

RNA profiles reveal patterns of circadian rhythm in pregnancy

Arkady Khodursky PhD, Mitsu Reddy PhD, Tiffany Brundage BSc, Maneesh Jain PhD, Eugeni Namsaraev PhD, Carrie Haverty MS CGC, Morten Rasmussen PhD

OBJECTIVE

Physiological systems vary from day to night in response to differing biological demands. In various nonpregnant populations, these changes may be reflected in RNA expression patterns measured in blood samples. This study sought to identify whether time of blood draw and circadian rhythm can be revealed through cell-free RNA analysis of maternal blood plasma in a pregnant population.

STUDY DESIGN

This was a prospective, observational study of a cell-free RNA platform utilizing direct-to-participant recruitment efforts from July 2020 to December 2021. The IRB-approved study was open to patients ages 18-45 with a singleton pregnancy in the United States.

Samples were grouped by time of non-fasting blood draw into morning (6am-10am, n=140), midday (10am-2pm, n=349) and afternoon (2pm-6pm, n=120). We ran differential gene expression (DGE) analyses to test the impact of time of day. A negative binomial model was fit to the count data using the edgeR package and DGE were discovered with an empirical Bayes quasi-likelihood F-test in all 3 possible pairwise comparisons.

Cell-free RNA captures daily biological rhythm in pregnancy

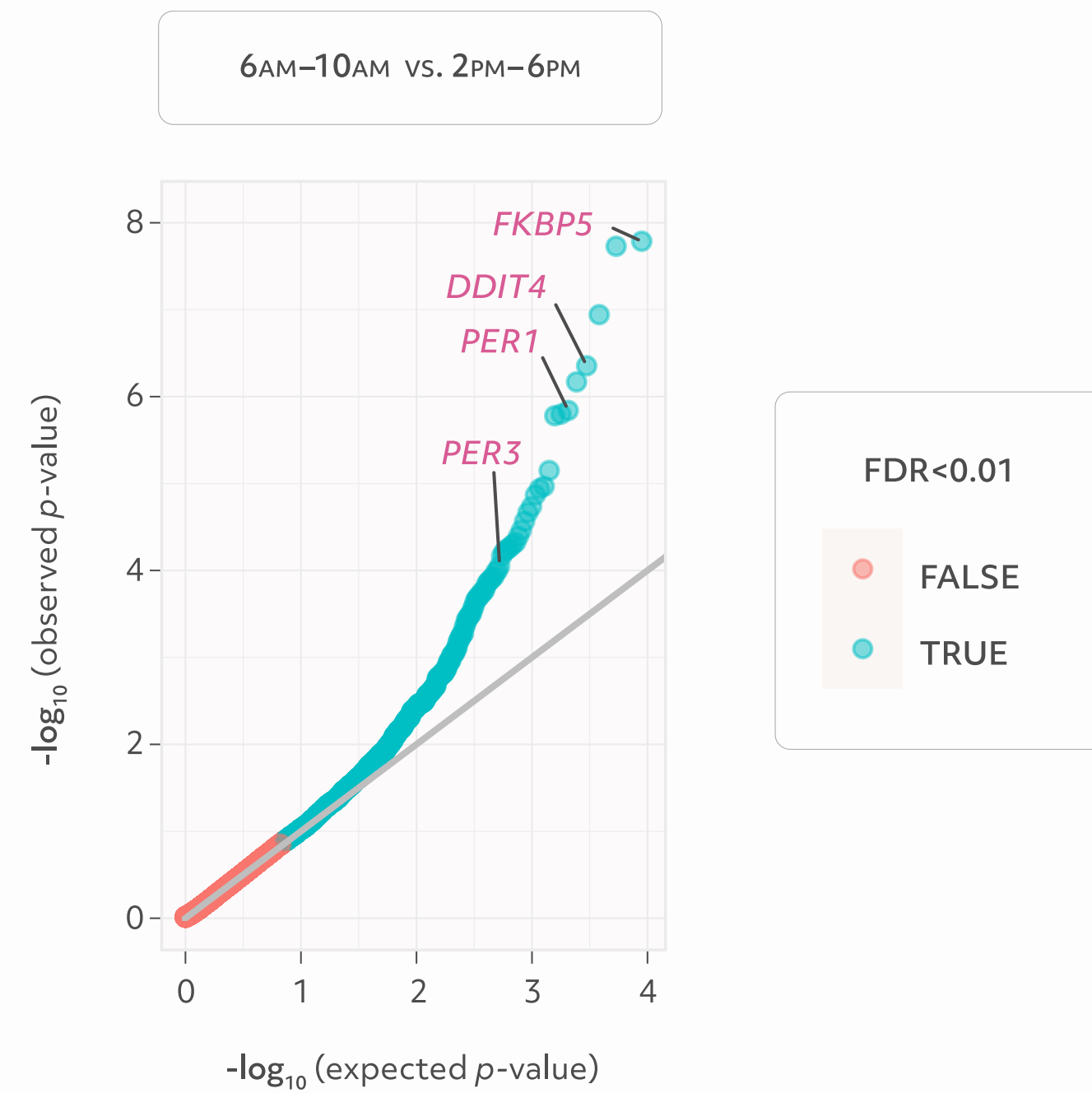


Figure 1: Q/Q Plot demonstrating differential gene expression between the morning blood draw and afternoon blood draw cohorts.

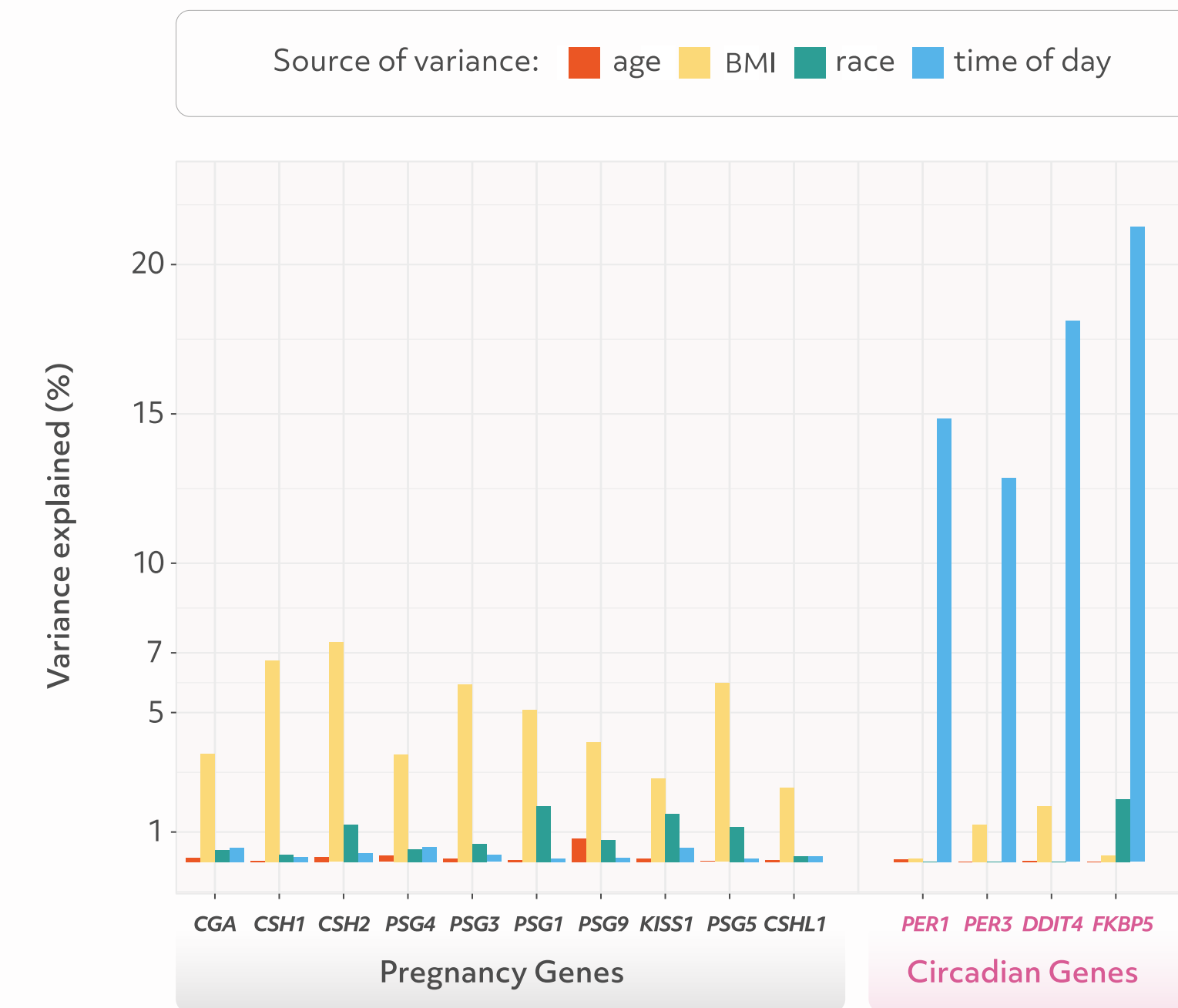


Figure 2: Proportion of variance explained in top 10 differentially expressed pregnancy genes and 4 circadian genes, by maternal age (red), maternal prepregnancy BMI (yellow), maternal race - Black or non-Black (green), or time of day (blue).

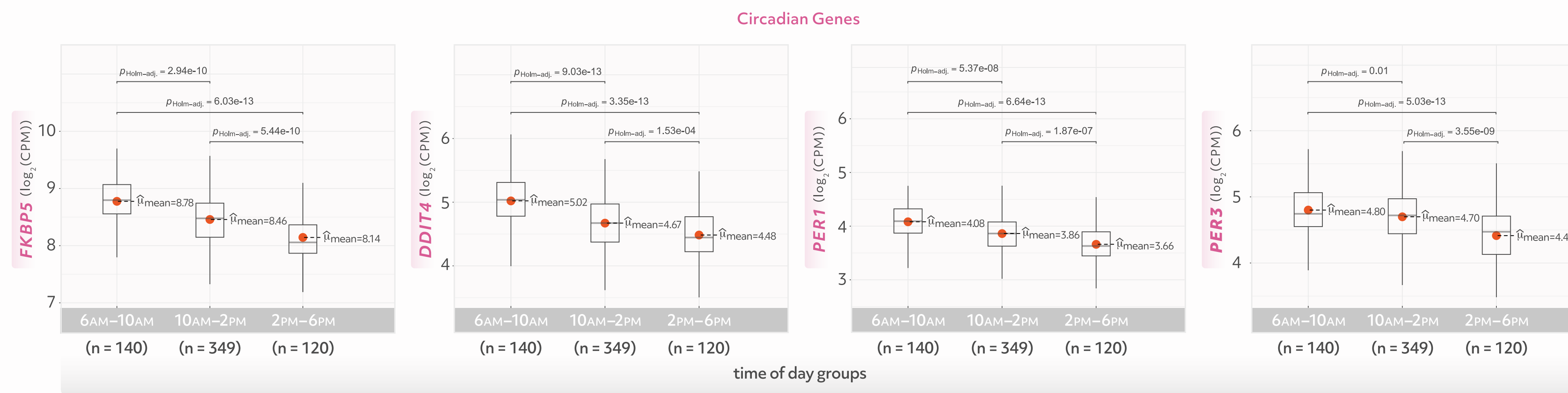


Figure 3: Expression of key circadian genes across time of day blood draws. CPM, counts per million.

RESULTS

When looking for DGE between the morning and afternoon groups we identify 1,858 genes (figure 1) or 14% of all analyzed genes as significantly differentially expressed. For morning vs midday the number is 2,167 (16%) and finally midday vs afternoon has 18 (0.1%) DGE. The three sets have very high overlap, ranging from 31% - 86% ($p < 10^{-10}$), and among the genes with highest separation we find those reported with circadian rhythm (*PER1*, *PER3*, *DDIT4*, *FKBP5*) (figure 3). When looking at top differentially expressed pregnancy genes, time of day explains less than 1% of variance unlike BMI which explains up to 7%, aligned with reported correlation to fetal fraction in blood (figure 2). Time of day is similar to the variance due to maternal age or race and much less than that attributed to BMI.

CONCLUSION

The cell-free RNA platform revealed that time of sample collection has a specific impact on RNA expression patterns. This is the first evidence of this phenomenon demonstrated in a pregnant population, and particularly the morning group is differentiated from later blood draws. The effect of circadian rhythm is an important consideration in current and future development of biomarkers making the time of sample collection an important variable to collect and analyze.

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