabolic syndrome, chronic inflammation diseases and complicated pregnancies. Gut microbiota can facilitate insulin resistance by inducing the chronic inflammation in the host and causing fat accumulation by regulating genes related to energy metabolism, which plays an important role in the development of core symptoms in metabolic disorders. Gut microbiome dysbiosis is a key factor in blood pressure (BP) regulation and can contribute to the development of proteinuria and subsequently lead to kidney disease.

The composition of the gut microbiota in patients with PE and the relationship between the gut microbiota and PE have not been clarified so far. Recent studies suggested a disrupted gut microbiota composition in patients with PE in late pregnancy, but there is lack of causative analysis. In this study, we performed 16S ribosomal RNA (rRNA) gene sequencing on faecal samples but there is lack of causative analysis. In this study, we performed 16S ribosomal RNA (rRNA) gene sequencing on faecal samples to investigate the detailed profile of the gut microbiome and to determine the crucial role of the disordered gut microbiome in triggering PE-like phenotypes by faecal microbiota transplantation (FMT).

MATERIALS AND METHODS

Study subjects

Women with PE and normotensive pregnant (NP) women without previous treatments were recruited in the third trimester from March 2017 to March 2018 in the Department of Obstetrics of the Nanfang Hospital, Southern Medical University, China. According to the current American College of Obstetricians and Gynaecologists criteria, PE was defined as hypertension (systolic blood pressure (SBP) or diastolic blood pressure (DBP) of ≥140 or ≥90 mm Hg on two occasions at least 4 hours apart) and significant proteinuria (≥300 mg protein per 24 hours’ urine collection or protein/creatinine ratio of ≥0.3 mg/dL or dipstick reading of 2+ in a random urine sample) after 20 weeks of gestation in a woman with a previously normal BP. In the absence of proteinuria, PE was defined when hypertension was associated with any features of end organ damage. PE with severe features was considered when either severe hypertension (SBP or DBP ≥160 or ≥110 mm Hg), thrombocytopenia, impaired liver function, renal insufficiency pulmonary oedema, headache or visual disturbance was present. The clinical characteristics of the faecal and placenta donors are summarised in online supplementary tables S1 and S2, respectively.

Animals and experimental protocol

C57BL/6 mice were housed with a 12 hours’ light–dark cycle in specific pathogen-free facilities. FMT was performed according to a modified method described previously. Briefly, female mice aged 6–8 weeks were administered antibiotics (vancomycin, 100 mg/kg; neomycin sulfate, metronidazole and ampicillin, 200 mg/kg) intragastrically once daily for five consecutive days. We randomly selected three patients with PE and three NP controls as faecal donors for FMT (online supplementary table S3). The faecal supernatants of three PE and three NP donors were mixed and used as a single source for the PE-FMT and NP-FMT mice, respectively. Following antibiotic treatment, the recipient mice were orally inoculated daily for three consecutive days and two times per week thereafter for 59 days with prepared faecal contents. Another group of female mice was gavaged as described previously, but the donor supernatant was replaced with phosphate-buffered saline (PBS). Six weeks after the first microbial administration, all female mice were housed with male mice at a 2–3:1 ratio overnight, and pregnancy was confirmed by the presence of vaginal spermatozoa. Mice harbouring microbiota from patients with PE and NP, as well as PBS, were referred to as PE-FMT, NP-FMT and control, respectively. The group and cage number of the mice involved in the study are shown in online supplementary table S4.

Protein expression and biochemical analysis

Protein was extracted from tissues with commercial lysis buffer. Western blotting was performed as described previously. Primary antibodies against zonula occluden (ZO)-1 (Invitrogen, Carlsbad, California, USA), ZO-2 (Abcam, Cambridge, UK), occludin (Invitrogen), claudin-4 (Abcam) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Invitrogen) were used. Faecal albumin and urinary protein concentrations were determined with a mouse albumin ELISA kit (Bethyl Labs, Montgomery, Alabama, USA) according to the manufacturer’s instructions. The endotoxin level was measured with a commercial kit (Bio-Swamp, Beijing, China).

Gene expression analysis

Total RNA was extracted with Trizol reagent (Invitrogen) according to the manufacturer’s instructions. The reverse transcription reaction was carried out with reverse transcription enzyme (Toyobo, Shanghai, China). Quantitative real-time PCR was carried out on an ABI Q5 real-time PCR system and the specific primers for quantitative PCR are shown in online supplementary table S5.

Flow cytometry

Isolated immune cells were resuspended and stained with anti-CD4-FITC and/or anti-CD25-APC monoclonal antibodies (mAbs) for surface antigens and anti-Foxp3-PE or anti-IL-17A-PE mAbs for intracellular antigens in accordance with the manufacturer’s instructions (eBioscience, San Diego, California, USA). Fluorescence minus one controls were stained in parallel. Flow cytometry data acquisition was conducted using a BD LSRSorter flow cytometer (BD Biosciences, San Jose, California, USA), and the analysis was performed by BD FACSDiva Software V 8.0.1 (BD Biosciences). The gating strategies are shown in online supplementary figure S1.

In situ hybridisation

In situ hybridisation (ISH) was performed following a modified protocol described by Choi et al. Briefly, paraffin-embedded sections were subjected to deparaffinisation, rehydration and permeabilisation. The probes used in this study were as follows: Eubacterial probes (EUB338 I- and EUB338 II-) mixture complementary to a 16S rRNA region specific for most bacteria; NON338, an oligonucleotide complementary to the probe EUB338, serving as a scrambled control for nonspecific binding; Fusobacterium-specific probe, a genus-specific probe complementary to a 16S rRNA region of Fusobacterium. All probes were 5'-labelled with digoxigenin, and antidigoxigenin/horseradish peroxidase antibodies were used as secondary antibodies. Tyramide signal amplification was performed to increase the detection sensitivity of bacterial signals. Six areas of each section were randomly photographed under a fluorescence microscope. The specific probes for ISH are shown in online supplementary table S6.

Chen X, et al. Gut 2020;69:513–522. doi:10.1136/gutjnl-2019-319101
Bioinformatics processing
Using QIIME V1.9.1, we merged, applied quality control and clus-
tered the 16S RNA gene reads into operational taxonomic units
(OTUs). Taxonomic groups were based on the Greengenes Data-
base V1.13_8 using closed reference to perform referenced-based
OTU clustering.\textsuperscript{20} Values for alpha diversity (Chao1 Index,
Shannon’s Index, Phylogenetic Diversity (PD) Whole Tree Index
and observed OTUs), beta diversity (unweighted UniFrac distance
metrics) and principal coordinate analysis (PCoA) employed based
on the UniFrac metrics were generated by QIIME V1.9.1. Permu-
tational multivariate analysis of variance was performed to deter-
mine if the compositions of microbiota differed between groups.
Linear discriminant analysis effect size (LEfSe) was performed to
determine the features most likely to explain the differences
between groups.\textsuperscript{22} To identify and filter the potential contami-
nation if the compositions of microbiota differed between groups.
Dysbiosis of gut microbe in patients with PE
A total of 85 normotensive women and 67 patients with PE were
included in the present study. In the PE group, there were 40
patients with severe features (PESF) and 27 patients without
severe features (PETSF). The PE group was further divided
to an early onset of PE group (21 women) and a late onset
of PE group (46 women) according to whether the disease was
detected before 33 gestational weeks.
According to PCoA, the gut microbiome of the PE group
differed significantly from that of the NP group using the
unweighted UniFrac distance (figure 1A). Alpha-diversity indices,
including Chao1 and observed OTU, were markedly decreased in
the PE group (figure 1B,C and online supplementary figure
S2A,B). We did not observe significant differences between the PE
with and without severe features subgroups (online supplemen-
tary figure S3A–E) or between the early and late onset of PE
subgroups (online supplementary figure S4A–E) for both alpha
diversity and beta diversity.
The intestinal bacterial community consisted of 379 genera
belonging to 18 phyla in patients with PE and in normotensive
individuals. At both the phylum and genus levels, the micro-
bial composition of patients with PE differed from that of the
normotensive group (online supplementary figure S2C,D). To
further identify which bacterial taxa were distinct between the
PE and NP groups, we performed LEfSe analysis and identi-
fied seven genera showing significant differences (figure 1D).
Moreover, the abundance comparisons of predominant genera
showed that Clostridium, Dialister, Veillonella and Fusobacte-
rion were significantly enriched, whereas Lachnospira, Akkerm-
ansa and Faecalibacterium were depleted in patients with PE
(figure 1E–K). However, no significant differences of the above-
mentioned genera were observed among the PESF and PETSF
subgroups (online supplementary figure S3F).
Phylogenetic Investigation of Communities by Reconstruction
of Unobserved States (PICRUSt) analysis was performed to infer
microbial metabolites and metabolic pathways. Pathways
correlated with inflammation, including lipopolysaccharide
biosynthesis and bacterial toxins, were enriched in patients with
PE (online supplementary figure S2E,F). These key genera showed
significant correlations with BP proteinuria, impaired hepatic func-
tions or other clinical manifestations based on Spearman correla-
tion calculated for all participants (figure 1H). Notably, Veillonella
and Fusobacterium were significantly correlated with most clinical
parameters, including SBI, DBP, proteinuria, oedema levels,
alanine aminotransferase, aspartate aminotransferase, serum creat-
inine and albumin. The correlation coefficients for Lachnospira,
Akkermansia and Faecalibacterium were just opposite. However,
no bacteria were significantly correlated with clinical parameters
when testing only on the PE group, which could be due to reduced
sample size. These results reveal profound changes in the intestinal
microbiome structure of the PE group, indicating the importance
of gut microbes in the development of PE.
FMT induces the pre-eclamptic phenotype
To further test whether alterations in the gut microbiota can
cause the progression of PE in vivo, faecal microbiome from
patients with PE were transplanted into mice. Following antibi-
otics treatment, the recipient mice were randomly divided into
groups and orally inoculated with prepared faecal contents from
donors with PE or NP (figure 2A). The gut microbial profiles of
recipient mice were analysed by 16S RNA sequencing at 6 weeks
post-transplantation. As expected, PCoA based on unweighted
UniFrac distance matrices showed a significant effect in the FMT
group on the mouse faecal microbiome (online supplementary
figure S5A,B). From the donors, 71 of 128 genera successfully
colonised the intestine of recipient mice (online supplementary
figure S5C). Additionally, 55% of the shared genera were found
to vary with the same trend across the microbiota of donors and
recipient mice (online supplementary figure S5E). Moreover, at
the genus level, the abundance variations in Coprococcus, Fusos-
bacterium, Megamonas, Clostridium, Phascolarctobacterium and
Akkermansia showed the same trend between mice and donors
(online supplementary figure S5D,F). These results suggest that
the faecal microbiota composition of human donors was success-
fully reproduced in recipient mice.
At 6 weeks post-transplantation, PE-FMT mice exhibited
significantly higher pregestational SBP compared with NP-FMT
and control mice. After pregnancy, the SBP of the PE-FMT group
was further elevated from the fifth gestational day to the end of
gestation (figure 2B and online supplementary figure S6A). In
addition to worsening hypertension, the PE-FMT group showed
a significantly higher urine protein concentration at 17 days of
gestation, whereas urinary protein excretion was unchanged at
6 weeks post-FMT prior to mating (figure 2C). The number of
live pups, fetal weight and placental weight were also decreased
in the PE-FMT group, whereas the fetal resorption rate was
increased compared with that in the NP-FMT and control
groups (figure 2D,E). To determine how faecal bacteria from
patients with PE affect the placenta, we examined the structure
of the implantation sites. Histomorphological analysis revealed
a significant structural change in the PE-FMT placenta, as the
ratio between the two functional placental zones (labyrinth and
junctional zones) was increased in placentas from the PE-FMT

Chen X, et al. Gut 2020;69:513–522. doi:10.1136/gutjnl-2019-319101 515

Gut: first published as 10.1136/gutjnl-2019-319101 on 3 January 2020. Downloaded from http://gut.bmj.com/ on September 15, 2023 by guest. Protected by copyright.
Figure 1  Dysbiosis of gut microbiota patterns in patients with PE and NP women. (A) Principal coordinate analysis based on unweighted UniFrac distances revealed that the NP bacterial communities clustered separately from PE bacterial communities, which were more similar to each other. Each circle represents a single sample, colour by group. The eigenvalues of axe Principal coordinate (PC)1 and PC2 were 1.27 (6.56%) and 0.73 (3.53%), respectively. Difference in beta diversity between NP and PE were tested by permutational multivariate analysis of variance (Adonis).

(B,C) Comparison of alpha-diversity indices (Chao1 Index and observed operational taxonomic units) between PE and NP groups. ** indicates p<0.01 by Wilcoxon rank-sum test.

(D) linear discriminant analysis effect size identified the most differentially abundant taxa between the two groups. PE-enriched taxa are indicated with a positive LDA score, and taxa enriched in NP controls have a negative score. Only taxa meeting an LDA significant threshold of >3 are shown.

(E–K) Relative abundances of Clostridium, Dialister, Veillonella, Fusobacterium, Lachnospira, Akkermansia and Faecalibacterium between PE and NP groups. * indicates p<0.05; ** indicates p<0.01; and *** indicates p<0.001 by Wilcoxon rank-sum test.

(L) Correlations between the relative abundance of selected bacterial genera and clinical parameters. Correlations with p<0.05 are shown. Spearman’s rho statistic adjusted using the Benjamini and Hochberg method.

AT-III, antithrombin III; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Cr, creatinine; DBP, diastolic blood pressure; LDA, linear discriminant analysis; NP, normotensive pregnant; PE, pre-eclampsia; SBP, systolic blood pressure; TP, total protein; TT, thrombin time.

group compared with those from the NP-FMT and control groups (figure 2F). In addition, differences in placental labyrinth morphology were evident in the PE-FMT group (figure 2G). The PE-FMT mice exhibited infarction and increased stromal collagen deposition in the labyrinth layer of the placenta. However, in the NP-FMT group, labyrinth morphology resembled that in controls. Furthermore, no visible histopathological abnormalities were observed in the kidney tissues (online supplementary figure S6B). These results show that PE-like phenotypes were induced by faecal bacterial transfer from patients with PE.

FMT induces immunological imbalances

Decreased regulatory T (Treg) cells and/or increased T helper-17 (Th17) cells play a central role in the pathogenesis of PE.26 27 Thus, we investigated whether the adverse effect observed with FMT was associated with changes in Treg/Th17 proportions. Treg and Th17 cells were defined as coexpressing CD4+ CD25+ Foxp3+ and CD4+ IL-17A+, respectively. The number of Treg and Th17 cells was expressed as a percentage of CD4+ T cells for each subject and compared among different groups. Flow cytometric analysis of splenocytes and small intestinal lamina propria lymphocytes revealed a significant reduction in Treg cells and a marked increase in Th17 cells after FMT from patients with PE for 62 days compared with those in the NP-FMT and control groups (figure 3A–D). Moreover, the Treg/Th17 cell ratios were lower in PE-FMT mice than in NP-FMT and control mice (figure 3E).

We further examined inflammatory gene expression in the ileum to detect alterations in immunological responses. As shown in figure 3F, PE-FMT mice exhibited significantly higher Il1β, Ccl3, Cxcl1 and Vegf levels than NP-FMT or control mice. Collectively, these data indicate that the faecal microbiota from patients with PE disrupted the mucosal and systemic immunological response.

FMT in patients with PE microbiota impairs intestinal barrier function

Impaired immunological responses in the intestine are known to be associated with gut barrier dysfunction.28 We investigated if faecal bacteria from patients with PE affected gut barrier function. Intestinal permeability was monitored by faecal albumin content, which was significantly increased in the PE-FMT group.
Figure 2  Administration of faecal bacteria from patients with PE to mice induced a series of PE-like phenotypes. (A) Schematic representation of FMT. Fresh faecal from three PE and three NP donors were mixed and used as a single source for PE-FMT and NP-FMT mice, respectively. Following antibiotic treatment, the recipient mice were randomly divided and orally inoculated daily for three consecutive days and two times per week for 59 days with prepared faecal contents. (B) SBP of the three groups was evaluated pregestation and postgestation (n=8 for control and PE-FMT, n=10 for NP-FMT). α indicates p<0.01 versus NP-FMT group on the corresponding day; β indicates p<0.01 versus control group on the corresponding day. θ indicates p<0.05 for each corresponding gestational day versus the pregestational day (42 days post-FMT) by two-tailed Student’s t-test. (C) Urine protein concentration was measured at 42 days (prior to mating) and 62 days (17 days of gestation) post-FMT (n=8 for control and PE-FMT, n=10 for NP-FMT). (D) Number of pups and resorption rate per litter (n=8 for control and PE-FMT, n=10 for NP-FMT). (E) Placental weight and fetal weight significantly decreased in PE-FMT (n=41) compared with in NP-FMT (n=76) and controls (n=59) at 17 days of gestation. (F) Representative images of H&E-stained midsagittal placental tissue sections used in histomorphological analysis. Zones are marked and indicated by labyrinth zone and junctional zone. The ratio between the labyrinth/junctional zones was enhanced in PE-FMT (n=14) compared with in NP-FMT (n=16) and controls (n=16). (G) Representative H&E staining of the labyrinth zone of recipient mouse placentas. Typical lesions are marked with dotted line (original magnification, ×400; scale bar=50 µm). Data are presented as the mean±SEM (B–F). *P<0.05, **P<0.01, ***P<0.001 by one-way analysis of variance following Duncan’s multiple range test. ABX, antibiotics; BP, blood pressure; FMT, faecal microbiota transplantation; NP, normotensive pregnant; PE, pre-eclampsia; SBP, systolic blood pressure.

FMT markedly increased plasma endotoxin levels in PE-FMT mice (figure 4B). In addition, faecal microbiota from patients with PE decreased Claudin4 and Occludin gene expression in the colon compared with that in NP-FMT or control mice (figure 4C). Similarly, barrier-forming tight-junction protein ZO-1 and ZO-2, claudin-4 and occludin protein levels in the colon were markedly lower according to immunohistochemistry and western blot analyses (figure 4D–H).
Gut microbiota

Figure 3  FMT induced immunological imbalance. Treg and Th17 cells were analysed for coexpression of CD4⁺ CD25⁺ Foxp3⁺ and CD4⁺ IL-17A⁺ by flow cytometry, respectively. The number of Treg and Th17 cells was expressed as a percentage of CD4⁺ T cells for each subject and compared among different groups. (A–D) Percentages of Th17 cells in siLPL and spleen, and Treg cells in siLPL and spleen were determined (n=8 mice in each group). (E) Treg/Th17 cell ratios in siLPL and spleen of the three groups are shown (n=8 mice in each group). (F) Messenger RNA levels of key cytokines and chemokines in the ileum (n=7–8). Data are presented as the mean±SEM (A–F); *P<0.05, **P<0.01, ***P<0.001 by one way analysis of variance following Duncan’s multiple range test. FMT, faecal microbiota transplantation; NP, normotensive pregnant; PE, pre-eclampsia; siLPL, small intestinal lamina propria lymphocyte; Th17, helper 17 T; Treg, regulatory T.

the ileum and colon among the three groups, except for mild infiltration of inflammatory cells in the colon of PE-FMT mice (online supplementary figure S7). Furthermore, significant correlations between the abundance of the gut microbiome, gut barrier and immune index were observed (figure 4I). These data demonstrate that the intestinal barrier was disrupted by faecal bacteria from patients with PE.

Bacterial translocation is associated with inflammation in the placenta

Increased gut permeability can lead to gut bacteria or bacterial component translocation into the extraintestinal tissues.²⁹ ³⁰ We next detected bacteria in human and mouse placentas by quantitative PCR to evaluate the bacterial 16S rRNA gene. The levels of total bacterial DNA were obviously increased in the PE and PE-FMT placentas (figure 5A,C). To confirm these results, we performed ISH using the probe EUB338 targeting bacterial 16S rRNA. Probe specificity was also tested using sections treated with a sequence scrambled probe. More bacterial signals were visualised in the villous tissue of PE placenta and junction layer of PE-FMT placenta. No staining for scrambled probe controls was detected but was detected in the EUB338-probed sections (figure 5B,D and online supplementary figures S8 and S9). As shown in online supplementary figure S10A,B, the detection rate
of *Fusobacterium* DNA was higher in PE placental samples than in NP samples according to quantitative PCR and ISH probing for the *Fusobacterium* 16S rRNA gene.

During experimental processing, we monitored contamination by adding DNA-free water during extraction and amplification (DNA extraction from blank controls and no-template amplification controls), as well as a sterile swab during placental sampling (sampling blank controls). As shown in figure 5E, placental metagenomes from human and mouse samples were significantly distinct in their clustering from negative controls. To exclude the possibility that the placental microbiota occurrence was due to contamination by bacteria in the environment, 16S metagenomics decontamination analysis was rigorously performed using the R package decontam and SourceTracker (online supplementary figure S8C,D). According to the no-contaminant filtered data, PCoA revealed the significant separation of two clusters of PE and normotension groups and NP-FMT and PE-FMT groups (figure 5F,G). LEfSe analysis identified different taxa between the PE and NP groups, showing that *Pseudomonas* and Enterobacteriaceae were enriched, whereas Ruminococcaceae and Flavobacteriia were depleted in patients with PE (online supplementary figure S10E,F). These data suggest that a low but consistently detectable biomass of bacteria DNA present in the placenta is taxonomically distinct from potential environmental negative controls (online supplementary figure S10G).
Proinflammatory cytokine and chemokines are thought to play key roles in the pathophysiology of PE.\textsuperscript{31,32} We next examined alterations in chemokine and proinflammatory cytokine expression in the placenta. Particularly, the levels of IL6, IL1β, CCL3 or CCL4 messenger RNA were significantly increased in the PE group compared with those in the NP group for both human and mice placentas (figure 5H,I).

**DISCUSSION**

PE is one of the most severe diseases during pregnancy that not only leads to pregnancy abortion but also is life threatening.\textsuperscript{1} Effective methods for the prediction, prevention and treatment of this disease are lacking because its aetiology remains unclear.\textsuperscript{9} The present study aimed to seek these answers from the gut. We found that the gut microbiome was dramatically shifted in patients with PE. Thus, using FMT, we proved that the gut dysbiosis can cause pre-eclamptic systems. One possible mechanism through which this happens might be gut barrier destruction and the translocation of pathogenic bacteria from the gut to the placenta, causing abnormal immune responses, as we identified higher levels of total bacteria and *Fusobacterium* in the placenta of both human and mice with PE. Our study proposed that a ‘gut–placenta’ axis could play a crucial role in understanding the aetiology of PE.

The gut dysbiosis in PE observed in our study was obvious and echoes the findings of previous reports. Because the human gut microbiome is highly heterogeneous among individuals, microbiomes in healthy and diseased individuals are usually overlapped with partial differentiation when analysed with unsupervised learning.\textsuperscript{7} Nevertheless, in this study, the gut microbiome in PE clearly deviated from that in the controls and showed very little overlap, indicating severe dysbiosis in PE. More interesting
were the dysbiosis patterns, such that *Faecalibacterium* and *Akkrermansia* were depleted, whereas *Fusobacterium* and *Veillonella* were enriched in PE. Similar to the microbiota profile of non-pregnant hypertensive patients, *Faecalibacterium* and *Akkrermansia*, which are involved in producing intestinal epithelial nutrition, maintaining immune homeostasis and strengthening intestinal barrier functions, were depleted in patients with PE. *Fusobacterium* and *Veillonella* are mainly opportunistic pathogens and are associated with chronic inflammatory conditions and adverse pregnancy outcomes. Liu et al found that these bacteria were changed in the intestine of patients with PE in America and South China, indicating that the dysbiosis patterns for PE are consistent across populations and regions. Thus, the dysbiosis pattern in PE reported by our research is reliable.

*Fusobacterium* could be the key regulator of the gut-placenta axis during PE. The enrichment of *Fusobacterium* in PE is especially worth noting because it is a newly identified key pathogen involved in promoting colorectal cancer. The mechanism involved is that *Fusobacterium* can affect apoptosis, cellular proliferation and DNA repair through its attachment to epithelial cells via *Fusobacterium* adhesion A and its activation of the immune response via the nuclear factor-κB signaling pathways. In other words, *Fusobacterium* is especially good at disrupting intestinal epithelia leading to gut leakage. A possible consequence of a leaky gut is bacterial translocation, dysbiosis in PE can elevate the host's BP, which is a major manifestation of PE pathogenesis by an imbalance of T reg/Th17, is also a putative mechanism of dysbiosis patterns for PE. It is, thus, important to use multomics techniques, inactivated faeces and isolated bacteria/metabolites to affirm the key factors contributing to PE. Second, even though we have used three methods, including sequencing, ISH and quantitative PCR, and identified bacteria in the placenta, these methods do not indicate the liveness of the detected bacteria. Future studies that isolate live bacteria from placenta can certainly add credibility to our findings. Lastly, even though we have detected the enrichment of *Fusobacterium* in the gut and placenta and hypothesised its translocation from the gut to the placenta, the exact route through which *Fusobacterium* completes its translocation also needs further investigation.

Overall, we identified here obvious gut dysbiosis in patients with PE and proved that this dysbiosis can cause pre-eclamptic systems. The possible underlying mechanisms is that dysbiosis influences the host’s BP and that gut *Fusobacterium* translocates into the placenta, causing local placental inflammation. Cohort studies that follow up women from early pregnancy to observe their microbiome dynamics, BP changes and PE occurrence will offer critical evidence linking the gut microbiome to PE. In conclusion, our study indicates the role of the gut–placenta axis in PE, providing new insights into PE aetiology.

**Author affiliations**

1Department of Obstetrics and Gynecology, Southern Medical University Nanfang Hospital, Guangzhou, Guangdong, China
2Microbiome Medicine Center, Division of Laboratory Medicine, Zhongda Hospital, Southeast Medical University, Guangzhou, Guangdong, China
3Microbiome Research, St George and Sutherland Clinical School, University of New South Wales, Sydney, New South Wales, Australia
4Department of Obstetrics and Gynecology, First Affiliated Hospital of Nanhang University, Nanhang, Jiangxi, China
5Department of Internal Medicine, Affiliated Hospital, Chengdu University, Chengdu, Sichuan, China

**Contributors** YY, HZ, LH, YH and XC designed the study and prepared the manuscript. XC, ML, XY, YE, LZ, YC, YH, GK, MZ and ZW collected the samples and conducted the experiments. PL, HZ, YH, XC, WT and HH analysed the data. All authors approved the final version of the manuscript.

**Funding** This investigation was financially supported by the National Natural Science Foundation of China (81952052, 81730378, 81771609, 81800746 and 31570497), the National Key Research and Development Program of China (2019YFA0803000), the Natural Science Foundation of Guangdong Province (2017A010105025) and China Postdoctoral Science Foundation (2018M630377).

**Competing interests** None declared.

**Patient consent for publication** Not required.

**Ethics approval** Nanfang Hospital.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** Data are available in a public, open access repository.

**Open access** This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/.

**ORCID iD**

Xia Chen http://orcid.org/0000-0002-7915-4568

**REFERENCES**

1. Mol BW, Roberts CT, Thangaratnam S, et al. Pre-eclampsia. *Lancet* 2016;387:999–1011.
2. ACOG Practice Bulletin No. 202: Gestational hypertension and preeclampsia. *Obstet Gynecol* 2019;133:1–25.
3. Brown MA, Magee LA, Kenny LC, et al. Hypertensive disorders of pregnancy: ISSHP classification, diagnosis, and management recommendations for international practice. *Hypertension* 2018;72:24–43.

---

Chen X, et al. Gut 2020;69:513–522. doi:10.1136/gutjnl-2019-319101
Gut microbiota

4 Udenze IC. Association of pre-eclampsia with metabolic syndrome and increased risk of cardiovascular disease in women: a systemic review. *Niger J Clin Pract* 2016;19:431–5.

5 Jabali G, Ahmad M, Koushaein L, et al. Metabolic syndrome mediates proinflammatory responses of inflammatory cells in pre-eclampsia. *Am J Reprod Immunol* 2019;81:e13086.

6 Rodie Ver al. Pre-Eclampsia and cardiovascular disease: metabolic syndrome of pregnancy? *Atherosclerosis* 2004;175:189–202.

7 Hooijssuur MEC, Ghoossein-Doha C, Kroon AA, et al. Metabolic syndrome and pre-eclampsia. *Ultrasound Obstet Gynecol* 2019;5:64–71.

8 He Y, Wu W, Zheng H-M, et al. Regional variation limits applications of healthy gut microbiome reference ranges and disease models. *Nat Med* 2018;24:1532–5.

9 Wang J, Zheng J, Shi W, et al. Dysbiosis of maternal and neonatal microbiota associated with gestational diabetes mellitus. *Gut* 2018;67:1614–25.

10 Zhao L. The gut microbiota and obesity: from correlation to causality. *Nat Rev Microbiol* 2013;11:639–47.

11 Li J, Zhao F, Wang Y, et al. Gut microbiota dysbiosis contributes to the development of hypertension. *Microbiome* 2017;5.

12 Kim S, Goel R, Kumar A, et al. Balance of gut microbiome and intestinal epithelial barrier dysfunction in patients with high blood pressure. *Clin Sci* 2018;132:701–18.

13 Kanbay M, Onal EM, Afsar B, et al. The crosstalk of gut microbiota and chronic kidney disease: role of inflammation, proteinuria, hypertension, and diabetes mellitus. *Int Urol Nephrol* 2018;50:1453–66.

14 Liu J, Yang H, Yin Z, et al. Remodeling of the gut microbiota and structural shifts in pre-eclampsia patients in South China. *Eur J Clin Microbiol Infect Dis* 2017;36:713–9.

15 Lv L, Li S-H, Li S-C, et al. Early-Onset pre-eclampsia is associated with gut microbiota alterations in antepartum and postpartum women. *Front Cell Infect Microbiol* 2019;9:224.

16 Gong S, Yan Z, Liu Z, et al. Intestinal microbiota mediates the susceptibility to polymicrobial Sepsis-induced liver injury by granisetron generation in mice. *Hepatology* 2019;69:1751–67.

17 Chen X, Xu M, Xing J, et al. Lanthanum chloride inhibits LPS mediated expressions of pro-inflammatory cytokines and adhesion molecules in HUVECs: involvement of NF-kB-IκBj3 signaling. *Cell Physiol Biochem* 2017;42:1713–24.

18 Choi YS, Kim YC, Baek KJ, et al. <em>cet</em>-Inh in <em>cet</em>-inhibitor Detection of Bacteria within Paraffin-embedded Tissues Using a Diginax-labeled DNA Probe Targeting 16S rRNA. *J Virol* 2015;85:52856.

19 Daims H, Brühl A, Amann R, et al. The domain-specific probe EUB338 is insufficient for the detection of all bacteria: development and evaluation of a more comprehensive probe set. *Syst Appl Microbiol* 1999;22:434–44.

20 Edgar RC. Search and clustering orders of magnitude faster than blast. *Bioinformatics* 2010;26:2460–1.

21 McDonald D, Price MN, Goodrich J, et al. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *Int J Mol Evol* 2012;6:610–8.

22 Segata N, Izard J, Waldron L, et al. Metagenomic biomarker discovery and explanation. *Genome Biol* 2011;12:R60.

23 Davis NM, Proctor DM, Holmes SP, et al. Simple statistical identification and removal of contaminant sequences in marker-gen and metagenomics data. *Microbiome* 2018;6.

24 Knights D, Kuczynski J, Charlson ES, et al. Bayesian community-wide culture-independent microbial source tracking. *Nat Methods* 2011;8:761–3. 10.1038/nmeth.1650.

25 Benjamini Y, Yekutieli D. The control of the false discovery rate in multiple testing under dependency. *Ann Statist* 2001;29:1165–88.

26 Eghbal-Erd F, Youssel H, Haydari H, et al. The imbalance of Th17/Treg axis involved in the pathogenesis of pre-eclampsia. *J Cell Physiol* 2019;234:5106–16.

27 Zhang Y, Liu Z, Tian M, et al. The altered PD-1/PD-L1 pathway delivers the ‘one-two punch’ effects to promote the Treg/Th17 imbalance in pre-eclampsia. *Cell Mol Immunol* 2018;15:710–23.

28 Lu L, Nalle SC, Shen L, et al. Trnf2 activates MLC2-dependent tight junction dysregulation to cause apoptosis-mediated barrier loss and experimental colitis. *Gastroenterology* 2013;145:407–15.

29 Wu L-L, Peng-W-H, Kuo-W-T, et al. Commensal bacterial endocytosis in epithelial cells is dependent on myosin light chain kinase-activated brush border fanning by interferon-γ. *Am J Pathol* 2014;184:260–74.

30 Manfredo Vieira S, Hiltsperger M, Kumar V, et al. Translocation of a gut pathobiont drives autoimmunity in mice and humans. *Science* 2018;359:1156–61.

31 Raghupathy R. Cytokines as key players in the pathophysiology of pre-eclampsia. *Med Princ Pract* 2013;22:8–19.

32 Holder ES, Tower CL, Jones CJ, et al. Heightened pro-inflammatory effect of pre-eclamptic placental microvesicles on peripheral blood immune cells in Humans. *1 Biol Reprod* 2012;86:103.

33 Hlippala K, Jouhten H, Ronkainen A, et al. The potential of gut Commensals in reinforcing intestinal barrier function and alleviating inflammation. *Nutrients* 2018;10:988.

34 Roumy B, Gopalakrishnan V, Daillère R, et al. The gut microbiota influences anticancer immunosurveillances and general health. *Nat Rev Clin Oncol* 2018;15:382–96.

35 Kuppen M, Holm K, Anmarkrud JA, et al. The gut microbial profile in patients with primary sclerosing cholangitis is distinct from patients with ulcerative colitis without biliary disease and healthy controls. *Gut* 2017;66:611–9.

36 De Cruz P, Kang S, Wagner J, et al. Association between specific mucosa-associated microbiota in Crohn's disease at the time of resection and subsequent disease recurrence: a pilot study. *J Gastroenterol Hepatol* 2015;30:268–78.

37 Barak S, Oettinger-Barak O, Machtei EE, et al. Evidence of periopathogenic microorganisms in placenta of women with pre-eclampsia. *J Periodontol* 2007;78:670–6.

38 DiGiulio DB, Callahan BL, McMurdie PJ, et al. Temporal and spatial variation of the human microbiota during pregnancy. *Proc Natl Acad Sci U S A* 2015;112:11060–5.

39 Yu T, Guo F, Yu Y, et al. Fusobacterium nucleatum promotes chemoresistance to colorectal cancer by modulating autophagy. *Cell* 2017;170:e16:548–54.

40 Rubinstein MR, Wang X, Liu W, et al. Fusobacterium nucleatum promotes colorectal carcinogenesis by modulating E-Cadherin/J-Beta-Catenin signaling via its FadA adhesin. *Cell Host Microbe* 2013;14:195–206.

41 Han YW. Fusobacterium nucleatum: a commensal-turned pathogen. *Curr Opin Microbiol* 2015;23:141–7.

42 Amasekara R, Jayasekara RW, Senanayake H, et al. Microbiome of the placenta in pre-eclampsia supports the role of bacteria in the multifactorial cause of pre-eclampsia. *J Obstet Gynaecol Res* 2015;41:662–9.

43 Rustveit LO, Kelsey SF, Sharma R, Association between maternal infections and preeclampsia: a systematic review of epidemiologic studies. *Matern Child Health J* 2008;12:233–42.

44 Seferovic MV, Pace RM, Carroll M, et al. Visualization of microbes by 16S in situ hybridization in term and preterm placentas without intraamniotic infection. *Am J Obstet Gynecol* 2019;221:146.e1–146.e23.

45 de Goffau MC, Lager S, Salter SJ, et al. Recognizing the reagent microbiome. *Nat Methods* 2018;15:951–3.

46 de Goffau MC, Lager S, Sovio U, et al. Human placenta has no microbiome but can contain potential pathogens. *Nature* 2019;572:329–34.

47 Britton GA, Contijoch EJ, Mogno I, et al. Microbiota from humans with inflammatory bowel disease alter the balance of gut Th17 and RORγt+ regulatory T cells and exacerbate colitis in mice. *Immunity* 2019;50:212–24.

48 Koren O, Goodrich JK, Cullender TC, et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell* 2012;150:470–80.