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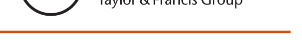

ORIGINAL ARTICLE

Maternal whole blood mRNA signatures identify women at risk of early preeclampsia: a longitudinal study

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ABSTRACT

Purpose: To determine whether previously established mRNA signatures are predictive of early preeclampsia when evaluated by maternal cellular transcriptome analysis in samples collected before clinical manifestation.

Materials and methods: We profiled gene expression at exon-level resolution in whole blood samples collected longitudinally from 49 women with normal pregnancy (controls) and 13 with early preeclampsia (delivery <34 weeks of gestation). After preprocessing and removal of gestational age-related trends in gene expression, data were converted into Z-scores based on the mean and standard deviation among controls for six gestational-age intervals. The average Z-scores of mRNAs in each previously established signature considered herein were compared between cases and controls at 9–11, 11–17, 17–22, 22–28, 28–32, and 32–34 weeks of gestation.

Results: (1) Average expression of the 16-gene *untargeted cellular mRNA* signature was higher in women diagnosed with early preeclampsia at 32–34 weeks of gestation, yet more importantly, also prior to diagnosis at 28–32 weeks and 22–28 weeks of gestation, compared to controls (all, $p < .05$). (2) A combination of four genes from this signature, including a long non-protein coding RNA [H19 imprinted maternally expressed transcript (*H19*)], fibronectin 1 (*FN1*), tubulin beta-6 class V (*TUBB6*), and formyl peptide receptor 3 (*FPR3*) had a sensitivity of 0.85 (0.55–0.98) and a specificity of 0.92 (0.8–0.98) for prediction of early preeclampsia at 22–28 weeks of gestation. (3) *H19*, *FN1*, and *TUBB6* were increased in women with early preeclampsia as early as 11–17 weeks of gestation (all, $p < .05$). (4) After diagnosis at 32–34 weeks, but also prior to diagnosis at 11–17 weeks, women destined to have early preeclampsia showed a coordinated increase in whole blood expression of several *single-cell placental signatures*, including the 20-gene signature of extravillous trophoblast (all, $p < .05$). (5) A combination of three mRNAs from the extravillous trophoblast signature (*MMP11*, *SLC6A2*, and *IL18BP*) predicted early preeclampsia at 11–17 weeks of gestation with a sensitivity of 0.83 (0.52–0.98) and specificity of 0.94 (0.79–0.99).

Conclusions: Circulating early transcriptomic markers for preeclampsia can be found either by untargeted profiling of the cellular transcriptome or by focusing on placental cell-specific mRNAs. The *untargeted cellular mRNA signature* was consistently increased in early preeclampsia after 22 weeks of gestation, and individual mRNAs of this signature were significantly increased as early as 11–17 weeks of gestation. Several *single-cell placental signatures* predicted future development of the disease at 11–17 weeks and were also increased in women already diagnosed at 32–34 weeks of gestation.

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Biomarker discovery; placental single-cell signatures; fibronectin 1 (*FN1*); formyl peptide receptor 3 (*FPR3*); *H19* imprinted maternally expressed transcript (non-protein coding) (*H19*); interleukin 18 binding protein (*IL18BP*); matrix metallopeptidase 11 (*MMP11*); solute carrier family 6 (neurotransmitter transporter); member 2 (*SLC6A2*); tubulin; beta 6 class V (*TUBB6*)

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Introduction

Early identification of patients at risk of obstetrical disease is required to improve health outcomes and develop new therapeutic interventions. Notwithstanding the progress made in the screening and treatment of women at risk for preterm delivery [1] and preeclampsia [2], a large proportion of pregnancies at risk to develop any of the "great obstetrical syndromes" [3] are still not detected by the current screening methods. Moreover, current screening procedures may require Doppler ultrasound measurements [4], a technique not readily available in all clinical settings; hence, more affordable alternatives are needed to better serve the at-risk patient population.

Studies aiming to develop molecular markers for early prediction of obstetrical syndromes were based on "omics" methods, such as transcriptomics [5,6], proteomics [7–15], and metabolomics [16–20]. Given that the gestational age when biomarkers may be predictive of an obstetrical syndrome is not known *a priori*, and that this may also vary among different biomarkers and syndromes, the collection of omics data in longitudinal, minimally invasive samples in large cohorts would be most suited for the discovery and validation of biomarkers [14,21,22].

The obstetrics literature showcases several strategies for the selection of candidate molecular markers that can predict obstetrical disease. An initial approach is the use of untargeted molecular profiling techniques to assess the transcriptome, proteome, or metabolome in samples collected by minimally invasive methods (e.g. maternal whole blood) from patients at the time of disease diagnosis and from gestational age-matched women who had a normal pregnancy [5,6]. A second approach is to narrow the search for molecules that are placental or fetal organ-specific, for which previous evidence of association with obstetrical disease was reported [23–25]. A third emerging strategy is a refinement of the latter approach: defining candidate markers among those molecules specific to subpopulations of placental cells identified by single-cell transcriptomics and for which there is evidence of significant changes in maternal plasma (cell-free RNA) at the time of disease diagnosis [26]. The assumption is that the latter two approaches will capture mechanisms specific to disorders of deep placentation [27–30] and, hence, are less susceptible to spurious findings that can affect untargeted studies.

However, it is unknown which of these approaches, based on evidence collected at the time of disease diagnosis, will lead to biomarkers predictive of obstetrical disease prior to clinical manifestation. To provide insight into this important question, we selected early preeclampsia (delivery <34 weeks of gestation) as a case study and profiled the cellular transcriptome in maternal circulation in longitudinal samples of women who subsequently were diagnosed with early preeclampsia and those with a normal pregnancy.

The goal was to determine whether a previously reported cellular mRNA signature of early preeclampsia defined at the time of diagnosis (*untargeted cellular RNA signature*) [5] is predictive of the disease before clinical manifestation. Moreover, we aimed at assessing whether *single-cell placental signatures*, such as that of extravillous trophoblast shown to be increased in maternal plasma at the time of a diagnosis of early preeclampsia [26], have predictive value prior to clinical manifestation.

Materials and methods

Study design

We conducted a prospective longitudinal study that enrolled pregnant women attending the Center for Advanced Obstetrical Care and Research of the Perinatology Research Branch, NICHD/NIH/DHHS, the Detroit Medical Center, and Wayne State University. Based on this cohort, we designed a retrospective study of 49 women with a normal pregnancy who delivered at term and had 4–6 whole blood samples collected before 40 weeks of gestation (median number of samples = 6, interquartile range = 6–6). The study also included 13 women who developed early preeclampsia (delivery <34 weeks of gestation) and had 3–7 whole blood samples collected before 34 weeks of gestation (median number of samples = 5, interquartile range = 4–6). All patients provided written informed consent for the use of biological specimens as well as clinical and ultrasound data for research purposes. The analysis presented in this manuscript is based on data and specimens collected under the protocol entitled Biological Markers of Disease in the Prediction of Preterm Delivery, Preeclampsia, and Intra-Uterine Growth Restriction: A Longitudinal Study. The study was approved by the Wayne State University Institutional Review Board (WSU IRB#110605MP2F) and by the Institutional Review Board of NICHD/NIH (OH97-CH-N067).

Clinical definitions

Preeclampsia was defined as new-onset hypertension and proteinuria after 20 weeks of gestation [31]. Hypertension was defined when systolic blood

pressure was ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg, measured on at least two occasions, 4 h to 1 week apart [31]. Proteinuria was defined as ≥300 mg of protein in a 24-h urine collection, or two random urine specimens obtained 4 h to 1 week apart demonstrating protein ≥1+ by dipstick or one dipstick demonstrating ≥2+ protein) [31]. Early preeclampsia was defined as preeclampsia diagnosed and delivered before 34 weeks of gestation [32]. Small-for-gestational-age neonate was defined as birthweight percentile <10th according to the Alexander birthweight standard [33].

RNA extraction

RNA was isolated from a PAXgene® Blood RNA collection tube (BD762165) as described in the PAXgene® Blood miRNA Kit Handbook (December 2015). Purified RNA was quantified by UV spectrophotometry using the DropSense96® microplate Spectrophotometer (Trinean) and quality-assessed by microfluidics using the RNA ScreenTape on the Agilent 2200 TapeStation.

Microarray analysis

One hundred nanograms of RNA was reverse-transcribed and amplified using the Affymetrix (Santa Clara, CA) GeneChip® WT Plus Reagent Kit, following the manufacturer's suggested protocol. A 5.5 µg sense-strand of cDNA was fragmented and labeled using the Affymetrix GeneChip® WT terminal Labeling Kit. A total of 200 µl of labeled targets were hybridized to the Affymetrix GeneChip® Human transcriptome Array 2.0 in an Affymetrix hybridization oven at 45 °C, 60 rpm for 16 h. Wash and stain steps were performed on an Affymetrix GeneChip® Fluidics Station 450 and scanned on an Affymetrix GeneChip® Scanner 3000. Raw intensity data were generated from array images using Affymetrix GeneChip™ Command Console Software.

Data analysis

Preprocessing

Data for 348 arrays, each containing >6.0 million probes, were preprocessed (background correction, normalization, and summarization) using the Robust Multi-array Average (RMA) approach [34] implemented in the *oligo* package [35]. Probes were assigned to transcript clusters using annotation from the *hta20-transcriptcluster.db* package of *Bioconductor* [36]. Given that the samples were profiled in several batches as a part of a larger study, correction for potential batch effects was performed using the *removeBatchEffect* function of the *limma* [37] package in *Bioconductor* [36]. Duplicate transcript clusters to unique genes were resolved by retaining the transcript cluster with the highest average expression across all samples.

Data transformation

Log_2 expression data in the control group were fit as a function of the gestational-age continuum using linear mixed-effects models. The adequate polynomial function was selected by minimizing the Akaike's information criterion for each gene. Data for all samples were then converted into $\log_2$ multiples of the mean (MoM) for gestational age by subtracting the predicted trend in the control group. Further, control samples ($n = 78$) collected after 34 weeks were excluded. The gestational-age continuum was then divided into intervals (9–11, 11–17, 17–22, 22–28, 28–32, 32–34 weeks) so that each interval included a comparable number of samples from cases while the number of duplicate samples from each woman in a given interval was minimal. In the few instances when there were duplicate samples from the same woman in a given gestational-age interval, the most recent sample (highest gestational age) was retained. The $\log_2$ MoM gene expression data for the remaining samples ($n = 257$) were transformed into a Z-score for each gene by subtracting the mean and dividing by the standard deviation calculated among controls in each gestational-age interval separately, hence accounting for the differences in expression variance among genes, as it was suggested by others [38].

Gene signatures

Two previously defined mRNA signatures were of primary interest in this study in relation to early preeclampsia:

Untargeted cellular RNA signature. The first signature considered was the set of 16 mRNAs present on the microarray platform from the 18 mRNAs reported by Chaiworapongsa et al. [5] to be significantly increased in whole blood of women diagnosed with early preeclampsia. The mRNAs included in this signature were *ANKRD28*, *CRIP1*, *ECT2*, *EFHC1*, *EMP1*, *FN1*, *FOSB*, *FPR3*, *H19*, *LMNA*, *S100A10*, *SERPINI2*, *TRNP1*, *TSHB*, *TUBB6*, and *VSIG4*.

Single-cell placental signature. The second mRNA signature of primary interest comprised 20 mRNAs deemed as specific to extravillous trophoblast cells by

Table 1. Demographic characteristics of the women included in the transcriptomics study.

	Normal pregnancy ($n = 49$)	Early preeclampsia ($n = 13$)	p-Value
Age	25 (21–28)	21 (19–27)	.35
Body mass index	25.8 (22.5–30.9)	31.8 (25.8–34.2)	.12
Nulliparity	16/49 (32.7%)	8/13 (61.5%)	.11
Race			1.00
African American	45/49 (91.8%)	13/13 (100%)	
Caucasian	2/49 (4.1%)	0/13 (0%)	
Other	2/49 (4.1%)	0/13 (0%)	
Gestational age at delivery	39.3 (38.6–39.9)	32.3 (30.4–33.4)	<.001
Birthweight (g)	3285 (3050–3495)	1395 (1255–1760)	<.001
Customized birthweight (percentile)	48.2 (31.8–63.4)	4.8 (1.3–9.9)	<.005
Customized birthweight (percentile) <10th	1/49 (2%)	10/13 (76.9%)	<.001
Customized EFW (percentile)[a]	63.40 (28.6–76.8)	10.7 (6.98–20.4)	<.001
Customized EFW (percentile) <10th[a]	1/47 (2.1%)	5/12 (41.7%)	<.001
Delivery route			.53
Vaginal	26/49 (53.1%)	5/13 (38.5%)	
Cesarean	23/49 (46.9%)	8/13 (61.5%)	

Continuous variables were compared between groups using Welch's t-test and are summarized as medians (interquartile range). Categorical variables are shown as number (%) and were compared using Fisher's exact test. EFW: Estimated Fetal Weight. [a]For 1 control and 2 cases, the customized EFW percentile could not be determined since the standard only applies after 22 weeks, while the last available scan for these three women was performed at 17–19 weeks.

Note. The one patient in the normal pregnancy group with customized EFW and birthweight percentile < 10th was considered to be appropriate-for-gestational-age according to non-customized standards used in clinical care.

single-cell RNA-Seq analysis [26]. The average expression of this signature was demonstrated to be increased in maternal plasma (cell-free RNA) at the time of diagnosis of early preeclampsia [26]. The member genes included in this signature were *AIF1L*, *BCAR4*, *CERCAM*, *CLDN19*, *EGLN3*, *GDPD3*, *HLA-G*, *IL18BP*, *INHA*, *ITGA5*, *LAIR2*, *LVRN*, *LY6D*, *MMP11*, *NOTUM*, *PAPPA2*, *PYCR1*, *RRAD*, *SLC6A2*, and *UPK1B*. We also considered secondary single-cell placental mRNA signatures specific to cytotrophoblasts, syncytiotrophoblast, decidual cells, dendritic cells, endothelial cells, erythrocytes, Hofbauer cells, stromal cells, and vascular smooth muscle cell populations described by Tsang et al. [26].

Differential expression of mRNA signatures. The expression of each mRNA signature in a given sample was determined as the average Z-score overall members' genes. Data were compared between cases and controls in each gestational-age interval using two-tailed equal variance t-tests, with $p < .05$ considered as a significant result.

Reduced mRNA signatures. To identify parsimonious sets of mRNAs that predict early preeclampsia with minimal or no loss of accuracy when compared to the full signatures, combinations of the genes part of the signatures were evaluated as predictors in linear discriminant analysis (LDA) models. To limit the chance of overfitting, only combinations of up to 4 mRNAs were tested. The sensitivity, specificity, and likelihood ratios (positive and negative) were obtained based on a posterior probability for early preeclampsia of .5 or greater. Such parsimonious LDA models were shown to lead to accurate predictions on independent test sets, as shown in multiple systems biology international initiatives [39–41].

Results

Clinical characteristics of the study population

We profiled the maternal blood cellular transcriptome in longitudinal samples collected in 49 women with a normal pregnancy and 13 women diagnosed with early preeclampsia. Demographic characteristics for this longitudinal case-control study population are shown in Table 1. There were no significant differences in maternal race, age, body mass index, or parity between the groups; however, women with early preeclampsia delivered earlier and had a lower customized estimated fetal weight and a lower birthweight percentile [42] (for all, $p < .001$). Among women with early preeclampsia, the rate of small-for-gestational-age (SGA; customized percentile <10th), determined based on last available ultrasound scan during the second or early third trimester, was 42% and the rate of SGA at birth was 77% (Table 1). Either a second or early third trimester Doppler measurement was available in 12 of the 13 early preeclampsia cases; of those cases, 16% had an abnormal umbilical artery pulsatility index, while 66% of the cases had an abnormal mean uterine artery pulsatility index, based on cutoff values reported elsewhere [43,44].

After microarray data preprocessing and transformation into MoM expression for gestational age values, data were converted into Z-scores and averaged over

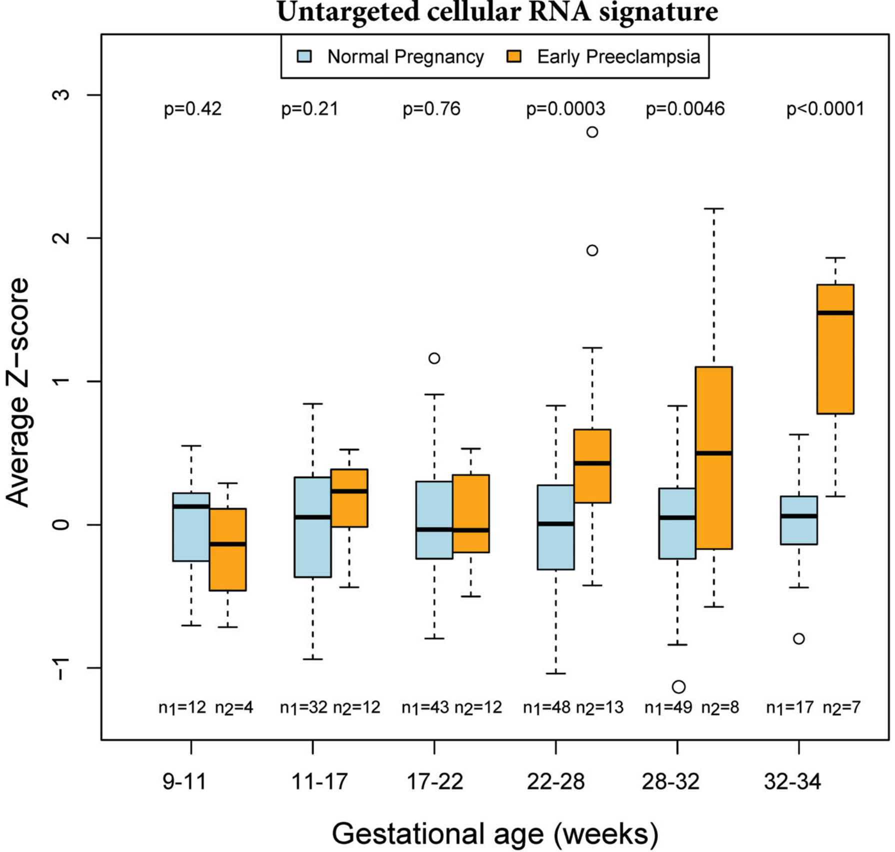

Figure 1. Cellular mRNA expression of the untargeted cellular mRNA signature in women with a normal pregnancy and in those who developed early preeclampsia. Log_2 gene expression data were corrected for gestational age and possible batch effects and then converted into Z-scores for each gene, using the mean and standard deviation calculated for the controls in each gestational-age interval separately. The distributions of the average Z-scores over genes in the untargeted cellular mRNA signature are shown using box plots; *n*1 refers to the number of controls while *n*2 refers to the number of cases. All case samples from the last interval (32–34 weeks of gestation) were taken at or after the diagnosis of preeclampsia. Only 2 of the 13 samples from cases at 22–28 weeks and 3 of the 8 samples at 28–32 weeks were collected after the diagnosis of early preeclampsia, respectively.

member genes of the *untargeted cellular RNA signature* and each *single-cell placental signature*.

Changes in the expression of the untargeted cellular RNA signature in early preeclampsia

Figure 1 shows the distribution of the expression of the *untargeted cellular RNA signature* as a function of the gestational-age interval and pregnancy outcome. As expected from our previous study [5]; after the diagnosis of early preeclampsia at 32–34 weeks of gestation, the average expression of this signature was increased compared to that of women with a normal pregnancy ($p < .0001$). When assessed in samples collected earlier in pregnancy at 28–32 and 22–28 weeks of gestation, the average expression of this signature was also significantly increased (for both intervals, $p < .005$). The differences between cases and controls remained significant at 22–28 weeks when only 11 of the 13 cases that were not already diagnosed by 28 weeks were included in the analysis ($p < .05$).

The sensitivity and false-positive rate for prediction of early preeclampsia by the *untargeted cellular RNA signature* at 22–28 weeks of gestation are shown by a Receiver Operating Characteristic (ROC) curve in Figure 2, with the area under the ROC curve (AUC) of 75%.

To determine whether the *untargeted cellular RNA signature* can be reduced to a smaller subset of genes without loss in prediction performance, we fitted linear discriminant models, using up to 4 of the original set of 16 genes, by analyzing data collected at 22–28 weeks of gestation. Ten 4-gene combinations were found to predict early preeclampsia with a positive likelihood ratio (LR+) >10.0 and a negative LR (LR−) <0.5 (Table 2). A majority of these gene combinations included H19 imprinted maternally expressed transcript (*H19*), a long nonprotein coding RNA.

The combination of *H19*, fibronectin 1 (*FN1*), tubulin beta-6 class V (*TUBB6*), and formyl peptide receptor 3 (*FPR3*) had a sensitivity of 85% and a specificity of 92% for prediction of early preeclampsia at 22–28 weeks of gestation. Of interest, *H19, FN1*, and *TUBB6* were each significantly increased in women with early preeclampsia as early as 11–17 weeks of gestation (for all, $p < .05$).

Changes in the expression of placenta-targeted RNA signatures in early preeclampsia

The cellular expression of the extravillous trophoblast mRNA signature is shown in Figure 3 as a function of gestational age-interval and pregnancy outcome. Not only was this signature increased in samples collected from women already diagnosed with early preeclampsia at 32–34 weeks ($p < .05$), but more importantly, it was also increased prior to the disease diagnosis at 11–17 weeks of gestation ($p < .05$). This increasing pattern (both at the time of disease diagnosis and at 11–17 weeks) was also true for gene signatures specific to other subpopulations of placental cells, including syncytiotrophoblast, decidual-, endothelial-, vascular smooth muscle-, and stromal cells (Supplementary Figure 1).

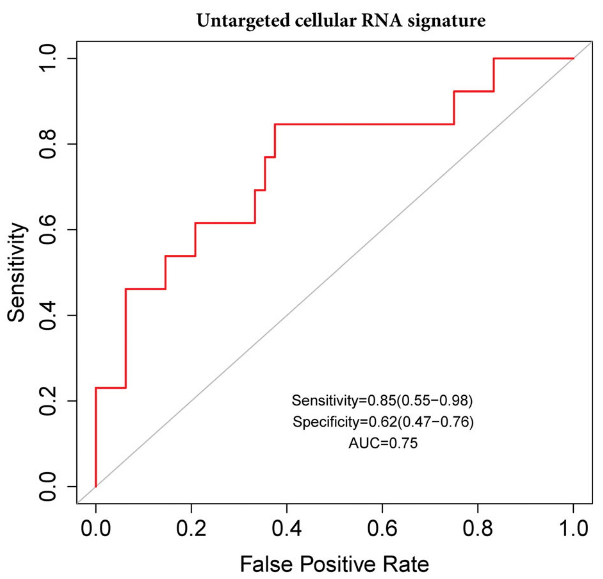

Figure 2. Prediction of early preeclampsia by untargeted cellular RNA signature at 22–28 weeks of gestation. Receiver Operating Characteristic (ROC) curve for the untargeted cellular RNA signature at 22–28 weeks of gestation. AUC: area under the ROC curve.

The ROC curves for prediction of early preeclampsia at 11–17 weeks of gestation by changes in *single-cell placental signatures* are shown in Figure 4. The area under the ROC curve ranged from 71% for the extravillous trophoblast signature to 81% for the dendritic cell signature. Table 3 provides additional prediction performance indices of these mRNA signatures.

To determine whether the extravillous trophoblast single-cell signature can be reduced from 20 mRNAs to a smaller subset without loss in prediction performance, we fitted linear discriminant analysis models using up to 4 of the original set of 20 genes. We found 11 3-gene combinations that predicted early preeclampsia at 11–17 weeks with a LR+ >10.0 and a sensitivity >60%. Most of these 11 extravillous trophoblast-specific mRNA combinations included *MMP11* (matrix metallopeptidase 11). For example, the combination of *MMP11*, *SLC6A2* (solute carrier family 6 member 2), and *AIF1L* (allograft inflammatory factor 1-like) mRNAs had a LR+ of 21.3 and a LR− of 0.34 for prediction of early preeclampsia at 11–17 weeks of gestation (see Table 3).

Comment

Principal findings of the study

(1) The average expression of the 16-gene *untargeted cellular mRNA signature* was higher in women already diagnosed with early preeclampsia at 32–34 weeks, but more importantly, also prior to diagnosis at

Table 2. Prediction performance for early preeclampsia at 22–28 weeks of gestation by combinations of 4 mRNAs part of the untargeted cellular mRNA signature.

Predictors	Sensitivity	Specificity	Likelihood ratio (+)	Likelihood ratio (−)
H19 + EFHC1 + TUBB6 + FPR3	0.85 (0.55–0.98)	0.94 (0.83–0.99)	13.5 (4.4–41.5)	0.16 (0.05–0.59)
H19 + FN1 + TUBB6 + FPR3	0.85 (0.55–0.98)	0.92 (0.8–0.98)	10.2 (3.9–26.7)	0.17 (0.05–0.6)
CRIP1 + VSIG4 + EFHC1 + FPR3	0.77 (0.46–0.95)	0.96 (0.86–0.99)	18.5 (4.6–74)	0.24 (0.09–0.65)
FOSB + H19 + FPR3 + ANKRD28	0.77 (0.46–0.95)	0.94 (0.83–0.99)	12.3 (4–38.3)	0.25 (0.09–0.67)
FOSB + H19 + FPR3 + TRNP1	0.77 (0.46–0.95)	0.94 (0.83–0.99)	12.3 (4–38.3)	0.25 (0.09–0.67)
EFHC1 + FN1 + TUBB6 + FPR3	0.77 (0.46–0.95)	0.94 (0.83–0.99)	12.3 (4–38.3)	0.25 (0.09–0.67)
EFHC1 + FN1 + FPR3 + ANKRD28	0.77 (0.46–0.95)	0.94 (0.83–0.99)	12.3 (4–38.3)	0.25 (0.09–0.67)
S100A10 + H19 + VSIG4 + ANKRD28	0.62 (0.32–0.86)	0.96 (0.86–0.99)	14.8 (3.6–61.3)	0.4 (0.2–0.8)
S100A10 + LMNA + VSIG4 + ANKRD28	0.62 (0.32–0.86)	0.96 (0.86–0.99)	14.8 (3.6–61.3)	0.4 (0.2–0.8)
H19 + VSIG4 + FN1 + ECT2	0.62 (0.32–0.86)	0.96 (0.86–0.99)	14.8 (3.6–61.3)	0.4 (0.2–0.8)

Results are shown for linear discriminant analysis models with 4 mRNAs from the original 16-gene signature reported by Chaiworapongsa et al. [5].

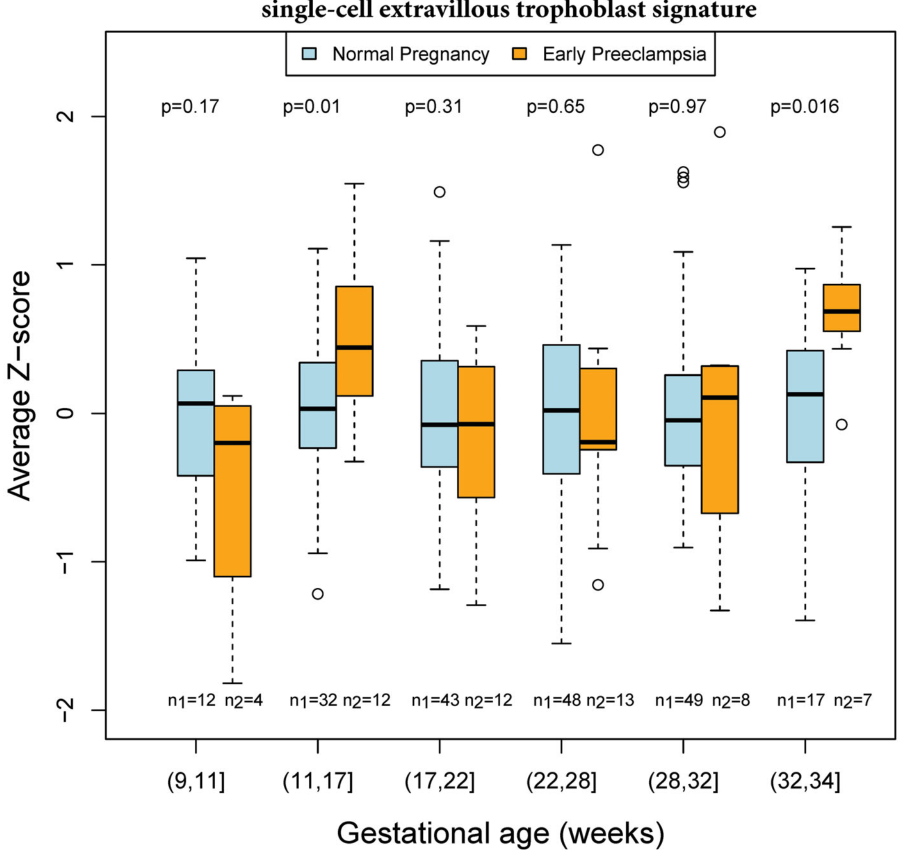

Figure 3. Cellular mRNA expression of the single-cell extravillous trophoblast signature. Log_2 gene expression data were corrected for gestational age and possible batch effects and then converted into Z-scores for each gene, using the mean and standard deviation calculated for the controls in each gestational age interval separately. The distribution of the average Z-scores overall 20 genes specific to extravillous trophoblast are shown using box plots; *n*1 refers to the number of controls while *n*2 refers to the number of cases. All samples from the cases in the last interval of 32–34 weeks were taken at or after the diagnosis. Only 2 of the 13 samples from cases at 22–28 weeks and 3 of the 8 samples at 28–32 weeks were collected after diagnosis with early preeclampsia, respectively.

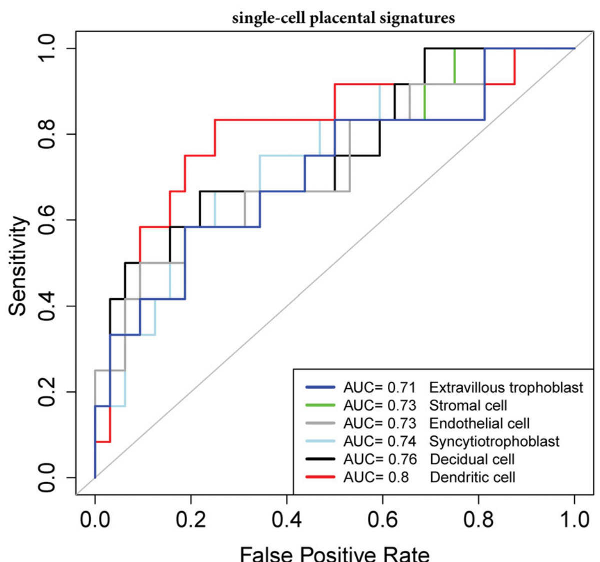

Figure 4. Prediction of early preeclampsia at 11–17 weeks of gestation by single-cell placental signatures in maternal whole blood. The area under the ROC curves (AUC) is shown in the legend. See Table 3 for other prediction performance metrics.

28–32 weeks and 22–28 weeks of gestation, compared to controls (all, $p < .05$). (2) A combination of 4 genes from this signature, including *H19*, a long nonprotein coding RNA, *FN1*, *TUBB6*, and *FPR3* had a sensitivity of 0.85 (0.55–0.98) and a specificity of 0.92 (0.8–0.98) for prediction of early preeclampsia at 22–28 weeks of gestation. (3) *H19, FN1*, and *TUBB6* were also separately increased in women with early preeclampsia as early as 11–17 weeks of gestation (all $p < .05$). (4) After diagnosis at 32–34 weeks, but also earlier at 11–17 weeks prior to diagnosis, women destined to have early preeclampsia showed a coordinated increase in whole blood expression of several *single-cell placental signatures*, including the 20-gene extravillous trophoblast signature (all, $p < .05$). (5) A combination of three mRNAs from the extravillous trophoblast signature (*MMP11, SLC6A2*, and *IL18BP*) predicted early preeclampsia at 11–17 weeks of gestation with a sensitivity of 0.83 (0.52–0.98) and specificity of 0.94 (0.79–0.99). Collectively, these findings

Table 3. Prediction performance for early preeclampsia at 11–17 weeks of gestation by cellular mRNA signatures defined by Tsang et al. [26]

Predictor	Sensitivity	Specificity	Likelihood ratio (+)	Likelihood ratio (−)
Syncytiotrophoblast signature	0.67 (0.35–0.9)	0.75 (0.57–0.89)	2.7 (1.3–5.5)	0.44 (0.19–1.01)
Dendritic cell signature	0.83 (0.52–0.98)	0.75 (0.57–0.89)	3.3 (1.7–6.4)	0.22 (0.06–0.8)
Stromal cell signature	0.58 (0.28–0.85)	0.81 (0.64–0.93)	3.1 (1.3–7.4)	0.51 (0.26–1.02)
Decidual cell signature	0.67 (0.35–0.9)	0.78 (0.6–0.91)	3 (1.4–6.6)	0.43 (0.19–0.97)
Endothelial cell signature	0.58 (0.28–0.85)	0.81 (0.64–0.93)	3.1 (1.3–7.4)	0.51 (0.26–1.02)
Extravillous trophoblast signature	0.58 (0.28–0.85)	0.81 (0.64–0.93)	3.1 (1.3–7.4)	0.51 (0.26–1.02)
Reduced extravillous trophoblast signature*				
MMP11 + *SLC6A2* + *AIF1L*	0.67 (0.35–0.9)	0.97 (0.84–1)	21.3 (3–153)	0.34 (0.15–0.77)
MMP11 + *SLC6A2* + *IL18BP*	0.83 (0.52–0.98)	0.94 (0.79–0.99)	13.3 (3.4–52.2)	0.18 (0.05–0.63)

*A parsimonious set of 3 mRNAs part of the original extravillous trophoblast signature identified herein by linear discriminant analysis.

indicate that early preeclampsia can be predicted before clinical manifestation, using information from the maternal cellular transcriptome.

Importance/significance of the study

Despite advances in prediction [45–47] and treatment [2] of preeclampsia, molecular markers that can predict early in pregnancy all women at risk of the different phenotypes of this syndrome are still lacking. Although most widely used maternal blood biochemical markers of preeclampsia are proteins, such as placental growth factor and pregnancy-associated plasma protein [48], transcriptomics has always been an important tool in understanding the etiology of preeclampsia [7,49–54]. The challenge of finding reliable transcriptomic markers of preeclampsia in maternal circulation was approached by different investigators using various strategies, as follows:

Untargeted transcriptomics approach to discover maternal blood biomarkers of preeclampsia

Our group previously compared the cellular transcriptome in maternal circulation at the time of diagnosis of preeclampsia to that of women who had a normal pregnancy, using gene expression microarrays [5]. This approach identified 18 genes increased in expression in early preeclampsia at the time of disease diagnosis, and four of these (*ANKRD28, ECT2*, *TSHB*, and *EMP1*) were also elevated in women with late preeclampsia. We, therefore, hypothesized that this mRNA signature may also have predictive value prior to clinical manifestation of the disease. Of note, of the genes included in this signature, *FOSB* [55,56], *FN1* [57,58], *VZIG4* [59], and *ANKRD28* [55] were also reported to be increased in placental tissue or in the maternal circulation of women with preeclampsia.

Placenta-targeted transcriptomics approach to discover maternal blood biomarkers of preeclampsia

Since preeclampsia is thought to be a disease of the mother, fetus, and placenta [3,25,60], we and other investigators sought to focus the search for transcriptomic markers of preeclampsia to placental-specific transcripts [25] or to those found to be dysregulated in the placental tissues at the time of delivery with preeclampsia [61]. Placental-specific transcripts are often defined based on higher placental expression compared to expression in other tissues [25,62], using the BioGPS Gene Expression Atlas (http://biogps.org). Yet, a novel approach to define placental specific transcripts has emerged based on the work of Tsang et al. [26] who used single-cell RNA sequencing in placental tissues from women with a normal pregnancy and those affected by early preeclampsia. The authors identified mRNAs highly expressed in subpopulations of cells, including some akin to extravillous trophoblasts, villous cytotrophoblasts and syncytiotrophoblast, decidual-, dendritic-, endothelial-, T-, and B-cells among others. Tsang et al. [26] reported that the maternal plasma expression of such single-cell signatures are modulated with gestational age and, hence, could be of value for noninvasive placental function monitoring. Interestingly, using 4 different transcriptomics platforms [63], we have shown that the gestational age-related trajectories of T-cell and B-cell signatures were similar in maternal whole blood (cellular RNA) to those reported in plasma (cell-free RNA). Since the extravillous trophoblast single-cell signature was shown to be significantly increased in the plasma of women diagnosed with early preeclampsia, we hypothesized that the cellular RNA counterpart could also be increased not only at the time of diagnosis but also earlier in gestation. Other studies have implicated mRNAs part of the extravillous trophoblast single-cell signature in the pathophysiology of preeclampsia, including *INHA* [56,64–67], *PAPPA2*

[56,64,66,68], *MMP11* [56], *HLAG* [55], *EGLN3* [55], and *ITGA5* [55,58].

Prediction of early preeclampsia by previously derived mRNA signatures

The result that our previously derived cellular mRNA signature of early preeclampsia [5] was increased in women already diagnosed with preeclampsia at 32–34 weeks of gestation confirms our initial report, yet the fact that this signature has predictive value as early as 22–28 weeks is novel. Although the average expression of all genes part of this signature was not significantly increased before 22 weeks, individual genes in this signature (*H19, FN1*, and *TUBB6*) were significantly increased in expression in women with early preeclampsia as early as 11–17 weeks of gestation. Of note, both *FN1* and *H19* were reported to have increased expression in the placental tissue of women with preeclampsia [69,70], while the latter was proposed to reduce cell viability but promote invasion and autophagy in trophoblast cells along with the activation of the PI3K/AKT/mTOR pathways [70].

The increase in circulating cellular expression of extravillous trophoblast-specific mRNAs in early preeclampsia is consistent also with the overall increase of extravillous trophoblast-specific gene expression in placenta [25]. The increase in extravillous trophoblast-specific mRNA expression with preeclampsia in maternal plasma [26] suggest that either (1) our whole blood gene expression quantification includes both cellular and cell-free mRNA fractions, (2) placenta-derived microparticles are contained by maternal white blood cells and, therefore, we can detect their expression in the circulation, or (3) placental-derived cells or microparticles release cell-free RNA into the circulation, which is conjugated to the maternal white blood cells. While Tsang et al. [26] demonstrated a significant plasma increase for the extravillous trophoblast single-cell signature at the time of disease diagnosis and reported only a trend of increase for decidual and endothelial single-cell signatures, the cellular transcriptome analysis herein revealed strong evidence that these and other single-cell placental signatures are increased at the time of and prior to the diagnosis of early preeclampsia. The prediction performance of these signatures at 11–17 weeks was similar (Figure 4). However, unlike with the untargeted cellular mRNA signature that showed a significant increase with early preeclampsia in 3 consecutive gestational-age intervals (Figure 1), the changes in placental single-cell signatures were observed only at 11–17 weeks and at 32–34 weeks after diagnosis (Figure 3). This observation is in line with our previous study demonstrating that the earliest gestational age when dysregulation of placenta-specific gene expression can be detected in the maternal circulation at the protein level is at 11–12 weeks after the opening of the plugged spiral arteries and the start of the maternal circulation of the placenta. Further studies will be needed to determine whether this window of gestation centered at the end of the first trimester (11–17 weeks) represents a particular phase in the development of early preeclampsia when more placental transcripts are released into the circulation than at any other time prior to clinical manifestation.

Strengths and limitations

Although gene expression measurements in this study were performed using genome-wide state-of-the-art microarrays, this study primarily involved a targeted hypothesis testing of two mRNA signatures for prediction of early preeclampsia in samples collected longitudinally prior to clinical manifestation and diagnosis. The moderate sample size and frequent sampling allowed us to pinpoint the timing when mRNA signatures in early preeclampsia diverge from the level expected in controls. An additional strength of this study is the availability of data on 1125 maternal plasma proteins collected from the longitudinal samples of all cases and from 39/49 normal pregnancies included herein (see Tarca et al. [71]). Among the protein changes previously implicated in the pathophysiology of preeclampsia, also reported in Tarca et al. [71], we noted the decrease in placental growth factor (PlGF) and the increase in sialic acid-binding immunoglobulin-like lectin 6 (siglec-6), fibronectin, and angiopoietin-1, among others (see Table S2 in Tarca et al. [71]).

Other important strengths of this work are attributed to the analytical aspects since data preprocessing and analysis were conducted to ensure that microarray batch effects and gestational age were not confounding factors, and that differences in expression variability across genes are accounted for when computing signature expression summaries.

Although the findings herein provided stronger evidence of an association between the cellular placental-cell mRNA signatures and early preeclampsia compared to what was presented by Tsang et al. [26] in maternal plasma, a direct evaluation of the best approach (cellular versus cell-free transcriptomics) would require both analyses to be conducted in the same set of blood samples. Among limitations, we

acknowledge the potential confounding effect of treatment, as 12/13 cases received $MgSO_4$, 3/13 received labetalol, and 2/13 received hydralazine and labetalol. However, such effects may have confounded only the changes observed in the maternal blood transcriptome after diagnosis, when treatment began, but not the changes reported herein based on data collected prior to diagnosis.

Summary

We characterized the cellular transcriptomic changes in maternal circulation associated with early preeclampsia prior to and at the time of disease diagnosis. We demonstrated that circulating early transcriptomic markers for preeclampsia can be identified either by untargeted profiling of the cellular transcriptome in samples collected at the time of diagnosis or by focusing on placental single-cell mRNA signatures dysregulated in plasma at the time of diagnosis. The *untargeted cellular mRNA signature* was consistently increased in early preeclampsia after 22 weeks of gestation, with individual mRNAs of this signature being increased as early as 11–17 weeks. Several *single-cell placental signatures* predicted future development of the disease at 11–17 weeks and were also increased in women already diagnosed at 32–34 weeks. Future studies are needed to determine which individual mRNAs or combinations thereof can add value to current prediction models so that more patients can benefit from treatment.

Disclosure statement

No potential conflict of interest was reported by the authors. Dr. Romero has contributed to this work as part of his official duties as an employee of the United States Federal Government.

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References

[1] Hassan SS, Romero R, Vidyadhari D, et al. Vaginal progesterone reduces the rate of preterm birth in women with a sonographic short cervix: a multicenter, randomized, double-blind, placebo-controlled trial. Ultrasound Obstet Gynecol. 2011;38(1):18–31.

[2] Poon LC, Wright D, Rolnik DL, et al. Aspirin for evidence-based preeclampsia prevention trial: effect of aspirin in prevention of preterm preeclampsia in subgroups of women according to their characteristics and medical and obstetrical history. Am J Obstet Gynecol. 2017;217(5):585.e1–585.e5.

[3] Brosens I, Pijnenborg R, Vercruysse L, et al. The "Great Obstetrical Syndromes" are associated with disorders of deep placentation. Am J Obstet Gynecol. 2011;204(3):193–201.

[4] Audibert F, Boucoiran I, An N, et al. Screening for preeclampsia using first-trimester serum markers and uterine artery Doppler in nulliparous women. Am J Obstet Gynecol. 2010;203(4):383.e1–383.e8.

[5] Chaiworapongsa T, Romero R, Whitten A, et al. 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 preeclampsiaa. J Perinat Med. 2013;41(5):485–504.

[6] Madan I, Than NG, Romero R, et al. The peripheral whole-blood transcriptome of acute pyelonephritis in human pregnancya. J Perinat Med. 2014;42(1):31–53.

[7] Romero R, Espinoza J, Gotsch F, et al. The use of high-dimensional biology (genomics, transcriptomics, proteomics, and metabolomics) to understand the preterm parturition syndrome. BJOG Int J Obstet Gynaecol. 2006;113(3):118–135.

[8] Bujold E, Romero R, Kusanovic JP, et al. Proteomic profiling of amniotic fluid in preterm labor using two-dimensional liquid separation and mass spectrometry. J Matern Fetal Neonatal Med. 2008;21(10):697–713.

[9] Romero R, Kusanovic JP, Gotsch F, et al. Isobaric labeling and tandem mass spectrometry: a novel approach for profiling and quantifying proteins differentially expressed in amniotic fluid in preterm labor with and without intra-amniotic infection/inflammation. J Matern Fetal Neonatal Med. 2010;23(4):261–280.

[10] Auer J, Camoin L, Guillonneau F, et al. Serum profile in preeclampsia and intra-uterine growth restriction revealed by iTRAQ technology. J Proteomics. 2010;73(5):1004–1017.

[11] Kacerovsky M, Lenco J, Musilova I, et al. Proteomic biomarkers for spontaneous preterm birth: a systematic review of the literature. Reprod Sci. 2014;21(3):283–295.

[12] Anand S, Bench Alvarez TM, Johnson WE, et al. Serum biomarkers predictive of pre-eclampsia. Biomarkers Med. 2015;9(6):563–575.

[13] Kolialexi A, Mavreli D, Tounta G, et al. Urine proteomic studies in preeclampsia. Prot Clin Appl. 2015; 9(5–6):501–506.

[14] Erez O, Romero R, Maymon E, et al. The prediction of late-onset preeclampsia: results from a longitudinal proteomics study. PLoS One. 2017;12(7):e0181468.

[15] Kolialexi A, Tsangaris GT, Sifakis S, et al. Plasma biomarkers for the identification of women at risk for early-onset preeclampsia. Expert Rev Proteomics. 2017;14(3):269–276.

[16] Romero R, Mazaki-Tovi S, Vaisbuch E, et al. Metabolomics in premature labor: a novel approach to identify patients at risk for preterm delivery. J Matern Fetal Neonatal Med. 2010;23(12):1344–1359.

[17] Bahado-Singh RO, Akolekar R, Mandal R, et al. First-trimester metabolomic detection of late-onset preeclampsia. Am J Obstet Gynecol. 2013;208(1): 58.e1–58.e7.

[18] Kuc S, Koster MP, Pennings JL, et al. Metabolomics profiling for identification of novel potential markers in early prediction of preeclampsia. PLoS One. 2014; 9(5):e98540.

[19] Austdal M, Tangerås LH, Skråstad RB, et al. First trimester urine and serum metabolomics for prediction of preeclampsia and gestational hypertension: a prospective screening study. IJMS. 2015;16(9): 21520–21538.

[20] Baraldi E, Giordano G, Stocchero M, et al. Untargeted metabolomic analysis of amniotic fluid in the prediction of preterm delivery and bronchopulmonary dysplasia. PLoS One. 2016;11(10):e0164211.

[21] Erez O, Romero R, Espinoza J, et al. The change in concentrations of angiogenic and anti-angiogenic factors in maternal plasma between the first and second trimesters in risk assessment for the subsequent development of preeclampsia and small-for-gestational age. J Matern Fetal Neonatal Med. 2008;21(5): 279–287.

[22] Aggarwal S, Sunderland N, Thornton C, et al. A longitudinal analysis of angiotensin II type 1 receptor antibody and angiogenic markers in pregnancy. Am J Obstet Gynecol. 2017;216(2):170.e1–170.e8.

[23] Than NG, Romero R, Xu Y, et al. Evolutionary origins of the placental expression of chromosome 19 cluster galectins and their complex dysregulation in preeclampsia. Placenta. 2014;35(11):855–865.

[24] Ngo TTM, Moufarrej MN, Rasmussen MH, et al. Noninvasive blood tests for fetal development predict gestational age and preterm delivery. Science. 2018; 360(6393):1133–1136.

[25] Than NG, Romero R, Tarca AL, et al. Integrated systems biology approach identifies novel maternal and placental pathways of preeclampsia. Front Immunol. 2018;9:1661.

[26] Tsang JCH, Vong JSL, Ji L, et al. Integrative single-cell and cell-free plasma RNA transcriptomics elucidates placental cellular dynamics. Proc Natl Acad Sci USA. 2017;114(37):E7786–E7795.

[27] Moldenhauer JS, Stanek J, Warshak C, et al. The frequency and severity of placental findings in women with preeclampsia are gestational age dependent. Am J Obstet Gynecol. 2003;189(4):1173–1177.

[28] Sebire NJ, Goldin RD, Regan L. Term preeclampsia is associated with minimal histopathological placental features regardless of clinical severity. J Obstet Gynaecol J Inst Obstet Gynaecol. 2005;25(2):117–118.

[29] van der Merwe JL, Hall DR, Wright C, et al. Are early and late preeclampsia distinct subclasses of the disease – what does the placenta reveal? Hypertens Preg. 2010;29(4):457–467.

[30] Ogge G, Chaiworapongsa T, Romero R, et al. Placental lesions associated with maternal underperfusion are more frequent in early-onset than in late-onset preeclampsia. J Perinat Med. 2011;39(6):641–652.

[31] ACOG Committee on Practice Bulletins–Obstetrics. ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. Obstet Gynecol. 2002;99(1):159–167.

[32] von Dadelszen P, Magee LA, Roberts JM. Subclassification of preeclampsia. Hypertens Preg. 2003;22(2):143–148.

[33] Alexander GR, Himes JH, Kaufman RB, et al. A United States national reference for fetal growth. Obstet Gynecol. 1996;87(2):163–168.

[34] Irizarry RA, Hobbs B, Collin F, et al. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics. 2003; 4(2):249–264.

[35] Carvalho BS, Irizarry RA. A framework for oligonucleotide microarray preprocessing. Bioinformatics. 2010; 26(19):2363–2367.

[36] Gentleman RC, Carey VJ, Bates DM, et al. Bioconductor: open software development for computational biology and bioinformatics. Genome Biol. 2004;5(10):R80.

[37] Smyth GK. Limma: linear models for microarray data. In: Gentleman R, Carey VJ, Huber W, editors. Bioinformatics and computational biology solutions using R and bioconductor. New York (NY): Springer; 2012.

[38] Hagenauer MH, Schulmann A, Li JZ, et al. Inference of cell type content from human brain transcriptomic datasets illuminates the effects of age, manner of death, dissection, and psychiatric diagnosis. PLoS One. 2018;13(7):e0200003.

[39] Tarca AL, Than NG, Romero R. Methodological approach from the best overall team in the sbv IMPROVER Diagnostic Signature Challenge. Syst Biomed. 2013;1(4):217–227.

[40] Tarca AL, Lauria M, Unger M, et al. Strengths and limitations of microarray-based phenotype prediction: lessons learned from the IMPROVER Diagnostic Signature Challenge. Bioinformatics. 2013;29(22): 2892–2899.

[41] Dayarian A, Romero R, Wang Z, et al. Predicting protein phosphorylation from gene expression: top methods from the IMPROVER Species Translation Challenge. Bioinformatics. 2015;31(4):462–470.

[42] Gardosi J, Francis A. A customized standard to assess fetal growth in a US population. Am J Obstet Gynecol. 2009;201(1):25.e1–25.e7.

[43] Gómez O, Figueras F, Fernández S, et al. Reference ranges for uterine artery mean pulsatility index at 11–41 weeks of gestation. Ultrasound Obstet Gynecol. 2008;32(2):128–132.

[44] Arduini D, Rizzo G. Normal values of Pulsatility Index from fetal vessels: a cross-sectional study on 1556 healthy fetuses. J Perinat Med. 1990;18(3):165–172.

[45] Romero R, Nien JK, Espinoza J, et al. A longitudinal study of angiogenic (placental growth factor) and anti-angiogenic (soluble endoglin and soluble vascular endothelial growth factor receptor-1) factors in normal pregnancy and patients destined to develop preeclampsia and deliver a small for gestational age neonate. J Matern Fetal Neonatal Med. 2008;21(1):9–23.

[46] Rolnik DL, Wright D, Poon LCY, et al. ASPRE trial: performance of screening for preterm pre-eclampsia. Ultrasound Obstet Gynecol. 2017;50(4):492–495.

[47] O'Gorman N, Wright D, Poon LC, et al. Accuracy of competing-risks model in screening for pre-eclampsia by maternal factors and biomarkers at 11–13 weeks' gestation. Ultrasound Obstet Gynecol. 2017;49(6):751–755.

[48] Akolekar R, Etchegaray A, Zhou Y, et al. Maternal serum activin a at 11–13 weeks of gestation in hypertensive disorders of pregnancy. Fetal Diagn Ther. 2009;25(3):320–327.

[49] Matrisian LM, Wright J, Newell K, et al. Matrix-degrading metalloproteinases in tumor progression. Int Symp Princess Takamatsu Cancer Res Fund. 1994;24:152–161.

[50] Cohen T, Gitay-Goren H, Sharon R, et al. VEGF121, a vascular endothelial growth factor (VEGF) isoform lacking heparin binding ability, requires cell-surface heparan sulfates for efficient binding to the VEGF receptors of human melanoma cells. J Biol Chem. 1995;270(19):11322–11326.

[51] Chung JY, Song Y, Wang Y, et al. Differential expression of vascular endothelial growth factor (VEGF), endocrine gland derived-VEGF, and VEGF receptors in human placentas from normal and preeclamptic pregnancies. J Clin Endocrinol Metab. 2004;89(5):2484–2490.

[52] Lian IA, Toft JH, Olsen GD, et al. Matrix metalloproteinase 1 in pre-eclampsia and fetal growth restriction: reduced gene expression in decidual tissue and protein expression in extravillous trophoblasts. Placenta. 2010;31(7):615–620.

[53] Conrad KP, Rabaglino MB, Post Uiterweer ED. Emerging role for dysregulated decidualization in the genesis of preeclampsia. Placenta. 2017;60:119–129.

[54] Rabaglino MB, Post Uiterweer ED, Jeyabalan A, et al. Bioinformatics approach reveals evidence for impaired endometrial maturation before and during early pregnancy in women who developed preeclampsia. Hypertension. 2015;65(2):421–429.

[55] Luo S, Cao N, Tang Y, et al. Identification of key microRNAs and genes in preeclampsia by bioinformatics analysis. PLoS One. 2017;12(6):e0178549.

[56] Vaiman D, Miralles F. An integrative analysis of preeclampsia based on the construction of an extended composite network featuring protein–protein physical interactions and transcriptional relationships. PLoS One. 2016;11(11):e0165849.

[57] Kim MS, Yu JH, Lee MY, et al. Differential expression of extracellular matrix and adhesion molecules in fetal-origin amniotic epithelial cells of preeclamptic pregnancy. PLoS One. 2016;11(5):e0156038.

[58] van Uitert M, Moerland PD, Enquobahrie DA, et al. Meta-analysis of placental transcriptome data identifies a novel molecular pathway related to preeclampsia. PLoS One. 2015;10(7):e0132468.

[59] Textoris J, Ivorra D, Ben Amara A, et al. Evaluation of current and new biomarkers in severe preeclampsia: a microarray approach reveals the VSIG4 gene as a potential blood biomarker. PLoS One. 2013;8(12):e82638.

[60] Friedman SA, Taylor RN, Roberts JM. Pathophysiology of preeclampsia. Clin Perinatol. 1991;18(4):661–682.

[61] Várkonyi T, Nagy B, Füle T, et al. Microarray profiling reveals that placental transcriptomes of early-onset HELLP syndrome and preeclampsia are similar. Placenta. 2011;32:S21–S29.

[62] Zwemer LM, Nolin SL, Okamoto PM, et al. Global transcriptome dysregulation in second trimester fetuses with FMR1 expansions. Prenat Diagn. 2017;37(1):43–52.

[63] Tarca AL, Romero R, Xu Z, et al. Targeted expression profiling by RNA-Seq improves detection of cellular dynamics during pregnancy and identifies a role for T cells in term parturition. Sci Rep. 2019;9(1):848.

[64] Kleinrouweler CE, van Uitert M, Moerland PD, et al. Differentially expressed genes in the pre-eclamptic placenta: a systematic review and meta-analysis. PLoS One. 2013;8(7):e68991.

[65] Buckberry S, Bianco-Miotto T, Bent SJ, et al. Placental transcriptome co-expression analysis reveals conserved regulatory programs across gestation. BMC Genom. 2017;18(1):10.

[66] Brew O, Sullivan MH, Woodman A. Comparison of normal and pre-eclamptic placental gene expression: a systematic review with meta-analysis. PLoS One. 2016;11(8):e0161504.

[67] Yong HE, Melton PE, Johnson MP, et al. Genome-wide transcriptome directed pathway analysis of maternal pre-eclampsia susceptibility genes. PLoS One. 2015;10(5):e0128230.

[68] Kaartokallio T, Cervera A, Kyllönen A, et al. Gene expression profiling of pre-eclamptic placentae by RNA sequencing. Sci Rep. 2015;5(1):14107.

[69] Zhao M, Li L, Yang X, et al. FN1, FOS, and ITGA5 induce preeclampsia: abnormal expression and methylation. Hypertens Preg. 2017;36(4):302–309.

[70] Xu J, Xia Y, Zhang H, et al. Overexpression of long non-coding RNA H19 promotes invasion and autophagy via the PI3K/AKT/mTOR pathways in trophoblast cells. Biomed Pharmacother. 2018;101:691–697.

[71] Tarca AL, Romero R, Benshalom-Tirosh N, et al. The prediction of early preeclampsia: results from a longitudinal proteomics study. PLoS One. 2019;14(6):e0217273.