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Differences and similarities in the transcriptional profile of peripheral whole blood in early and late-onset preeclampsia: insights into the molecular basis of the phenotype of preeclampsia^a

Abstract

Objective: Preeclampsia (PE) can be sub-divided into early- and late-onset phenotypes. The pathogenesis of these two phenotypes has not been elucidated. To gain insight into the mechanisms of disease, the transcriptional profiles of whole blood from women with early- and late-onset PE were examined.

Methods: A cross-sectional study was conducted to include women with: i) early-onset PE (diagnosed prior to 34 weeks, n=25); ii) late-onset PE (after 34 weeks, n=47); and iii) uncomplicated pregnancy (n=61). Microarray analysis of mRNA expression in peripheral whole blood was undertaken using Affymetrix microarrays. Differential gene expression was evaluated using a moderated t-test (false discovery rate <0.1 and fold change >1.5), adjusting for maternal white blood cell count and gestational age. Validation by real-time qRT-PCR was performed in a larger sample size [early PE (n=31), late PE (n=72) and controls (n=99)] in all differentially expressed genes. Gene ontology analysis and pathway analysis were performed.

Results: i) 43 and 28 genes were differentially expressed in early- and late-onset PE compared to the control group, respectively; ii) qRT-PCR confirmed the microarray results for early and late-onset PE in 77% (33/43) and 71% (20/28) of genes, respectively; iii) 20 genes that are involved in coagulation (SERPINI2), immune regulation (VSIG4, CD24), developmental process (H19) and inflammation (S100A10) were differentially expressed in early-onset PE alone. In contrast, only seven genes that encoded proteins involved in innate immunity (LTF, ELANE) and cell-to-cell recognition in the nervous system (CNTNAP3) were differentially expressed in late-onset PE alone. Thirteen genes that encode proteins involved in host defense (DEFA4, BPI, CTSG, LCN2), tight junctions in blood-brain barrier (EMPI) and liver regeneration (ECT2) were differentially expressed in both early- and late-onset PE.

Conclusion: Early- and late-onset PE are characterized by a common signature in the transcriptional profile of whole blood. A small set of genes were differentially regulated in early- and late-onset PE. Future studies of the biological function, expression timetable and protein expression of these genes may provide insight into the pathophysiology of PE.

Keywords: Affymetrix; gene expression; H19; microarray; PAX gene; PCR transcriptomic; pregnancy; white blood cell count.

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Introduction

Preeclampsia (PE), one of the most common obstetrical syndromes [19–31, 35, 37, 40–42, 52, 54, 61, 63, 65, 68, 80, 89, 94, 97, 98, 106, 110, 111, 115, 117, 118, 126, 133, 134, 141, 147–151, 153, 164, 173, 174, 176, 179, 181, 187, 188, 201], is a
leading cause of maternal death and perinatal morbidity and mortality [13, 88, 129, 155, 158, 166, 199]. Women with a previous history of PE are at increased risk of death as a result of cardiovascular disease [53, 82, 140, 143, 160].

The mechanisms of disease responsible for PE remain elusive. High dimensional biology techniques including genomics [92, 114, 121, 193], transcriptomics [138, 175, 180, 184], proteomics [9, 18, 24, 51, 64, 84, 96, 105, 108, 119, 139, 156], metabolomics [7, 8, 74, 87, 125] and other –omic sciences are being utilized to gain insight into the pathophysiology of disease state and the identification of biomarkers in many disciplines including obstetrics [46, 62, 70, 77, 113, 130, 152, 165].

Transcriptome analysis has been used to classify lymphomas [5, 157] and cancer [15, 16, 44, 66, 79, 90, 122, 131, 132, 136, 161, 183, 194]. Several investigators have used this approach to examine the placental transcriptome in PE [69, 78, 124, 142, 159, 203]. The gene expression profiles of peripheral blood have shown promising results in elucidating the mechanisms of other disorders [1, 11, 116, 145, 170–172, 182]. This approach may be helpful in gaining understanding of the mechanism of disease in early- and late-onset PE.

The objective of this study was to characterize the transcriptional profiles of whole blood from women with early- and late-onset PE.

Patients and methods

Study design

A prospective cross-sectional study was conducted and included women in the following groups: i) early-onset PE (diagnosed prior to 34 weeks, n=25); ii) late-onset PE (after 34 weeks, n=47); and iii) uncomplicated pregnancy (n=61) (for microarray experiment). Exclusion criteria were the following: i) chronic hypertension; ii) known major fetal or chromosomal anomaly; iii) multiple gestations; and iv) patients receiving medication other than iron, stool softener, or vitamins prior to venipuncture. All women were enrolled at Hutzel Women’s Hospital, Detroit, MI and followed until delivery.

Clinical definition

Preeclampsia was defined as hypertension (systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg on at least two occasions, 4 h to 1 week apart) and proteinuria [2, 143]. Proteinuria was defined as protein of >300 mg in a 24-h urine specimen. If a 24-h urine collection was not available, the diagnosis of PE required ≥1+ proteinuria on two separate occasions 4 h to 1 week apart or ≥2+ proteinuria on a urine dipstick. Early- and late-onset PE were defined as a diagnosis of PE at ≤34 and >34 weeks of gestation, respectively [186, 190]. Pregnancy was considered uncomplicated if the women had no major medical, obstetrical or surgical complications, and delivered a normal term (≥37 weeks) infant whose birthweight was appropriate for gestational age (10–90th percentile) [6].

All patients provided written informed consent for the collection and use of samples for research purposes under the protocols approved by the Institutional Review Boards of Wayne State University and the Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Department of Health and Human Services (NICHD/NIH/DHHS).

Sample collection and preparation

Venipunctures were performed and 2.5 mL of whole blood was collected into PAXgene Blood RNA tubes (PreAnalytiX GmbH, distributed by Becton, Dickinson and Company, Franklin Lakes, NJ, USA), which contain a proprietary cell lysis and RNA stabilizing solution. PAXgene Blood RNA tubes were kept at room temperature for 24 h to ensure complete cellular lysis, then frozen at -70°C until further processing. Blood samples were also collected to determine white blood cell (WBC) count. For the patients with PE, maternal whole blood was collected at the time of diagnosis. Samples for the control group were collected at the prenatal clinic where patients had regular prenatal care, at the labor reception center where patients visited for minor complaints (such as headache, asymptomatic short cervix, itching, pelvic pressure, minor accident, etc.) or at the labor delivery unit for a scheduled cesarean section at term. All patients were followed until delivery.

RNA isolation

Intracellular total RNA was isolated from whole blood using the PAXgene Blood RNA Kit (Qiagen, Valencia, CA, USA). Blood lysates were reduced to pellets by centrifugation, washed, and re-suspended in buffer. Proteins were removed by proteinase K digestion and cellular debris removed by centrifugation through a PAXgene Shredder spin column. RNA was semi-precipitated with ethanol and selectively bound to the silica membrane of a PAXgene spin column. The membrane was treated with DNase I to remove any residual DNA, washed, and the purified total RNA was eluted in nuclease-free water. Purified total RNA was quantified by UV spectrophotometry using the DropSense96 Microplate Spectrophotometer (Trinean, Micronic North America LLC, McMurray, PA, USA) and the was purity assessed based on the A260/A280 and A260/A230 ratios. An aliquot of the RNA was assessed using the RNA 6000 nano assay for the Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Santa Clara, CA, USA). The electrophoretogram, RNA integrity number (RIN), and the ratio of the 28S:18S RNA bands were examined to determine overall quality of the RNA.

Whole blood transcriptome

The transcriptome of peripheral blood samples was profiled using Affymetrix GeneChip HG-U133 PLUS 2.0 arrays (Affymetrix Inc., Santa Clara, CA, USA). Briefly, isolated RNA was amplified using the Ovation RNA Amplification System V2 (NuGEN Technologies, Inc., San Carlos, CA, USA). Complementary DNA (cDNA) was synthesized.
using the Ovation buffer mix, first strand enzyme mix, and first strand primer mix with 5 μL (20 ng) of total RNA in specified thermal cycler protocols according to the manufacturer’s instructions. Amplification and purification of the generated cDNA were performed by combining SPIA buffer mix, enzyme mix, and water with the products of the second strand cDNA synthesis reactions in pre-specified thermal cycler programs. Optical density of the amplified cDNA product was obtained to demonstrate product yield and verify purity. Fragmentation and labeling was done using the FL-Ovation cDNA Biotin Module V2 (NuGEN Technologies, Inc.). In the primary step, a combined chemical and enzymatic fragmentation process was used to produce cDNA products in the 50 to 100 bases range. Fragmented cDNA products were then biotin-labeled using the Encore Biotin Module (NuGEN Technologies, Inc.). All reactions were carried out according to the manufacturer’s protocols. Amplified, fragmented and biotin-labeled cDNAs were used for hybridization cocktail assembly, then hybridized to the Affymetrix GeneChip HG-U133 PLUS 2.0 arrays according to the Affymetrix standard protocol.

Real-time quantitative polymerase chain reaction (qRT-PCR)

Validation of mRNA levels of selected genes was performed on a larger group of cases and controls using real-time qRT-PCR. The catalog number for each RNA primer is presented in Table S1. Total RNA was isolated as described above. Biomark™ System (Fluidigm, San Francisco, CA, USA) was used for high-throughput PCR. Briefly, an Invitrogen Superscript III 1st Strand Kit was used to generate cDNA. Pre-amplification procedures included combining 1.25 μL cDNA with 2.5 μL TaqMan PreAMP Mastermix and 1.25 μL pooled assay mix. The reaction was performed with a thermal cycler for one cycle at 95°C for 10 min and 14 cycles at 95°C for 15 s and 60°C for 4 min. After cycling, the reaction was diluted 1:5 by double-distilled water to a final volume of 25 μL. Fluidigm 96.96 Dynamic Array chip was used to perform the next step qRT-PCR assays. The 96.96 array chip was primed in an integrated fluidic circuit (IFC) controller with control fluid. After priming, 2.5 μL 20X TaqMan gene expression assays (Applied Biosystems) were mixed with 2.5 μL 2X assay loading reagent (Fluidigm) and loaded into the assay inlet on the 96.96 array chip. 2.25 μL preamplified cDNA was mixed with 2.5 μL TaqMan Universal PCR master mix (Applied Biosystems) and 0.25 μL 20X sample loading reagent (Fluidigm) and loaded into the sample inlet on the chip. The chip was returned to the IFC controller for loading. After loading, the chip was placed in the Biomark System to run the reactions. The cycle threshold (Ct) value of each reaction was obtained with the Fluidigm RT-PCR analysis software.

Analysis for microarray and qRT-PCR data

For microarray analysis, 25 patients with early-onset PE and 47 patients with late-onset PE were compared with 61 controls. To confirm the results from microarray, all differentially expressed genes which met the criteria for significances in microarray experiment were tested with qRT-PCR assays in a subset of the original sample set (early-onset PE n=25; late-onset PE n=44 and controls n=61) and in a new set of samples (early-onset PE n=6; late-onset PE n=28 and controls n=38). The selection criteria for the new specimens were the same as those used to select patients for the microarray analysis. Four patients in the late-onset PE group did not have enough RNA available after the microarray experiment and were not included in the qRT-PCR experiments.

Statistical analysis

Gene expression data pre-processing, that included background correction, normalization and summarization, was performed with the robust multi-array average algorithm (RMA) [43] implemented in the affy package of Bioconductor [2, 60]. Further analysis was performed only on the probe sets that were called “present” (expressed) in at least half of the number of samples in the smaller groups that were compared. The Affymetrix MAS 5.0 detection call method implemented in the affy package was used for determining the “present” calls of a given probe set in a given sample. Comparisons between early-onset PE and control samples and between late-onset PE and controls were performed by fitting a linear model on the gene expression levels using disease status, gestational age and WBC count of patients as variables in the model. The significance of the disease status coefficient was tested via a moderated t-statistic. The resulting P-values were adjusted to account for multiple testing, using the false discovery rate (FDR) algorithm. Genes with adjusted P-values (Q-values) <0.1 and fold change >1.5 were considered significant.

Gene ontology analysis was conducted to determine the “biological processes”, which are enriched in the list of differentially expressed genes as described elsewhere [1] and implemented in the GO stats package [3, 48]. Pathway analysis was performed on the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database using an overrepresentation analysis as well as the signaling pathway impact analysis [5].

The differential mRNA expression between early-onset PE and control samples and between late-onset PE and controls were compared using qRT-PCR data. The ΔΔCt values, surrogates for log gene expression, were fitted using the same linear model described for the microarray analysis. A nominal P-value <0.05 for the disease status coefficient in the linear model was considered as significant (validated).

The Mann-Whitney U and χ²-tests were used to compare the differences in demographics and clinical characteristics between PE and controls. SPSS (version 12.0; SPSS Inc, Chicago, IL, USA) was used for the analysis of demographic and clinical characteristic data. A probability value of P<0.05 was considered significant.

Results

Table 1 displays the demographic and clinical characteristics of the study population for both microarray and qRT-PCR procedures. Patients with early-onset PE had a higher median WBC count than those in the control groups (P=0.002). Among patients with uncomplicated pregnancy, there were 56 genes (70 probe sets) whose expression levels were correlated with WBC count after adjustment for gestational age (Table S2) and only two
Table 1  Demographics and clinical characteristics of the study populations.

<table>
<thead>
<tr>
<th></th>
<th>Control patients (n=61)</th>
<th>Early-onset pre eclampsia (n=25)</th>
<th>P-value</th>
<th>Late-onset pre eclampsia (n=47)</th>
<th>P-value</th>
<th>Control patients (n=99)</th>
<th>Early-onset pre eclampsia (n=31)</th>
<th>P-value</th>
<th>Late-onset pre eclampsia (n=72)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>24.1(16–39)</td>
<td>24.0 (16–37)</td>
<td>0.38</td>
<td>21.7 (16–36)</td>
<td>0.07</td>
<td>24.3 (16–39)</td>
<td>22.7 (16–37)</td>
<td>0.21</td>
<td>21.8 (16–36)</td>
<td>0.01</td>
</tr>
<tr>
<td>Nulliparity</td>
<td>19 (31.1)</td>
<td>14 (56.0)</td>
<td>0.03</td>
<td>27 (57.4)</td>
<td>0.006</td>
<td>29 (29.3)</td>
<td>19 (61.3)</td>
<td>0.001</td>
<td>41 (56.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>African American</td>
<td>56 (91.8)</td>
<td>21 (84.0)</td>
<td>0.28</td>
<td>41 (87.2)</td>
<td>0.44</td>
<td>85 (85.9)</td>
<td>25 (80.6)</td>
<td>0.48</td>
<td>66 (91.7)</td>
<td>0.24</td>
</tr>
<tr>
<td>Tobacco Use</td>
<td>4 (6.6)</td>
<td>4 (16.0)</td>
<td>0.17</td>
<td>9 (19.1)</td>
<td>0.046</td>
<td>17 (17.2)</td>
<td>5 (16.1)</td>
<td>0.89</td>
<td>10 (13.9)</td>
<td>0.56</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.1 (16.6–47.7)</td>
<td>24.6 (13.8–35.9)</td>
<td>0.04</td>
<td>26.4 (17.7–48.3)</td>
<td>0.69</td>
<td>26.2 (16.4–54.9)</td>
<td>24.7 (13.8–36.0)</td>
<td>0.22</td>
<td>27.9 (16.5–52.4)</td>
<td>0.37</td>
</tr>
<tr>
<td>Gestational age at blood draw (weeks)</td>
<td>33.7 (20.0–40.1)</td>
<td>31.1 (25.0–33.9)</td>
<td>0.004</td>
<td>37.9 (34.3–41.4)</td>
<td>&lt;0.001</td>
<td>34.7 (20.0–40.1)</td>
<td>31.1 (24.7–34.0)</td>
<td>&lt;0.001</td>
<td>38.1 (34.3–42.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WBC cells/mm³</td>
<td>8.0 (4.2–13.8)</td>
<td>9.7 (4.7–18.7)</td>
<td>0.002</td>
<td>8.3 (4.8–15.1)</td>
<td>0.19</td>
<td>8.8 (2.7–17.8)</td>
<td>9.7 (3.8–18.7)</td>
<td>0.04</td>
<td>8.5 (4.8–23.6)</td>
<td>0.63</td>
</tr>
<tr>
<td>Severe pre eclampsia</td>
<td>22 (88.0)</td>
<td>28 (88.0)</td>
<td>0.997</td>
<td>28 (89.6)</td>
<td>0.79</td>
<td>28 (88.0)</td>
<td>28 (88.0)</td>
<td>0.997</td>
<td>28 (88.0)</td>
<td>0.997</td>
</tr>
<tr>
<td>Gestational age at delivery (weeks)</td>
<td>39.1 (37.0–41.7)</td>
<td>31.7 (26.1–34.0)</td>
<td>&lt;0.001</td>
<td>38.0 (35.0–41.4)</td>
<td>&lt;0.001</td>
<td>39.1 (37.0–42.1)</td>
<td>31.7 (24.9–35.4)</td>
<td>&lt;0.001</td>
<td>38.3 (35.0–42.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Birthweight (grams)</td>
<td>3390 (2575–4010)</td>
<td>1235 (550–1910)</td>
<td>&lt;0.001</td>
<td>2880 (1670–4180)</td>
<td>&lt;0.001</td>
<td>3340 (2575–4010)</td>
<td>1330 (474–2730)</td>
<td>&lt;0.001</td>
<td>2968 (1670–4180)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Birthweight &lt;10%ile</td>
<td>0 (0)</td>
<td>11 (44.0)</td>
<td>&lt;0.001</td>
<td>15 (31.9)</td>
<td>&lt;0.001</td>
<td>14 (45.2)</td>
<td>18 (25.0)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as median (range).
Data are expressed as count (% of group).
BMI=body mass index; WBC=white blood cell counts.

When comparing the late-onset PE group and controls, 32 probe sets that represented 28 genes were differentially expressed in women with late-onset PE compared to those with uncomplicated pregnancy. A volcano plot for these probe sets is displayed in Figure 2. A total of six genes had higher and 22 genes had lower expression in women with late-onset PE. GO term enrichment analysis demonstrated that 49 probe sets (corresponding with 43 unique genes) were differentially expressed in maternal whole blood between early-onset PE and uncomplicated pregnancy after adjustment for WBC count and gestational age (Table 2; without adjustment, there were 31 genes differentially expressed).

A total of 18 genes had higher expression and 25 genes had lower expression in the early-onset disease group than in the control group.

A "volcano plot" shows the differential expression of all the annotated probe sets on the Affymetrix GeneChip HG-U133 PLUS 2.0 array with the log (base 10) of the FDR-adjusted probability values (y-axis) plotted against the log (base 2) fold changes (-axis) between the early-onset PE and control groups (Figure 1). Gene ontology analysis of the differentially expressed genes was performed and 41 biological processes were enriched (pFDR < 0.05 and at least two significant genes per GO term); the top 20 significant processes (ranking based on odds ratio) are presented in Table 3 (see full list in Table S3) and include negative regulation of T cell activation, defense response to bacterium, acute inflammatory response and negative regulation of cell activation.

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Late-onset PE vs. controls

When comparing the early-onset PE group and controls, 49 probe sets (corresponding with 43 unique genes) were differentially expressed in maternal whole blood between early-onset PE and uncomplicated pregnancy after adjustment for WBC count and gestational age (Table 2; without adjustment, there were 31 genes differentially expressed).

A total of 18 genes had higher expression and 25 genes had lower expression in the early-onset disease group than in the control group. A "volcano plot" shows the differential expression of all the annotated probe sets on the Affymetrix GeneChip HG-U133 PLUS 2.0 array with the log (base 10) of the FDR-adjusted probability values (y-axis) plotted against the log (base 2) fold changes (-axis) between the early-onset PE and control groups (Figure 1). Gene ontology analysis of the differentially expressed genes was performed and 41 biological processes were enriched (pFDR < 0.05 and at least two significant genes per biological process); the top 20 significant processes (ranking based on odds ratio) are presented in Table 3 (see full list in Table S3) and include negative regulation of T cell activation, defense response to bacterium, acute inflammatory response and negative regulation of cell activation.

Microarray analysis demonstrated that 49 probe sets (corresponding with 43 unique genes) were differentially expressed in maternal whole blood between early-onset PE and uncomplicated pregnancy after adjustment for WBC count and gestational age. A total of 18 genes had higher expression and 25 genes had lower expression in the early-onset disease group than in the control group.
### Table 2  Differentially expressed genes between early-onset preeclampsia and controls based on microarray data.

<table>
<thead>
<tr>
<th>Entrez gene</th>
<th>Symbol</th>
<th>Gene name</th>
<th>Fold change</th>
<th>Adjusted P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Higher expression</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>EMP1</td>
<td>Epithelial membrane protein 1</td>
<td>2.33</td>
<td>0.0001</td>
</tr>
<tr>
<td>2354</td>
<td>FOSB</td>
<td>FBJ murine osteosarcoma viral oncogene homolog B</td>
<td>2.06</td>
<td>0.0000</td>
</tr>
<tr>
<td>23243</td>
<td>ANKR28</td>
<td>Ankyrin repeat domain 28</td>
<td>1.72</td>
<td>0.0233</td>
</tr>
<tr>
<td>1894</td>
<td>ECT2</td>
<td>Epithelial cell transforming sequence 2 oncogene</td>
<td>1.72</td>
<td>0.0077</td>
</tr>
<tr>
<td>6281</td>
<td>S100A10</td>
<td>S100 calcium binding protein A10</td>
<td>1.69</td>
<td>0.0000</td>
</tr>
<tr>
<td>7252</td>
<td>TSHB</td>
<td>Thyroid stimulating hormone, β</td>
<td>1.68</td>
<td>0.0002</td>
</tr>
<tr>
<td>283120</td>
<td>H19</td>
<td>H19, imprinted maternally expressed transcript (non-protein coding)</td>
<td>1.66</td>
<td>0.0011</td>
</tr>
<tr>
<td>283120</td>
<td>H19</td>
<td>H19, imprinted maternally expressed transcript (non-protein coding)</td>
<td>1.66</td>
<td>0.0011</td>
</tr>
<tr>
<td>11326</td>
<td>VSIG4</td>
<td>V-set and immunoglobulin domain containing 4</td>
<td>1.63</td>
<td>0.0026</td>
</tr>
<tr>
<td>2359</td>
<td>FPR3</td>
<td>Formyl peptide receptor 3</td>
<td>1.63</td>
<td>0.0139</td>
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<tr>
<td>84617</td>
<td>TUBB6</td>
<td>Tubulin, β 6</td>
<td>1.62</td>
<td>0.0106</td>
</tr>
<tr>
<td>2335</td>
<td>FN1</td>
<td>Fibronectin 1</td>
<td>1.6</td>
<td>0.0074</td>
</tr>
<tr>
<td>1396</td>
<td>CRIP1</td>
<td>Cysteine-rich protein 1 (intestinal)</td>
<td>1.59</td>
<td>0.0000</td>
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<td>2335</td>
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<td>Fibronectin 1</td>
<td>1.55</td>
<td>0.0204</td>
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<tr>
<td>388610</td>
<td>TRNP1</td>
<td>TGFβ1-regulated nuclear protein 1</td>
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<tr>
<td>257177</td>
<td>C1orf192</td>
<td>Chromosome 1 open reading frame 192</td>
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<tr>
<td>79992</td>
<td>C6orf59</td>
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<td>5276</td>
<td>SERPIN2</td>
<td>Serpin peptidase inhibitor, clade I (panc), member 2</td>
<td>1.53</td>
<td>0.0077</td>
</tr>
<tr>
<td>4000</td>
<td>LMNA</td>
<td>Lamin A/C</td>
<td>1.52</td>
<td>0.0011</td>
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<tr>
<td>114327</td>
<td>EFHC1</td>
<td>EF-hand domain (C-terminal) containing 1</td>
<td>1.52</td>
<td>0.0047</td>
</tr>
<tr>
<td>4000</td>
<td>LMNA</td>
<td>Lamin A/C</td>
<td>1.51</td>
<td>0.0012</td>
</tr>
<tr>
<td><strong>Lower expression</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1669</td>
<td>DEFA4</td>
<td>Defensin, α 4, corticostatin</td>
<td>2.36</td>
<td>0.0110</td>
</tr>
<tr>
<td>4680</td>
<td>CEACAM6</td>
<td>Carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen)</td>
<td>2.1</td>
<td>0.0409</td>
</tr>
<tr>
<td>4680</td>
<td>CEACAM6</td>
<td>Carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen)</td>
<td>2.06</td>
<td>0.0334</td>
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<td>671</td>
<td>BPI</td>
<td>Bactericidal/permeability-increasing protein</td>
<td>2</td>
<td>0.0214</td>
</tr>
<tr>
<td>23254</td>
<td>RP1-21O18.1</td>
<td>Kazrin</td>
<td>1.97</td>
<td>0.0166</td>
</tr>
<tr>
<td>1511</td>
<td>CTS5</td>
<td>Cathepsin G</td>
<td>1.93</td>
<td>0.0093</td>
</tr>
<tr>
<td>820</td>
<td>CAMP</td>
<td>Cathelicidin antimicrobial peptide</td>
<td>1.93</td>
<td>0.0002</td>
</tr>
<tr>
<td>1088</td>
<td>CEACAM8</td>
<td>Carcinoembryonic antigen-related cell adhesion molecule 8</td>
<td>1.9</td>
<td>0.0508</td>
</tr>
<tr>
<td>1232</td>
<td>CCR3</td>
<td>Chemokine (C-C motif) receptor 3</td>
<td>1.84</td>
<td>0.0002</td>
</tr>
<tr>
<td>932</td>
<td>MS4A3</td>
<td>Membrane-spanning 4-domains, subfamily A, member 3 (hematopoietic cell-specific)</td>
<td>1.83</td>
<td>0.0962</td>
</tr>
<tr>
<td>23254</td>
<td>RP1-21O18.1</td>
<td>Kazrin</td>
<td>1.82</td>
<td>0.0051</td>
</tr>
<tr>
<td>6947</td>
<td>TNC1</td>
<td>Transcobalamin I (vitamin B12 binding protein, R binder family)</td>
<td>1.75</td>
<td>0.0120</td>
</tr>
<tr>
<td>7053</td>
<td>TGM3</td>
<td>Transglutaminase 3 (E polypeptide, protein-glutamine-γ-glutamyltransferase)</td>
<td>1.7</td>
<td>0.0539</td>
</tr>
<tr>
<td>23462</td>
<td>HEY1</td>
<td>Hairy/enhancer-of-split related with YRPW motif 1</td>
<td>1.66</td>
<td>0.0543</td>
</tr>
<tr>
<td>100133941</td>
<td>CD24</td>
<td>CD24 molecule</td>
<td>1.63</td>
<td>0.0387</td>
</tr>
<tr>
<td>6518</td>
<td>SLC2A5</td>
<td>Solute carrier family 2 (facilitated glucose/fructose transporter), member 5</td>
<td>1.63</td>
<td>0.0614</td>
</tr>
<tr>
<td>4318</td>
<td>MMP9</td>
<td>Matrix metalloepidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)</td>
<td>1.62</td>
<td>0.0390</td>
</tr>
<tr>
<td>8993</td>
<td>PGLYRP1</td>
<td>Peptidoglycan recognition protein 1</td>
<td>1.59</td>
<td>0.0065</td>
</tr>
<tr>
<td>2280</td>
<td>FKBP1A</td>
<td>FK506 binding protein 1A, 12kDa</td>
<td>1.59</td>
<td>0.0104</td>
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<tr>
<td>1053</td>
<td>CEBPE</td>
<td>CCAAT/enhancer binding protein (C/EBP), epsilon</td>
<td>1.59</td>
<td>0.0036</td>
</tr>
<tr>
<td>100133941</td>
<td>CD24</td>
<td>CD24 molecule</td>
<td>1.59</td>
<td>0.0508</td>
</tr>
<tr>
<td>3934</td>
<td>LCN2</td>
<td>Lipocalin 2</td>
<td>1.58</td>
<td>0.0984</td>
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<tr>
<td>5806</td>
<td>PTX3</td>
<td>Pentraxin-related gene, rapidly induced by IL-1 β</td>
<td>1.57</td>
<td>0.0448</td>
</tr>
<tr>
<td>154664</td>
<td>ABCA13</td>
<td>ATP-binding cassette, subfamily A (ABCA1), member 13</td>
<td>1.56</td>
<td>0.0855</td>
</tr>
<tr>
<td>116448</td>
<td>OLIG1</td>
<td>Oligodendrocyte transcription factor 1</td>
<td>1.54</td>
<td>0.0166</td>
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<tr>
<td>762</td>
<td>CA4</td>
<td>Carbonic anhydrase IV</td>
<td>1.52</td>
<td>0.0398</td>
</tr>
<tr>
<td>8851</td>
<td>CDK5R1</td>
<td>Cyclin-dependent kinase 5, regulatory subunit 1 (p35)</td>
<td>1.51</td>
<td>0.0315</td>
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<tr>
<td>660</td>
<td>BMX</td>
<td>BMX non-receptor tyrosine kinase</td>
<td>1.5</td>
<td>0.0332</td>
</tr>
</tbody>
</table>
Pathway analysis of the differentially expressed genes was undertaken with an overrepresentation method and the signaling pathway impact analysis method.

No pathways emerged as significantly enriched or impacted based on either method in both comparisons (between both early-onset vs. controls and between late-onset vs. controls).

**qRT-PCR analysis**

Figure 3 summarizes the numbers of genes differentially expressed in microarray and qRT-PCR experiments. For the comparison between early-onset PE and controls, qRT-PCR confirmed the microarray results for both the direction of fold change and the significance in 77% (33/43) of genes. Specifically, qRT-PCR confirmed increased expression of 15 genes and decreased expression of 18 genes in early-onset PE (Table S5) when compared to uncomplicated pregnancies. Similarly, qRT-PCR validated the microarray results for the comparison between late-onset PE and controls in 71% (20/28) of genes. qRT-PCR confirmed up-regulation of four genes and down-regulation of 16 genes (see supplementary Table 6).

Genes differentially expressed by both microarray and qRT-PCR analysis are summarized in Table 6. Twenty genes (12 higher expressions and eight lower expression) were differentially expressed in early-onset PE alone. Among these genes, H19 – an imprinted

**Table 3** Top 20 biological processes (ranking by odds ratio) significantly enriched in comparison between early-onset preeclampsia and controls.

<table>
<thead>
<tr>
<th>GOBPID</th>
<th>Biological process</th>
<th>Genes in differentially expressed list (n)</th>
<th>Genes in GOBPID list</th>
<th>Odds ratio</th>
<th>Adjusted P-value</th>
</tr>
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<tbody>
<tr>
<td>GO:0031424</td>
<td>Keratinization</td>
<td>2</td>
<td>8</td>
<td>101.47</td>
<td>0.0080</td>
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<tr>
<td>GO:0030225</td>
<td>Macrophage differentiation</td>
<td>2</td>
<td>12</td>
<td>60.86</td>
<td>0.0133</td>
</tr>
<tr>
<td>GO:0018149</td>
<td>Peptide cross-linking</td>
<td>2</td>
<td>16</td>
<td>43.45</td>
<td>0.0179</td>
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<tr>
<td>GO:0042742</td>
<td>Defense response to bacterium</td>
<td>4</td>
<td>39</td>
<td>39.11</td>
<td>0.0005</td>
</tr>
<tr>
<td>GO:0032945</td>
<td>Negative regulation of mononuclear cell proliferation</td>
<td>2</td>
<td>27</td>
<td>24.31</td>
<td>0.0192</td>
</tr>
<tr>
<td>GO:0009913</td>
<td>Epidermal cell differentiation</td>
<td>2</td>
<td>35</td>
<td>18.40</td>
<td>0.0241</td>
</tr>
<tr>
<td>GO:0002695</td>
<td>Negative regulation of T cell activation</td>
<td>2</td>
<td>37</td>
<td>17.35</td>
<td>0.0241</td>
</tr>
<tr>
<td>GO:0030855</td>
<td>Epithelial cell differentiation</td>
<td>4</td>
<td>83</td>
<td>16.23</td>
<td>0.0057</td>
</tr>
<tr>
<td>GO:0002695</td>
<td>Negative regulation of leukocyte activation</td>
<td>2</td>
<td>44</td>
<td>14.87</td>
<td>0.0305</td>
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<tr>
<td>GO:0051707</td>
<td>Response to other organism</td>
<td>8</td>
<td>227</td>
<td>13.15</td>
<td>0.0001</td>
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<tr>
<td>GO:0042129</td>
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<td>52</td>
<td>12.13</td>
<td>0.0329</td>
</tr>
<tr>
<td>GO:0007398</td>
<td>Ectoderm development</td>
<td>3</td>
<td>100</td>
<td>9.61</td>
<td>0.0233</td>
</tr>
<tr>
<td>GO:0043627</td>
<td>Response to estrogen stimulus</td>
<td>2</td>
<td>69</td>
<td>9.03</td>
<td>0.0386</td>
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<tr>
<td>GO:0002526</td>
<td>Acute inflammatory response</td>
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<td>70</td>
<td>8.90</td>
<td>0.0386</td>
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<tr>
<td>GO:0070663</td>
<td>Regulation of leukocyte proliferation</td>
<td>2</td>
<td>71</td>
<td>8.77</td>
<td>0.0391</td>
</tr>
<tr>
<td>GO:0007204</td>
<td>Elevation of cytosolic calcium ion concentration</td>
<td>2</td>
<td>77</td>
<td>8.06</td>
<td>0.0405</td>
</tr>
<tr>
<td>GO:0050865</td>
<td>Regulation of cell activation</td>
<td>3</td>
<td>147</td>
<td>6.44</td>
<td>0.0329</td>
</tr>
<tr>
<td>GO:0008624</td>
<td>Induction of apoptosis by extracellular signals</td>
<td>2</td>
<td>97</td>
<td>6.35</td>
<td>0.0476</td>
</tr>
<tr>
<td>GO:0045666</td>
<td>Regulation of neuron differentiation</td>
<td>2</td>
<td>97</td>
<td>6.35</td>
<td>0.0476</td>
</tr>
<tr>
<td>GO:0043123</td>
<td>Positive regulation of I-kappab kinase/NF-kappab cascade</td>
<td>2</td>
<td>98</td>
<td>6.29</td>
<td>0.0477</td>
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</table>
Table 4  Differentially expressed genes between late-onset preeclampsia and controls based on microarray data.

<table>
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<tr>
<th>Entrez gene</th>
<th>Symbol</th>
<th>Gene name</th>
<th>Fold change</th>
<th>Adjusted P-value</th>
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<tr>
<td>Higher expression</td>
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<td>728577</td>
<td>CNTNAP3B</td>
<td>Contactin associated protein-like 3B</td>
<td>1.78</td>
<td>0.05735</td>
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<tr>
<td>2012</td>
<td>EMP1</td>
<td>Epithelial membrane protein 1</td>
<td>1.63</td>
<td>0.02499</td>
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<tr>
<td>79937</td>
<td>CNTNAP3</td>
<td>Contactin associated protein-like 3</td>
<td>1.63</td>
<td>0.09549</td>
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<tr>
<td>79937</td>
<td>CNTNAP3</td>
<td>Contactin associated protein-like 3</td>
<td>1.61</td>
<td>0.03953</td>
</tr>
<tr>
<td>23243</td>
<td>ANKRD28</td>
<td>Ankyrin repeat domain 28</td>
<td>1.57</td>
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<tr>
<td>1894</td>
<td>ECT2</td>
<td>Epithelial cell transforming sequence 2 oncogene</td>
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<td>0.02362</td>
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<tr>
<td>7252</td>
<td>TSHB</td>
<td>Thyroid stimulating hormone, β</td>
<td>1.5</td>
<td>0.00116</td>
</tr>
<tr>
<td>Lower expression</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1669</td>
<td>DEFA4</td>
<td>Defensin, α, 4, corticostatin</td>
<td>2.49</td>
<td>0.00116</td>
</tr>
<tr>
<td>1088</td>
<td>CEACAM8</td>
<td>Carcinoembryonic antigen-related cell adhesion molecule 8</td>
<td>2.09</td>
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<td>4057</td>
<td>LTF</td>
<td>Lactotransferrin</td>
<td>2.03</td>
<td>0.00099</td>
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<tr>
<td>671</td>
<td>BPI</td>
<td>Bactericidal/permeability-increasing protein</td>
<td>2.01</td>
<td>0.00519</td>
</tr>
<tr>
<td>4317</td>
<td>MMP8</td>
<td>Matrix metallopeptidase 8 (neutrophil collagenase)</td>
<td>1.99</td>
<td>0.03667</td>
</tr>
<tr>
<td>4680</td>
<td>CEACAM6</td>
<td>Carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen)</td>
<td>1.99</td>
<td>0.02288</td>
</tr>
<tr>
<td>4317</td>
<td>MMP8</td>
<td>Matrix metallopeptidase 8 (neutrophil collagenase)</td>
<td>1.98</td>
<td>0.02162</td>
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<tr>
<td>6037</td>
<td>RNASE3</td>
<td>Ribonuclease, mase A family, 3 (eosinophil cationic protein)</td>
<td>1.89</td>
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<tr>
<td>4070</td>
<td>TACSTD2</td>
<td>Tumor-associated calcium signal transducer 2</td>
<td>1.89</td>
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<tr>
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<td>OLFM4</td>
<td>Olfactomedin 4</td>
<td>1.88</td>
<td>0.07735</td>
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<tr>
<td>4680</td>
<td>CEACAM6</td>
<td>Carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen)</td>
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<td>3934</td>
<td>LCN2</td>
<td>Lipocalin 2</td>
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<td>10321</td>
<td>CRISP3</td>
<td>Cysteine-rich secretory protein 3</td>
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<tr>
<td>1991</td>
<td>ELANE</td>
<td>Elastase, neutrophil expressed</td>
<td>1.78</td>
<td>0.02841</td>
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<tr>
<td>260429</td>
<td>PRSS33</td>
<td>Protease, serine, 33</td>
<td>1.73</td>
<td>0.08714</td>
</tr>
<tr>
<td>5806</td>
<td>PTK3</td>
<td>Pentraxin-related gene, rapidly induced by IL-1 β</td>
<td>1.69</td>
<td>0.00206</td>
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<td>1511</td>
<td>CTSG</td>
<td>Cathepsin G</td>
<td>1.69</td>
<td>0.09089</td>
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<td>6947</td>
<td>TCN1</td>
<td>Transcobalamin I (vitamin B12 binding protein, R binder family)</td>
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<td>0.00847</td>
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<td>820</td>
<td>CAMP</td>
<td>Cathelicidin antimicrobial peptide</td>
<td>1.66</td>
<td>0.00178</td>
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<td>1232</td>
<td>CCR3</td>
<td>Chemokine (C-C motif) receptor 3</td>
<td>1.64</td>
<td>0.001</td>
</tr>
<tr>
<td>932</td>
<td>M5A4A3</td>
<td>Membrane-spanning 4-domains, subfamily A, member 3 (hematopoietic cell-specific)</td>
<td>1.63</td>
<td>0.08534</td>
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<tr>
<td>260429</td>
<td>PRSS33</td>
<td>Protease, serine, 33</td>
<td>1.57</td>
<td>0.08534</td>
</tr>
<tr>
<td>4973</td>
<td>OLR1</td>
<td>Oxidized low density lipoprotein (lectin-like) receptor 1</td>
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<td>0.0919</td>
</tr>
<tr>
<td>56729</td>
<td>RETN</td>
<td>Resistin</td>
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<td>0.05967</td>
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<tr>
<td>100133941</td>
<td>CD24</td>
<td>CD24 molecule</td>
<td>1.52</td>
<td>0.03254</td>
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</tbody>
</table>

maternally expressed gene that is involved in developmental processes – had the highest change (5.7-fold). Genes involved in coagulation/fibrinolytic system (SERPIN2), immune regulation (VSIG4, CD24), and inflammation (SIOOA10) were detected as differentially regulated. In contrast, only seven genes (one higher expression and five lower expressions) that are involved in innate immunity (LTF, ELANE) and cell-to-cell recognition in the nervous system (CNTNAP3) were differentially expressed in late-onset PE alone. Thirteen genes (three higher expressions and ten lower expression) that are essential to host defense [DEFA4, bactericidal/permeability-increasing (BPI), CTSG, LCN2], tight junctions in blood-brain barrier (EMP1) and liver regeneration (ECT2) were differentially expressed in late-onset PE alone compared to uncomplicated pregnancies.

Discussion

Principal findings of the study

1. Microarray analysis in maternal whole blood revealed 43 and 28 differentially expressed genes in early-onset and late-onset PE, respectively, compared to uncomplicated pregnancies.
Table 5  Top 20 biological processes (ranking by odds ratio) significantly enriched in the comparison between late-onset preeclampsia and controls.

<table>
<thead>
<tr>
<th>GOBPID</th>
<th>Biological process</th>
<th># Differentially expressed genes in GO term</th>
<th># Genes in GO term</th>
<th>Odds ratio</th>
<th>Adjusted P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GO:0042742</td>
<td>Defense response to bacterium</td>
<td>5</td>
<td>51</td>
<td>60.76</td>
<td>0.0000</td>
</tr>
<tr>
<td>GO:0007159</td>
<td>Leukocyte cell-cell adhesion</td>
<td>2</td>
<td>25</td>
<td>40.26</td>
<td>0.0129</td>
</tr>
<tr>
<td>GO:0051707</td>
<td>Response to other organism</td>
<td>8</td>
<td>227</td>
<td>22.46</td>
<td>0.0000</td>
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<td>GO:0050866</td>
<td>Negative regulation of cell activation</td>
<td>2</td>
<td>48</td>
<td>20.09</td>
<td>0.0163</td>
</tr>
<tr>
<td>GO:0002683</td>
<td>Negative regulation of immune system process</td>
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<td>68</td>
<td>13.97</td>
<td>0.0255</td>
</tr>
<tr>
<td>GO:0043627</td>
<td>Response to estrogen stimulus</td>
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<td>69</td>
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<td>0.0256</td>
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<tr>
<td>GO:006874</td>
<td>Cellular calcium ion homeostasis</td>
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<td>117</td>
<td>12.63</td>
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</tr>
<tr>
<td>GO:0055066</td>
<td>Di-, tri-valent inorganic cation homeostasis</td>
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<tr>
<td>GO:0007204</td>
<td>Elevation of cytosolic calcium ion concentration</td>
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</tr>
<tr>
<td>GO:0043406</td>
<td>Positive regulation of MAP kinase activity</td>
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<td>11.96</td>
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<tr>
<td>GO:0030003</td>
<td>Cellular cation homeostasis</td>
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<td>175</td>
<td>11.70</td>
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<tr>
<td>GO:0055065</td>
<td>Metal ion homeostasis</td>
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<td>131</td>
<td>11.23</td>
<td>0.0142</td>
</tr>
<tr>
<td>GO:0008624</td>
<td>Induction of apoptosis by extracellular signals</td>
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<td>97</td>
<td>9.68</td>
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<td>GO:0006955</td>
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<td>GO:0031347</td>
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<td>GO:0055082</td>
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<td>GO:0050801</td>
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<td>Membrane invagination</td>
<td>3</td>
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<td>6.86</td>
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3. Twenty genes that are involved in coagulation, immune regulation, developmental process and inflammation were differentially expressed in early-onset PE alone. In contrast, only seven genes implicated in innate immunity and cell-to-cell recognition in the nervous system were differentially expressed in late-onset PE alone. Thirteen genes that are essential to host defense, tight junctions in blood-brain barrier and liver regeneration were differentially expressed in both early- and late-onset PE compared to uncomplicated pregnancy.

The results of our study were reported after adjustment for gestational age and WBC count, as variation in the proportion of peripheral blood cell types could be responsible for differences of gene expression profiles observed among groups [5, 195]. Among women with uncomplicated pregnancies, the expression of 56 genes was correlated with WBC count, whereas the expression of only two genes changed as a function of gestational age after adjustment for the WBC count. After adjustment for WBC count and gestational age, the differentially expressed genes between PE and controls increased from 31 to 43 genes and from 22 to 28 genes for early- and late-onset PE, respectively.

In the current study, we did not compare transcriptomic profiles of early- and late-onset PE directly, because...
we could not reasonably adjust for gestational age differences (by definition, these two conditions have completely different ranges of gestational age at blood sampling). It is noteworthy that several differentially expressed genes in early- and late-onset PE change in the same direction. One interpretation of these findings is that early- and late-onset PE are characterized by a common signature in the transcriptional profile of whole blood. However, a small set of genes were differentially expressed in early- or late-onset PE alone when compared to uncomplicated pregnancies. Selected differentially expressed genes are discussed below.

**H19, imprinted maternally expressed transcript (non-protein coding) (H-19)**

The H-19 gene produces a non-coding RNA which may have growth suppressive function. This gene is located in an imprinted region of chromosome 11 near the insulin-like growth factor-2 (IGF2) gene. Expression of H-19 and IGF2 are imprinted so that H-19 is only expressed from the maternal allele, and IGF2 is only expressed from the paternal allele [67, 85, 198]. Experiments in mice have demonstrated that placental IGF2 knockout leads to fetal growth restriction, while H-19 silencing results in fetal overgrowth [47]. These phenotypes are also observed in humans, in Silver Russell syndrome [17, 43, 101, 124] and Beckwith-Wiedemann syndrome [34, 36, 38], respectively.

H-19 is highly expressed in cytotrophoblasts [67]. Hypomethylation, along with increased expression of H-19, has been observed in the placenta of pregnancies complicated by fetal growth restriction [91], while hypermethylation with decreased expression of H-19 has been reported in the placenta of patients with early-onset PE [57]. Furthermore, H-19 could inhibit trophoblast proliferation through miR-675 and the lower expression of H-19 in the placenta may result in an excessive proliferation of trophoblasts in early-onset PE [58].

Another study also reports biallelic expression (loss of imprinting: LOI) of the H-19 gene in 46% (6/13) of the placentas from patients with PE whereas there is no bialleic expression in 26 cases of normal pregnancy [198]. The clinical symptoms of patients with LOI on the H-19 gene
### Table 6  Summary of genes that are differentially expressed in both microarray analysis and by qRT-PCR.

<table>
<thead>
<tr>
<th>Number</th>
<th>Entrez gene</th>
<th>Symbol</th>
<th>Gene name</th>
<th>Microarray</th>
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<td>Adjusted P-value</td>
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<td>Early-onset preeclampsia alone</td>
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<tr>
<td>Higher expression</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>224997_x_at</td>
<td>H19</td>
<td>H19, imprinted maternally expressed transcript (non-protein coding)</td>
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<td>0.0011</td>
</tr>
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<td>VSIG4</td>
<td>V-set and immunoglobulin domain containing 4</td>
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<td>0.0026</td>
</tr>
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<td>Serpin peptidase inhibitor, clade I (pancin), member 2</td>
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<td>0.0077</td>
</tr>
<tr>
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<td>202768_at</td>
<td>FOSB</td>
<td>FBJ murine osteosarcoma viral oncogene homolog B</td>
<td>2.06</td>
<td>0.0000</td>
</tr>
<tr>
<td>Lower expression</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
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<td>Tubulin, β 6</td>
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<td>Olfactomedin 4</td>
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<td>CCAAT/enhancer binding protein (C/EBP)</td>
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<td>0.0065</td>
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<tr>
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<td>Carbonic anhydrase IV</td>
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<td>Resistin</td>
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<td>Both early- and late-onset preeclampsia</td>
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<tr>
<td>Higher expression in early-onset</td>
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<td></td>
</tr>
<tr>
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<tr>
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<td>Defensin, α 4, corticostatin</td>
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<td>Carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen)</td>
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<td>0.0334</td>
</tr>
<tr>
<td>3</td>
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<td>CAMP</td>
<td>Cathelicidin antimicrobial peptide</td>
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are more severe than those of patients without LOI [198]. Moreover, H-19 expression may be related to hypoxia since over-expression of HIF-1α and suppression of p53, a tumor suppressor gene, induces H-19 expression in response to hypoxia in several tumor cell lines [109]. We found a 5.7-fold higher mRNA expression of H-19 in the whole blood of patients with early-onset, but not late-onset PE, compared to uncomplicated pregnancies. The biological consequence of increased expression of H-19 genes on leukocytes deserves further investigation.

**V-set and immunoglobulin domain containing 4 (VSIG4)**

This gene encodes a v-set and immunoglobulin domain containing protein, also referred to as complement receptor of the immunoglobulin superfamily (CRIg). This protein is a receptor for the complement component 3 (C3), fragments C3b, iC3b and has been implicated in the clearance of systemic pathogens and autologous cells [75]. The activation of the complement system resulting in the generation of split products with pro-inflammatory properties has been observed in PE [71, 162]. In the current study, a 2.7-fold higher expression of VSIG4 gene was observed in early-onset PE, but not in the late-onset disease. However, VSIG4 is believed to play a role in tissue homeostasis and resolution rather than initiation of inflammatory response [73]. In mice, this protein inhibits both cytotoxic T and B cell responses to viral antigen, and thus, may also be a negative regulator of T cell responses [189].

**CD24 molecule (CD24)**

CD24, a cell-surface sialoglycoprotein, is expressed on multiple cell types and disappears after these cells have reached their final stage of differentiation [49, 81]. Recently, CD24 has been proposed to be a genetic check point in T cell homeostasis and autoimmune disease as CD24 expression in T cells is necessary for optimal T cell homeostatic proliferation in the lymphopenic host, while CD24 expressed by non-T cells acts as a negative regulator that control the pace of T cell proliferation [103]. CD24 polymorphisms are also associated with multiple sclerosis [200] and systemic lupus erythematosus [154].

CD24 also plays a role in the innate immune response. In a mouse model of acetaminophen-induced liver necrosis, CD24 interacts with sialic acid binding

---

### Table 6 (Continued)

<table>
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**Lower expression in late-onset**

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</tr>
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immunoglobulin-like lectins (Siglecs) and decreases the host response to danger-associated molecular patterns (i.e., high mobility group box 1, heat shock protein 70 and heat shock protein 90), but not to pathogen-associated molecular pattern (e.g., Toll-like receptor), by selectively repression of NF-κB activation [32]. Furthermore, a recent study reported that cytotoxic lymphocytes and the syncytiotrophoblasts expressed CD24, and treating placental explants with adiponectin (an adipokine that has been reported to be elevated in circulation of patients with PE [123]) increased expression of Siglec10 (both mRNA and protein) [112], suggesting that CD24/Siglec interaction may be one mechanism whereby trophoblasts protect themselves against a danger signal [32, 112]. However, the consequences of decreased CD24 expression in the peripheral blood of patients with early-onset PE reported herein remains to be determined.

**ATP-binding cassette, subfamily A (ABC), member 13 (ABCA13)**

ABCA 13 is a new member of the ATP-binding cassette (ABC) gene subfamily A (ABCA). The 12 members of this subfamily share a high degree of sequence conservation and have been mostly related to lipid trafficking [3, 32, 72, 86, 102, 120, 168, 169, 192, 202]. For example; ABCA 2, which is predominantly expressed in the brain [191], plays an important role in neuronal lipid transport [169, 202] and is associated with Alzheimer’s disease [33, 107]. ABCA 3 is predominantly expressed in alveolar type II cells [197], and plays a role in the excretion of the lipid fraction of the pulmonary surfactant [6, 120]. However, the function of ABCA 13, which is expressed in trachea, testis and bone marrow [137], remains to be determined. The murine ABCA13 promoter region contains transcription factor-binding sites associated with myeloid and lymphoid cell types, and its ubiquitous expression in blood-derived cells suggests a role for ABCA 13 in hematopoiesis [12]. In the current study, ABCA13 gene expression in peripheral whole blood was lower in early-onset PE than in uncomplicated pregnancies.

**Contactin associated protein-like 3 (CNTNAP3)**

The protein encoded by this gene belongs to the Neuroxin-IV/CNTNAP/Paranodin (NCP) family, the biological function of which is thought to be the mediation of neuron-glial cell interactions [14]. Thus, this protein may play a role in cell recognition within the nervous system. At least five CNTNAP genes, with expression restricted primarily to the brain and peripheral nerves, have been cloned so far and are presumed to play a role in cell-to-cell interactions [135, 144, 163, 178]. CNTNAP3 was the only gene that up-regulated significantly in late-onset PE alone.

**Epithelial membrane protein 1 (EMP1)**

This gene encodes a protein that is integral to the formation of tight junctions in the blood-brain barrier [10]. This protein is a liver cell-junction protein [99] and is expressed in tissues that have significant epidermal growth factor (EGF) receptor expression [83, 100]. EMP1 gene expression was higher in the peripheral blood of patients with early- and late-onset PE than in uncomplicated pregnancies.

**Preeclampsia: a condition with down-regulation of anti-inflammatory genes in peripheral whole blood**

An interesting finding of this study is that several genes involved in the innate immune response of neutrophils and monocytes/macrophages, such as neutrophil elastase (ELANE), cathepsin G (CTSG), BPI, defensin α (DEFA)-4, and lactotransferrin (LTF), were down-regulated either in both subtypes of PE (DEFA4, BPI, CTSG) or in late-onset PE (ELANE, LTF). As the nature of systemic inflammation in PE is chronic [55, 56, 150], it is not surprising that these genes (which are involved in the acute inflammatory response and participated in the first line of defense against pathogens) are not up-regulated in whole blood. However, the fact that their expression was down-regulated in PE may seem paradoxical. Nonetheless, recent evidence may provide a possible explanation for this phenomenon.

Previous studies indicate that neutrophil elastase and cathepsin G have not only pro-inflammatory, but also anti-inflammatory properties [59, 177, 195]. A study by Gardiner et al. demonstrated that when both proteins were applied to neutrophils, they were capable of cleaving P-selectin glycoprotein ligand-1 (PSGL-1) on neutrophils, which led to the loss of PSGL-1 protein from neutrophil surfaces, and rapidly abolished their capacity to bind P-selectin, a PSGL-1 ligand on activated endothelium [59]. This study suggested that the cathepsin G and neutrophil elastase-mediated PSGL-1 proteolysis is part of an autocrine mechanism for the down-regulation of neutrophil adhesion to activated endothelium. Similarly, another study reported that elastase and cathepsin G containing
fractions (derived from neutrophil lysates and degranulation supernatants), as well as purified elastase and cathepsin G, inhibited C5a-dependent neutrophil functions [177]. These findings indicate that elastase and cathepsin G play an important role in the down-regulation of acute inflammation.

Neutrophil cathepsin G may also exert its anti-inflammatory functions on monocytes because it is capable of cleaving CD14 (endotoxin receptor) from monocyte surfaces, thus limiting inflammation [95]. As the activation of the endothelium and complement system is part of the exaggerated systemic maternal inflammation in PE [71, 141, 146, 153, 162], the down-regulation of neutrophil elastase and cathepsin G may indicate defective up-regulation of important anti-inflammatory molecules in peripheral leukocytes that is intended to limit inflammation.

Of interest, a microarray study by Williams et al. found that genes encoding for α-defensin A3, cathelicidin, cathepsin G, and lactotransferrin were down-regulated in monocytes that had a transmigratory phenotype [196]. It is possible that our findings showing down-regulation of three out of four of these genes, as well as of DEFA-4, may reflect a phenotypic change in monocytes and/or neutrophils, in which this phenomenon may be related to their altered migratory potential in PE.

Previous microarray studies of the transcriptome of peripheral blood of patients with preeclampsia

Four studies have used microarray to examine transcriptomic signatures in peripheral blood of patients with PE. Sun et al. reported 72 differentially regulated genes between six patients with early-onset PE and five uncomplicated pregnant women [167]. The authors reported the up-regulation of the VSIG4 gene, which is the only finding that is consistent with the current study. However, this gene was found to be down-regulated in their qRT-PCR validation. In contrast to our study, Sun et al. used Trizol and isolated lymphocytes prior to conducting microarray analysis. Rajakumar et al. compared the transcriptomic profiles of peripheral blood mononuclear cells isolated from five patients with PE and women with uncomplicated pregnancies (n=5) [138]. Matrix metalloproteinase (MMP)-9 was found to be differentially expressed in this study, but was not confirmed in our qRT-PCR analysis. Dahlstrom et al., using the PAX gene Blood RNA system, reported 19 differentially expressed genes between eight early-onset PE and eight normal pregnant women [39]. Only lower expression of CCR3 (CC chemokine receptor 3) gene in late-onset PE was confirmed in our study. Although this study was the only one that evaluated gene expression at the time of diagnosis in whole blood of PE using the PAX gene Blood RNA system (as in our study), the difference in analytic approach (principal component analysis) and the inclusion of patients who received medications may explain the discrepancy between their results and our findings. A recent study by Enquobahrie et al. examined gene expression profiles in peripheral whole blood with the PAX gene Blood RNA system at 16 weeks of gestation in 16 patients destined to develop PE [45]. Differential expressed genes included COLIA1, IKKB and RBL1, none of which was significantly differentially expressed between uncomplicated pregnancies and PE at the time of diagnosis in our study. However, Enquobahrie et al. did not stratify patients into early- or late-onset PE.

Advantage and disadvantage of whole blood transcriptome

The current study used microarray technology, which allows unbiased genome-wide examination of the transcriptome of peripheral blood in patients with PE. In order to minimize alteration in RNA expression after blood sampling, the PAX gene Blood RNA system, which contains a stabilizing additive in a blood collection tube to immediately capture the transcriptional profile, was used. This approach allows analysis of intracellular RNA expression with reduced laboratory complexities and less artificial activation compared to other approaches, which isolated each cellular subpopulation prior to microarray experiments. Results of microarray analysis with the use of the PAX gene Blood RNA system have been reported to provide useful information in several studies [6, 93, 185]. However, the microarray hybridization rates reported in whole blood are usually lower than those using individual cell types [8, 50]. The Encore™ Biotin Module and Ovation® RNA Amplification System V2 were used in this study for amplification of whole blood total RNA to increase hybridization rates. We did not use globin reduction procedures in order to avoid the interference with the observed transcriptional profiles caused by these methods [104].

As the PAXgene blood system stabilizes intracellular RNA, the transcriptomic profiles of whole blood observed in this study mainly originated from maternal leukocytes (especially neutrophils) and other cellular components, including reticulocytes and immature platelets. However, we also observed transcripts of placental specific or
pregnancy specific genes such as PLAC4, PLAC1, Placental lactogen (CSH1), chorionic gonadotropin, β popy-peptide (CGB) genes in some samples (data not shown). This observation is consistent with a study reported by Okazaki et al. – that the mRNA expression of trophoblasts can be observed through analysis of the cellular component of maternal blood [127, 128] – although PLAC4, and PLAC1 may not be specific to placenta [76] as they could be detectable in non-pregnant whole blood.

Strengths and limitations

This is the largest study to date that used microarray for the transcriptomic profiling of whole blood in patients with PE. Patients with early- and late-onset PE were included in the study. Moreover, all differentially expressed genes from microarray analysis were validated by qRT-PCR. The limitations of the study include: i) the differentially expressed genes reflect the changes in total intracellular mRNA components of whole blood and cannot be traced to the subpopulation of leukocytes or reticulocytes that are responsible for the observed differences; and ii) since blood samples were obtained at the time of diagnosis, it remains to be determined whether the differentially expressed genes reported herein are causally related to PE.

Conclusion

Microarray analysis in maternal whole blood revealed several differentially expressed genes in early- and late-onset PE compared to uncomplicated pregnancies. Differentially expressed genes are involved in coagulation, immune regulation, growth/developmental process, host defense and tight junctions in the blood-brain barrier. However, early- and late-onset PE are characterized by a common signature in the transcriptional profile of whole blood. Future studies of the biological function, expression timetable and protein expression of differentially expressed genes may provide insight into the pathophysiology of PE.

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