

RESEARCH ARTICLE

Comparative analysis of gut microbiome alterations in early- and late-onset preeclampsia: A case control study

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Abstract

Preeclampsia (PE) is a complication during pregnancy characterized by hypertension, organ damage, and systemic inflammation. Increasing evidence suggests that the gut microbiome may play a role in the pathophysiology of PE. However, previous studies on the gut microbiome have generally overlooked the distinction between subgroups of PE, although clinical manifestations may differ. Also, most studies have not used deep sequencing techniques. Therefore, this study aimed to explore further potential differences in gut dysbiosis in different PE subgroups compared to controls using shotgun metagenomics. We studied the bacterial gut microbiome using shotgun metagenomic sequencing in 37 pregnant patients in the third trimester from a Swedish cohort, separating patients according to subtype (healthy controls N=21, late-onset PE N=8, early-onset PE N=8). Differential relative abundances and alpha diversity were evaluated using Wilcoxon rank sum test, and beta diversity was evaluated using PERMANOVA. Multiple linear regression was used to study associations between gut microbiome composition differences and clinical parameters. Late-onset PE and early-onset PE were both associated with significantly different beta diversity compared to controls. Differences remained significant after adjusting for age, and were not affected by gestational age, BMI or parity. Alpha diversity was lower in late-onset PE compared to controls. While no significant differences in taxonomic abundances were seen after correcting for multiple testing, several interesting leads were identified, including a higher abundance of genus *Blautia* in late-onset PE, and lower abundance of *Coproccoccus catus* and unclassified *Lachnospiraceae* in early-onset PE. Functional analysis did not reveal any significant differences after false discovery rate (FDR) correction. In conclusion, our results showed subgroup-specific gut microbiome differences in PE with more pronounced associations in late-onset PE, despite limited power due to the observational design and small

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Data availability statement: The metagenomics datasets generated and analyzed during the current study are available in the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB79379 (<https://www.ebi.ac.uk/ena/browser/view/PRJEB79379>). The clinical data for “Comparative analysis of gut microbiome alterations in early- and late-onset preeclampsia: a case control study” (Meijer & Hansson, 2026) contains information that could compromise the privacy of research participants. The data is a confidential document according to Swedish law and cannot be made openly available. A description of the data and information about how to request access are available at <https://doi.org/10.5878/xhz2-m560>.

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cohort. Accordingly, our results highlight the importance of subgroup analysis when studying PE.

Introduction

Preeclampsia (PE) is a pregnancy-related inflammatory disease characterized by hypertension and organ damage, manifesting after 20 gestational weeks (gw). It is a leading cause of maternal and fetal morbidity and mortality worldwide [1]. The clinical manifestations and disease severity can vary greatly, and severe PE is defined as the following features: early-onset PE (onset <34 gw); blood pressure (BP) >160/110 mmHg; hemolysis, elevated liver enzymes and low platelets (HELLP) syndrome; severe organ dysfunction; and/or eclampsia [1]. The etiology is partly unknown, but the placenta is central to pathophysiology, as described in the two-stage model of PE [2]. In early-onset PE, shallow invasion of the trophoblast into the decidual spiral arterioles leads to poor placentation and malperfusion, causing oxidative- and syncytiotrophoblast stress [3]. Late-onset PE (≥34 gw) is thought to be more related to maternal risk factors, syncytiotrophoblast stress and eventually senescence of the placenta. A dysfunctional placenta results in the maternal syndrome of PE, with an increased inflammatory response and oxidative stress as part of the systemic reaction causing the general endothelial damage pathognomonic for PE [2,4]. Early-onset PE seems to be more related to an increased inflammatory response than late-onset PE, including a decrease in regulatory T (Treg) cell activity [5–8].

The gut microbiome has gained increasing attention as a possible factor in the pathophysiology of PE, as well as in many other diseases involving low-grade inflammation such as obesity, diabetes, and hypertension [9–12]. Maternal obesity is also considered a risk factor for PE. Several PE studies, mainly performed in Asian populations, have shown that PE is associated with a less favorable gut microbiome composition [13–18]. Among others, the probiotic genus *Bifidobacteria* has been shown to be depleted in PE [17]. Miao et al showed a negative correlation between *Bifidobacterium* abundance and systolic blood pressure (SBP), diastolic blood pressure (DBP), cholesterol levels, and aspartate aminotransferase (ASAT), and a highly significant negative correlation with triglyceride levels [17].

The gut microbiome diversity and composition are affected by both environmental and host factors such as diet, and ethnicity, causing a high variation in the gut microbiome between populations [19]. Using 16S rRNA sequencing, our previous study showed that PE is associated with dysbiosis of the gut microbiome in a Swedish cohort, characterized by higher levels of phylum *Bacteroidetes* and lower levels of phylae *Verrucomicrobia*, *Synergistetes*, and genera *Cloacibacillus* and *Akkermansia*. We also showed a tendency towards lower abundance of the class *Actinobacteria* in PE, a class mainly consisting of *Bifidobacteria*. In addition, a correlation between gastrointestinal symptoms and PE was shown, suggesting that the gut microbiota and gastrointestinal symptoms might be a part of the maternal syndrome [20].

Concentrations of lipopolysaccharide (LPS) have been found to be elevated in plasma and feces in PE, indicating that gut dysbiosis might lead to endotoxemia that

contributes to systemic inflammation through toll-like receptor 4 (TLR4) signaling [15,21]. Increased plasma levels, of the gut microbiota-derived metabolite trimethylamine-N-oxide (TMAO), which is a strong prognostic marker for chronic kidney disease and adverse cardiac events [22–24], have also been found to be elevated in PE [15]. Gut bacteria influence their host by producing various short-chain fatty acids (SCFAs). Notably, lower levels of SCFAs, such as butyrate, along with a reduction in butyrate-producing bacterial taxa, have been observed in PE [25]. Some taxa have been correlated with PE parameters such as BP, proteinuria, oedema, and inflammatory markers such as IL-6 [7,9]. Consumption of probiotic milk during pregnancy is associated with lower risk of severe PE, including late-onset PE with severe features [26], although randomized controlled studies are lacking. The gut microbiome might be a potential target for therapeutics in PE.

Although there is increasing evidence that the gut microbiome plays a role in PE, previous studies have generally overlooked the distinction between subgroups of PE. Gut dysbiosis may exacerbate inflammation, and thereby clinical manifestations and symptoms. However, as the clinical phenotype can vary greatly between PE subgroups, we hypothesize that the role of the gut microbiome may also differ. Additionally, most studies have utilized 16S rRNA sequencing rather than deep sequencing techniques. Therefore, this study aimed to explore further potential differences in gut dysbiosis in different PE subgroups compared to controls using shotgun metagenomics.

Materials and methods

Ethics statement

The study was approved by the Regional Ethics Committee at Lund University, Sweden (Study number 2017/419), and conducted in accordance with the Declaration of Helsinki. All subjects gave their written informed consent before entering the study. All samples were coded before analysis.

Study cohort and sample collection

In our previous study [20], a larger cohort of women with singleton normotensive pregnancy or newly diagnosed PE in the third trimester were recruited between 04/10/2017 and 10/02/2020 at Skåne University Hospital and antenatal care centers in Scania, Sweden. Subjects were matched according to gestational length and recruited in the same area of Scania. The 16S rRNA gene amplicon sequencing was successfully performed on a total of 15 PE (7 early-onset, 8 late-onset), and 24 controls (C) (8 before 34 gw, 16 after 34 gw). For this study, subjects were chosen based on availability of samples, where there were remaining feces stored from a subset of the original cohort (16 PE patients and 21 C), hence there is an overlap (11 PE and 19 C) with samples used for 16S sequencing in our previous study [20]. The remaining samples were analysed using shotgun metagenomics, aiming to further evaluate potential differences between subgroups of PE. The demographic data of the groups was compared to ensure the groups were comparable.

Preeclampsia was defined according to the International Society for the Study of Hypertension in Pregnancy (ISSHP) 2018 diagnostic criteria [1], i.e., *de novo* hypertension ($\geq 140/90$ mmHg) after gestational week 20, and proteinuria and/or signs of organ dysfunction. Early-onset PE was defined as manifesting before gw 34, and late-onset PE after gw 34. Exclusion criteria were diabetes (including gestational diabetes), celiac disease, inflammatory bowel disease, BMI > 30 before pregnancy, and antibiotic treatment within the previous 2 weeks. The BMI threshold was set due to known differences in the gut microbiota composition in obese pregnant women [27].

Demographic data for all three groups and results from routine blood sample analyses performed on PE patients (platelets, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), creatinine, uric acid, albumin) were acquired from medical records. A questionnaire regarding diet and gastrointestinal symptoms was filled in at inclusion, and plasma and feces samples were collected and stored at -80°C . Plasma (P) and feces (F) calprotectin levels were analysed using a two-site sandwich ELISA technique (Immundiagnostik AG, Bensheim, Germany). Details regarding data and sample collection, as well as lab analyses, are described in our previous study [20].

Shotgun metagenomic sequencing and metagenomic analyses

Fresh-frozen feces samples were sent on dry ice to CosmosID (Germantown, MD, USA) for DNA extraction and shotgun metagenomic sequencing. DNA from samples was isolated using the QIAGEN DNeasy PowerSoil Pro Kit, according to the manufacturer's protocol. Extracted DNA samples were quantified using Qubit Flex fluorometer and Qubit™ dsDNA HS Assay Kit (ThermoFisher Scientific). DNA libraries were prepared using the Nextera XT DNA Library Preparation Kit (Illumina) and Nextera Index Kit (Illumina) with total DNA input of 1 ng. Genomic DNA was fragmented using a proportional amount of Illumina Nextera XT fragmentation enzyme. Combinatory dual indexes were added to each sample followed by 12 cycles of PCR to construct libraries. DNA libraries were purified using AMPure magnetic Beads (Beckman Coulter) and eluted in QIAGEN EB buffer. DNA libraries were quantified using Qubit fluorometer and Qubit™ dsDNA HS Assay Kit. Libraries were then sequenced on an Illumina HiSeq X platform at 2x150bp.

Raw taxonomic reads were filtered with fastp [28] for the removal of adapters and low-quality bases and depleted of human DNA with kraken2 [29] using the hg19 reference human genome. The remaining reads were annotated taxonomically with Metaphlan4 [30] against the CHOCOPHiAn vOct22 database and functionally with HUMAnN3 [31]. Functional assignments were then converted to gut metabolic modules with Omixer-rpmR [32].

All further bioinformatic analyses were performed in R v.4.3.1 using the vegan package v.2.7–1. Taxa were excluded if they did not meet at least one of the following criteria: a relative abundance of 0.005% in at least 7 samples, or a relative abundance of at least 0.1% in at least 2 samples. Of 990 taxa originally detected, 348 were kept. After exclusion, a row called “other taxa” was added to the matrix to preserve compositionality after the exclusion of low abundance taxa; the median relative abundance of “other taxa” was 2%. Alpha (within-sample) diversity indices were calculated as observed species number, Shannon's entropy and Pielou's evenness. Beta (between-samples) diversity was based on Bray-Curtis dissimilarities. Homogeneity of dispersion was assessed with the betdisper function. The Wilcoxon rank sum test was used for significance testing in relative abundance and alpha diversity indices, and a PERMANOVA analysis with 999 permutations was performed for beta diversity significance testing. Results with p values < 0.05 were considered significant. Differential abundance of species, genera, metabolic and gut-brain modules was calculated in Maaslin3, considering only differential abundance, but not prevalence, and adjusting for age, sequencing depth and gut transit time, defined as slow (defecate less than 5 times per week, and never soft stools), regular, or fast (defecate at least daily, of which at least half of stools are soft). Although no features were significant after FDR, we have further explored features with a nominal p -value < 0.05 for at least one PE subgroup, and where the direction of change was the same for total PE and at least one PE subgroup. These candidate features were correlated with clinical parameters with generalized linear models adjusting for the same factors as in the Maaslin3 analysis.

Additional statistical analyses

The IBM SPSS Statistics software version 27 was used for analysis of demographic data, calprotectin values, and data collected from questionnaires and medical records. Demographic data was compared pairwise using Mann-Whitney U-test for quantitative variables, and Fisher's exact test for categorical variables.

Results

Study cohort characteristics

A total of 21 C patients, and 16 PE patients with singleton pregnancies in the third trimester were included in the study. The PE patients were divided into two subgroups: late-onset PE (N=8); and early-onset PE (N=8), previously defined in the material and methods section. Patient demographics, selected questionnaire answers (diet, probiotic food consumption, and gastrointestinal symptoms), and PE routine blood sample analyses are summarized in [Table 1](#). All three groups were similar in pre-pregnancy BMI, age, parity, smoking habits, diet, and probiotic food consumption, according

Table 1. Patient demographics, questionnaire answers, and biochemical lab results.

Parameter	C	Late-onset PE	Early-onset PE	p values (Late-onset PE/Early-onset PE)
N =	21	8	8	
Age (years)	31 (28 – 33.5)	32.5 (27.5 – 35)	30.5 (29 – 32.5)	0.624/1.000
Pre-pregnancy BMI (kg/m ²)	23.3 (21.4 – 24.1)	22.9 (21.2 – 25.6)	20.9 (20.2 – 23.7)	0.919/0.234
Nulliparous, n/N (%)	16/21 (76.2 %)			0.448/0.557
Gestational age at inclusion (weeks + days)	35+4 (31+5 – 37+0)	36+4 (35+3 – 38+2)	32+0 (29+6 – 34+0)	0.213/0.032*
Gestational age at delivery (weeks + days)	40+0 (39+3 – 41+1)	37+2 (36+2 – 38+6)	33+4 (30+2 – 34+1)	0.001**/<0.001***
Birth weight (g)	3656 (3252 – 4012)	2755 (2476 – 3045)	1738 (1069 – 2071)	0.001**/<0.001***
Smoker, n/N (%):				
No	20/21 (95.2%)	7/8 (87.5%)	8/8 (100%)	
Yes	0/21 (0%)	0/8 (0%)	0/8 (0%)	
Quit before pregnancy	1/21 (4.8%)	1/8 (12.5%)	0/8 (0%)	0.685
Diet, n/N (%):				
Vegan	1/21 (4.8%)	0/8 (0%)	0/8 (0%)	
Lacto-ovo vegetarian	5/21 (23.8%)	0/8 (0%)	2/8 (25%)	
Omnivorous	15/21 (71.4%)	8/8 (100%)	6/8 (75%)	0.594
Probiotic food items consumed				
> 1 time/week (number)	1.0 (1.0 – 2.0)	1.5 (1.0 – 2.0)	1.0 (1.0 – 2.8)	0.444/0.614
Stool frequency, n/N (%):				
<3 times/week	2/21 (9.5%)	0/8 (0%)	0/8 (0%)	
3-5 times/week	8/21 (38.1%)	2/8 (25%)	2/8 (25%)	
1 time/day	9/21 (42.9%)	3/8 (37.5%)	5/8 (62.5%)	
>1 time/day	2/21 (9.5%)	3/8 (37.5%)	1/8 (12.5%)	0.102/0.277
Loose/watery stool frequency, n/N (%):				
Never/rarely	15/21 (71.4%)	3/7 (42.9%)	2/8 (25%)	
Around 25% of the time	2/21 (9.5%)	3/7 (42.9%)	3/8 (37.5%)	
Around 50% of the time	2/21 (9.5%)	0/7 (0%)	1/8 (12.5%)	
Around 75% of the time	2/21 (9.5%)	1/7 (14.3%)	2/8 (25%)	
Always, 100% of the time	0/21 (0%)	0/7 (0%)	0/8 (0%)	0.290/0.042*
SBP (mmHg)	117 (108 – 127)	145 (140 – 156)	155 (150–160)	<0.001***/<0.001***
DBP (mmHg)	70 (68 – 76)	100 (95 – 100)	103 (96 – 109)	<0.001***/<0.001***
Plasma calprotectin (µg/L)	1540 (948 – 2450)	13639 (3415 – 24342)	3109 (2442 – 4157)	<0.001***/0.004**
Feces calprotectin (mg/kg)	32 (22 – 58)	12 (3 – 26)	22 (9 – 230)	0.071/0.884
Platelets (x 10 ⁹ /L)	-	213 (156 – 257)	196 (139 – 232)	0.563
ASAT (µkat/L)	-	0.38 (0.27 – 0.52)	0.36 (0.30 – 0.51)	0.958
ALAT (µkat/L)	-	0.28 (0.21 – 0.42)	0.35 (0.17 – 0.42)	0.753
Creatinine (µmol/L)	-	65 (56 – 69)	56 (46 – 65)	0.113
Uric acid (µmol/L)	-	347 (300 – 367)	371 (327 – 391)	0.156
Albumin (g/L)	-	24.5 (23.0 – 28.5)	30.0 (25.8 – 31.8)	0.042*

Numbers presented as medians (interquartile ranges, Q1–Q3). The Mann–Whitney U-test was used for pairwise significance testing compared with C except for smoking, and diet, where Fisher's exact test was used. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. ALAT = alanine amino transferase, ASAT = aspartate amino transferase, BMI = body mass index, C = control, DBP = diastolic blood pressure, PE = preeclampsia, SBP = systolic blood pressure.

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to Mann-Whitney U-test (quantitative variables) and Fisher's exact test (categorical variables). Gestational age at inclusion was overlapping, although the median gestational age was significantly lower in early-onset PE (median 32 weeks, 0 days) compared to controls (median 35 weeks, 4 days). Frequency of loose/watery stool was significantly higher in the early-onset PE, but not late-onset PE. Gestational age at delivery, and fetal birth weight were both significantly lower in the PE subgroups compared to C. P-calprotectin was significantly higher in both PE subgroups, while no difference was seen in F-calprotectin. No significant differences were seen in routine blood samples between the two PE subgroups, except for plasma albumin, which was significantly lower in late-onset PE compared to early-onset PE.

Diversity Indices

Alpha and beta diversity analyses were performed to evaluate if there were overall gut microbiome compositional differences in PE subgroups compared with C. Late-onset PE had lower microbial diversity than C (Richness: C: 134 (IQR 116–152); Late-onset PE: 101 (IQR 93.25–121); $p=0.042$; Shannon's entropy: C: 3.39 (IQR 3.34–3.80), Late-onset PE: 3.68 (IQR 3.58–3.78); $p=0.036$; Pielou's evenness: C: 0.749 (IQR 0.728–0.765); Late-onset PE: 0.727 (IQR 0.709–0.736); $p=0.08$) (Fig 1). There were no significant differences between the alpha-diversity in C compared to early-onset PE (Richness: 134 (IQR 109.5–146); Shannon's: 3.5 (IQR 3.31–3.65); Pielou's: 0.746 (IQR: 0.727–0.763); all $p>0.6$).

Beta diversity was significantly different between C and subgroups of PE (adonis2: $R^2=0.091$, $p=0.003$; beta-disper: $p=0.09$) (Fig 2). Since the low p -value of the beta-dispersion analysis suggested an effect of group size, we also assessed controls against all PE cases combined, which was also significant and had no effect of group size (adonis2: $R^2=0.060$, $p=0.002$; beta-disper $p=0.60$). Beta diversity was correlated with the age of participants ($R^2=0.041$, $p=0.047$), but not with parity, BMI or gestational week. Both PE and PE subgroups remained significant after adjusting for age.

Gut microbiome taxa abundances

The gut microbiome composition was analysed in subgroups of PE compared with C, adjusting for the effects of age, gut transit time and sequencing depth, to evaluate potential differences between different phenotypes of PE. This analysis was performed at the species and genus levels, as well as for a set of 119 bacterial pathways with known metabolic or neuroactive activity. While no taxa or pathways were significant past multiple testing correction, promising leads include higher abundance of genera *Blautia* and *Eggerthella*, and species *Roseburia inulinivorans* in late-onset PE, and lower

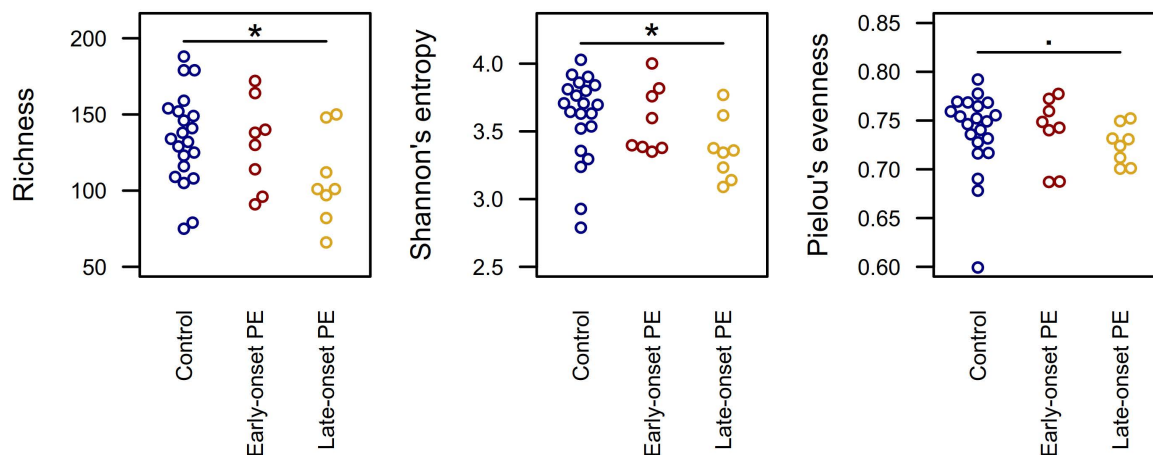


Fig 1. Alpha diversity in PE subgroups vs C. a) Richness, b) Shannon's entropy, c) Pielou's evenness.

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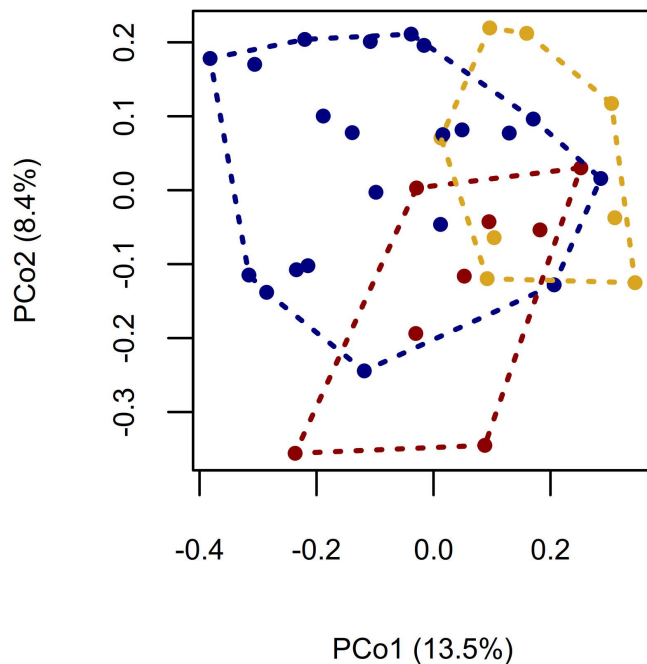


Fig 2. Beta diversity based on Bray-Curtis dissimilarities. The first two principal coordinates are shown. Dark blue dots: controls; yellow dots: late-onset preeclampsia; red dots: early-onset preeclampsia. Dashed lines mark the observed limit of each group.

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abundance of unclassified *Lachnospiraceae* family members GGB3653 and GGB3653 SGB4964, and species *Coprococcus catus*, and higher abundance of species *Eggerthella lenta* in early-onset PE. Potentially interesting functional pathways include increased glutamate synthesis II in late-onset PE, and increased pectine degradation II, serine degradation II, and butyrate synthesis I in early-onset PE. Promising leads are listed in [Table 2](#).

Multiple linear regression analyses

Multiple linear regression analyses were performed to evaluate associations between potentially interesting taxa (significantly differentially abundant before correcting for multiple testing with the false discovery rate (FDR) procedure) in any subgroup of PE, and clinical parameters relevant to PE ([Table 3](#)). The variables SBP, DBP, gestational age at birth, birth weight, P- and F-calprotectin were analysed across the entire cohort (N=37), while routine blood samples were only available for PE patients (N=16). All analyses were adjusted for age and BMI. Significant associations were found most often with systolic blood pressure and various taxa, while gut metabolic modules had one weak correlation each ([table 3](#)). After adjusting for multiple testing, four correlations remained, all between two genera (*Eggerthella* and the uncultured genus GGB3653), and both SBP and DBP ([Table 3](#), [Fig 3](#)).

Discussion

In this study, we showed that patients with early-onset PE and late-onset PE display subgroup-specific differences in gut microbiome compositions when compared to C, with larger differences in late-onset PE, including a lower microbial diversity. Both late-onset PE and early-onset PE were associated with a significant difference in beta diversity compared with C. Importantly, beta diversity did not correlate with gestational age, BMI, or parity, and remained significantly different

Table 2. Summary of taxa with differential relative abundances in subgroups of PE, compared with C.

Feature	Feature type / description	C, median abundance (prevalence)	Late-onset PE, median abundance (prevalence)	Early-onset PE, median abundance (prevalence)	Control vs. Late-onset PE, p-value (q-value)	Control vs. Early-onset PE, p-value (q-value)	Control vs. All PE p-value (q-value)
GGB9760 SGB15374	Phylum <i>Firmicutes</i>	11% (60%)	0 (37.5%)	0.5% (50%)	0.12 (0.88)	0.034 (0.71)	0.017 (0.66)
<i>Blautia</i>	Family <i>Lachnospiraceae</i>	10.1% (100%)	17.1% (100%)	12.5% (100%)	0.012 (0.71)	0.12 (0.84)	0.012 (0.72)
GGB3653	Family <i>Lachnospiraceae</i>	0.25% (85%)	0 (12.5%)	0.01% (50%)	0.49 (0.86)	0.044 (0.71)	0.041 (0.72)
GGB3653 SGB4964	Family <i>Lachnospiraceae</i>	24.7% (85%)	0 (12.5%)	1.8% (50%)	0.49 (0.89)	0.044 (0.71)	0.041 (0.72)
<i>Coprococcus catus</i>	Family <i>Lachnospiraceae</i>	42.5% (85%)	39.7% (87.5%)	30.5% (100%)	0.42 (0.88)	0.006 (0.70)	0.031 (0.69)
<i>Roseburia inulinivorans</i>	Family <i>Lachnospiraceae</i>	0% (45%)	12.0% (62.5%)	20.1% (87.5%)	0.031 (0.71)	0.017 (0.70)	0.006 (0.66)
<i>Eggerthella</i>	Family <i>Eggertellaceae</i>	0.004% (55%)	0.11% (50%)	0.019% (50%)	0.041 (0.71)	0.066 (0.71)	0.012 (0.72)
<i>Eggerthella lenta</i>	Family <i>Eggertellaceae</i>	0.42% (55%)	11.4% (50%)	1.9% (50%)	0.066 (0.81)	0.041 (0.73)	0.012 (0.66)
MF0004	pectine degradation II	3.37 (50%)	13.20 (87.5%)	15.14 (100%)	0.08 (0.8)	0.045 (0.7)	0.013 (0.76)
MF0048	Serine degradation	43.06 (100%)	57.66 (100%)	58.31 (100%)	0.3 (0.9)	0.039 (0.7)	0.057 (0.81)
MGB007	Glutamate synthesis II	50.68 (100%)	69.71 (100%)	67.00 (100%)	0.049 (0.7)	0.29 (0.92)	0.058 (0.81)
MGB009	Histamine synthesis	0 (35%)	0 (37.5%)	0 (37.5%)	0.91 (0.98)	0.6 (0.98)	0.011 (0.76)
MGB022	GABA synthesis II	0.46 (60%)	1.05 (75%)	0.59 (62.5%)	0.09 (0.8)	0.057 (0.69)	0.031 (0.76)
MGB052	Butyrate synthesis I	2.39 (55%)	4.32 (87.5%)	9.92 (87.5%)	0.125 (0.84)	0.001 (0.38)	0.053 (0.76)

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after adjusting for age. Our results suggest that PE is associated with an altered gut microbiome composition at the global level, and these alterations may play different roles in the pathophysiology of PE subgroups.

A well-known issue in high dimensional data such as metagenomics is multiple comparisons, resulting in an increased rate of false positive results. We chose to adjust for multiple comparisons using FDR correction, resulting in no taxa showing significantly differential relative abundances despite differences in alpha and beta diversity. It is possible that the sample size was too small to show significant differences in specific taxa. Although non-significant after FDR correction, interesting leads for taxa possibly involved in PE include several members of the *Lachnospiraceae* family. These leads may primarily be seen as hypothesis generating, and some are discussed further below. Further studies with larger sample sizes are needed to determine any true differences in taxa abundances. It is possible that other factors mediate microbiome differences as well, such as diet change and medications related to PE.

Late-onset PE was associated with a higher median relative abundance of family *Lachnospiraceae* member *Blautia*, compared with controls. These differences are in line with previous studies mainly performed in China [14,17,33,34], except from one study showing lower *Blautia* in PE [34], and previous studies showing lower unclassified *Lachnospiraceae* in PE [35,36].

Blautia is a known butyrate-producer within the family *Lachnospiraceae*, and although the genus is commonly regarded as beneficial [37], it has also been indicated in diseases such as IBS, gestational diabetes mellitus, and PE [14,33,38,39]. Miao et al showed a positive correlation in PE between *Blautia* and age, pregestational weight, C-reactive protein, triglyceride, and low-density lipoprotein-cholesterol [17]. In non-pregnant individuals, high abundance of *Blautia* is also

Table 3. Significant results from multiple linear regression models on clinical parameters and potentially interesting taxa and functional pathways in late-onset or early-onset PE, including values after FDR correction.

Dependent variable	Independent variable	Partial slope coefficient (SE)	p-value	After FDR
<i>Eggerthella</i>	SBP	41.4 (10.3)	0.00028	0.0043
<i>Eggerthella</i>	DBP	27.3 (8.3)	0.0039	0.029
GGB3653	SBP	−20.6 (5.1)	0.00029	0.0043
GGB3653	DBP	−13.9 (4.4)	0.0030	0.029
<i>Blautia</i>	Platelets	45.0 (19.5)	0.036	0.21
<i>Eggerthella lenta</i>	SBP	22.8 (10.3)	0.034	0.36
<i>Eggerthella lenta</i>	Albumin	−5.6 (2.5)	0.042	0.36
GGB3653 SGB4964	SBP	−26.5 (12.8)	0.046	0.39
GGB9760 SGB15374	SBP	−18.2 (8.1)	0.032	0.36
GGB9760 SGB15374	Albumin	5.6 (1.8)	0.009	0.36
MGB007	Uric acid	181 (76.9)	0.034	0.66
MF0004	Albumin	12.6 (5.5)	0.039	70.65
MGB052	Platelets	−1065 (363)	0.011	0.65

SBP=systolic blood pressure, DBP=diastolic blood pressure, FDR=false discovery rate, SE=standard error

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associated with glucose metabolism disturbances, arterial hypertension, low consumption of resistant starch, and high-fat diet [40]. Low dietary fiber intake has also been associated with PE [41], and a high-fat diet has been shown to aggravate PE symptoms in a PE mouse model [42].

Our results also indicated a strong positive correlation between the genus *Eggerthella*, SBP and DBP. Higher abundance of *Eggerthella* has been reported in non-pregnant hypertensive patients previously [43], but has not been indicated in PE, to the best of our knowledge. Interestingly, our results also showed a significant negative correlation between unclassified *Lachnospiraceae* member GGB3653 and SBP as well as DBP. Previous studies have shown depleted unclassified *Lachnospiraceae* in PE [35,36], in line with our results. Members of this taxon are suggested to have a protective effect against PE [44], being among the main producers of SCFAs including butyrate [37]. In fact, lower SCFA levels have been indicated in PE [25,34,45]. However, the role of *Lachnospiraceae* is controversial, and different members of *Lachnospiraceae* have also been associated with diseases such as obesity [46]. Worth mentioning is also that the probiotic species *Coprococcus catus* was lower in early-onset PE, which has been observed in previous studies of PE as well, even before clinical onset [13,25]. Further studies are needed to correlate dysbiosis, clinical parameters and SCFAs levels in PE.

Our previous study showed that PE was associated with significantly more frequent bowel movements, and a tendency towards more loose/watery stool [20]. Our current subgroup analysis showed that early-onset, but not late-onset, PE was associated with a higher rate of loose/watery stool. It is possible that gastrointestinal manifestations differ between PE phenotypes, although further studies in larger cohorts are needed.

Although none remained significant after FDR correction, analyses of the functional potential of the gut microbiome in PE versus normal pregnancy revealed several potentially interesting pathways with known metabolic or neuroactive activity. However, very little is known about what effects these pathways might have in PE. Further studies are needed to elucidate which pathways are potentially involved in PE.

Previous studies have generally overlooked the distinction between subgroups of PE, often failing to examine them separately. As PE is a heterogeneous condition with diverse clinical manifestations, this lack of differentiation may partly account for inconsistencies in the literature. Additionally, variations in study populations may contribute to the discrepancies observed across studies. Our results indicate that both late-onset PE and early-onset PE are associated with gut

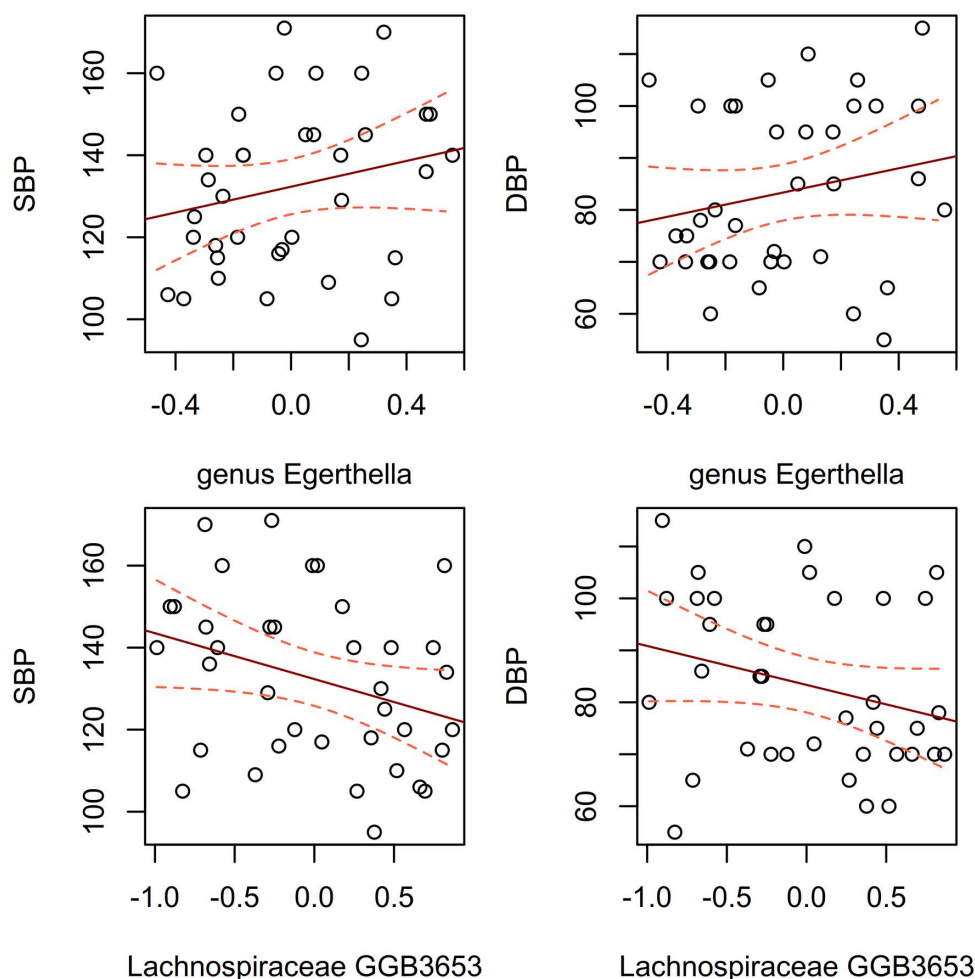


Fig 3. Genus *Eggerthella* has strong positive correlations with systolic blood pressure (SBP) and diastolic blood pressure (DBP), while uncultured *Lachnospiraceae* genus GGB3653 has negative correlations with both.

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dysbiosis, although possibly with larger differences in late-onset PE, as suggested by significantly lower alpha diversity. We hypothesize that late-onset PE might be more driven by a combination of host and environmental risk factors, such as gut dysbiosis, while early-onset PE is more associated with early defects in placentation. This might have important implications for interventional strategies as well. To date, low-dose acetyl salicylic acid is used as a prophylactic treatment during pregnancy in individuals with an increased risk of PE, but treatment mainly lowers the risk of early-onset disease [47]. We speculate that interventions targeting the gut microbiome, such as probiotics, might have a larger effect on late-onset PE.

A key strength of this study is the focus on distinct subgroups of PE, acknowledging that underlying mechanisms and clinical manifestations might differ between them. However, the relatively small size of these subgroups represents a limitation, reducing statistical power and potentially affecting the robustness of the findings. Future studies aimed at confirming and expanding these findings should include larger, well-characterized cohorts and clearly defined subgroups. In addition, longitudinal studies are warranted to investigate whether microbiome differences can be detected as early as in the first trimester, as suggested by previous reports [25].

Conclusions

Our findings reveal robust differences in the gut microbiome composition in both early-onset and late-onset PE, despite limited power due to the observational design and small cohort. Late-onset PE was associated with more significant differences, characterized by a significantly lower alpha diversity in addition to differences in beta diversity. Based on our results, we hypothesize that host and environmental factors such as the gut microbiome might be more involved in the pathophysiology of late-onset PE. Collectively, our results highlight the importance of subgroup-specific analysis and consideration of population-related differences in PE research. However, validation in larger and more diverse cohorts is essential to substantiate these findings and clarify the role of the gut microbiome in the development and progression of PE.

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