Cell Reports Medicine, Volume *1*

Supplemental Information

Discovery and Verification of Extracellular miRNA Biomarkers for Non-invasive Prediction of Pre-eclampsia in Asymptomatic Women

Srimeenakshi Srinivasan, Ryan Treacy, Tiffany Herrero, Richelle Olsen, Trevor R. Leonardo, Xuan Zhang, Peter DeHoff, Cuong To, Lara G. Poling, Aileen Fernando, Sandra Leon-Garcia, Katharine Knepper, Vy Tran, Morgan Meads, Jennifer Tasarz, Aishwarya Vuppala, Soojin Park, Clara D. Laurent, Tony Bui, Pike See Cheah, Rachael Tabitha Overcash, Gladys A. Ramos, Hilary Roeder, Ionita Ghiran, Mana Parast, The PAPR Study Consortium, Xandra O. Breakefield, Amir J. Lueth, Sharon R. Rust, Max T. Dufford, Angela C. Fox, Durlin E. Hickok, Julja Burchard, J. Jay Boniface, and Louise C. Laurent

Supplementary Figures and Supplementary Table Legends

Figure S1A. Enrollment Criteria	Figure S1B. Case/Control Selection Criteria

UCSD Placental Dysfunction Clinic
 	
Inclusion Criteria
• At least 18 years old and can provide informed consent
• Patient is planning a hospital delivery
• Singleton gestation
• Gestational age between 17w0d and 28w0d inclusive at time of enrollment
• Increased risk of placental dysfunction based on one or more of the following:
  • Abnormal serum analytes
  • PAPP-A <0.3 MoM (first trimester)
  • AFP >2.5 MoM (second trimester)
  • hCG>3.0 MoM (second trimester)
  • Inhibin > 2.0 MoM (second trimester)
  • Unconjugated estriol < 0.3 MoM (second trimester)
  • Previous adverse pregnancy outcome
  • Severe preeclampsia, eclampsia, or HELLP
  • Birthweight <5th percentile
  • Fetal loss, idiopathic or with known placental dysfunction
  • Placental abruption
  • Maternal comorbidity
  • Chronic hypertension requiring medication
  • Lupus or other autoimmune disease requiring medication
  • Chronic renal insufficiency
 	
Exclusion Criteria
• Has active or history of malignancy requiring major surgery or systemic chemotherapy
• Multiple gestation (including history of twin demise including reduction, spontaneous or elective)
 	
Sera Prognostics Repository
 	
Inclusion Criteria
• At least 18 years old and can provide informed consent
• Singleton gestation
• Gestational age between 17w0d and 28w0d inclusive at time of enrollment
 	
Exclusion Criteria
• Multiple gestation
• Known or suspected fetal anomaly
	UCSD Placental Dysfunction Clinic
	
	Case Selection Criteria
	• New-onset hypertension **OR**
	• Chronic hypertension and new-onset proteinuria **OR**
	• Chronic proteinuria and new-onset hypertension **OR**
	• New-onset or chronic hypertension and new-onset severe feature (elevated liver function tests (> 2x upper limit of the normal range, elevated Creatinine >1.2, low platelet count <100,000/uL, and/or intrauterine growth restriction <10th percentile).
	• New-onset or chronic proteinuria and new-onset severe feature (elevated liver function tests (> 2x upper limit of the normal range, elevated Creatinine >1.2, low platelet count <100,000/uL, and/or intrauterine growth restriction <10th percentile).
	
	Control Selection Criteria
	• No hypertensive disease
	
	Sera Prognostics Repository
	
	Case Selection Criteria
	• New-onset hypertension with or without severe features **OR**
	• Superimposed preeclampsia with or without severe features
	
	Control Selection Criteria
	• No hypertensive disease

Figure S1: Enrollment and Selection Criteria. Related to Figure 1. **A.** Enrollment Criteria. **B.** Selection Criteria for Cases and Controls.

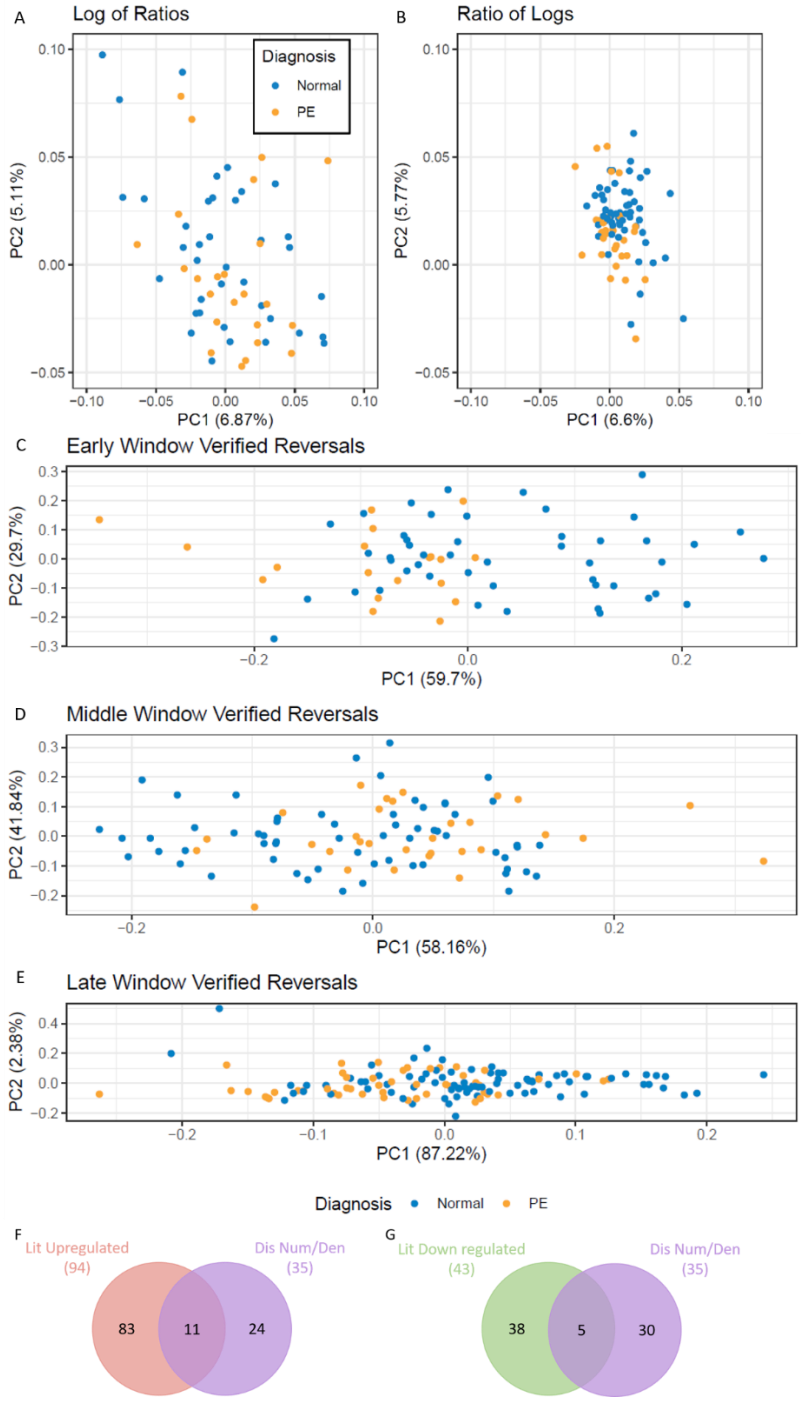

Figure S2. Principal Component Analysis Plots. Related to Table 3. **A-B.** Principal Component Analysis for the extracellular miRNA data (all possible reversals) using the log values of the ratios (**A**) or the ratios of the log values (**B**) as the features. **C-E. Principal Component Analysis of Verified Reversals.** Principal component analysis was performed on normalized reversal scores for verified reversals calculated for both Discovery and Verification subjects. **C.** Early GABD window. **D.** Middle GABD window. **E.** Late GABD window. All PCA plots show unsupervised clustering of preeclampsia cases (gold) and non-case controls (blue). **F-G. Overlap in Results from Our Study with Prior Literature.** Venn diagrams indicating the overlap between extracellular miRNA predictors identified in our study (Lab's Num/Den) with extracellular miRNA biomarkers identified in previous studies to be expressed higher in preeclampsia than control (Lit-upregulated, **F**) or lower in preeclampsia than control (Lit-downregulated, **G**).

Table S3. Tally of Candidate Univariate Predictors and Reversals Selected in Discovery and Passing Verification. Related to Table 3.

	Univariate Analysis		Bivariate Analysis	
GABD window (days)	**#Pass-filter Discovery, χ^2 p<0.05**	**#Pass-filter Verification (Lower 95% CI AUC > 0.5)**	**#Selected Discovery**	**#Pass-filter Verification (Lower 95% CI AUC > 0.5)**
Full (119-202)	14	0	50	1
Early (119-152)	14	1	50	4
Middle (138-172)	11	1	50	2
Late (156-196)	21	0	50	23