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## Title: Differences in prenatal psychological distress and hair cortisol concentration associations with placental methylation: evidence of sensitive periods.

Background. Epigenetic characterization can elucidate how maternal prenatal stress contributes to offspring physiology and behavior. Maternal prenatal psychological distress (PD) did not predict cord blood methylation in a recent Epigenome-Wide Association Study (EWAS), but maternal lifetime stress predicted differential methylation in placental tissue. Maternal hair cortisol concentrations (HCC) are a plausible biological mechanism of maternal PD influence on fetal development, however, mothers' self-reported PD during pregnancy is inconsistently linked to her HCC and to date, HCC has not been examined in relation to placental methylation.

Objective. To test whether methylation in placental tissue is associated with PD and HCC, and whether methylation at the same sites are associated with birthweight.

Design/methods. Women aged 18+ who were <12 weeks pregnant were enrolled (n=34) and completed questionnaires and provided hair samples for HCC at 12 (T1), 26 (T2), and 38 (T3) weeks of pregnancy. At birth, placentas (n=28) were collected, dissected (maternal and fetal side), and assayed for methylation on the Illumina EPIC array. We examined correlations of PD (i.e., pregnancy-related anxiety) with HCC at each trimester. Differential methylation analyses tested a) maternal vs. fetal side methylation differences, and b) associations of PD, HCC, and birthweight (n=26) with each CpG site (EWAS).

Results. Maternal and fetal sides were differentially methylated (fetal-maternal side comparison showed hypomethylation at 6,604 sites and hypermethylation at 15,559 sites). PD and HCC were uncorrelated across pregnancy; neither was associated with lower birthweight. T2 HCC was associated with the most differential methylation of the maternal side – at 4,493 sites (Fig 1; T2: fetal side=96, T1: maternal=43, fetal=77; T3: maternal=15, fetal=1). T1 PD was associated with the most differential methylation of the fetal side – at 19,418 sites (Fig. 1; T1: maternal side=17; T2: fetal=15,755, maternal=3; T3: fetal=0, maternal=0). Placental methylation was related to birthweight (maternal=7,156; fetal=38; Fig. 2); 6 CpG site hits for T2 HCC were also related to birthweight. The top 10 gene ontologies significantly enriched for CpG site hits for T1 PD were primarily cellular components related to plasma membrane, but for T2 HCC were biological processes related to biosynthetic process generally, and negative regulation of aspects of biosynthetic processes. Sensitivity analyses also examined different measures of PD: the state-trait anxiety inventory assessing general anxiety, and the perceived stress scale - a general measure of stress. Neither of these measures were related to maternal or fetal side methylation. The effects found here are specific to anxiety about the pregnancy specifically.

Conclusions. This pilot study suggests that T2 may be a sensitive period for the influence of maternal HCC on placental methylation, particularly on the maternal side, whereas T1 pregnancy-related anxiety was most predictive of fetal side methylation. Pregnancy-related anxiety likely influences methylation through non-cortisol or non-stress-related pathways (e.g., immune, diet, or lifestyle changes in response to feeling anxiety about the pregnancy and upcoming life changes). Follow-up analyses will include probes of the functional relevance of significant CpG sites and differentially methylated regions.

Fig 1. Number of CpG site hits for hair cortisol concetrations across trimesters.

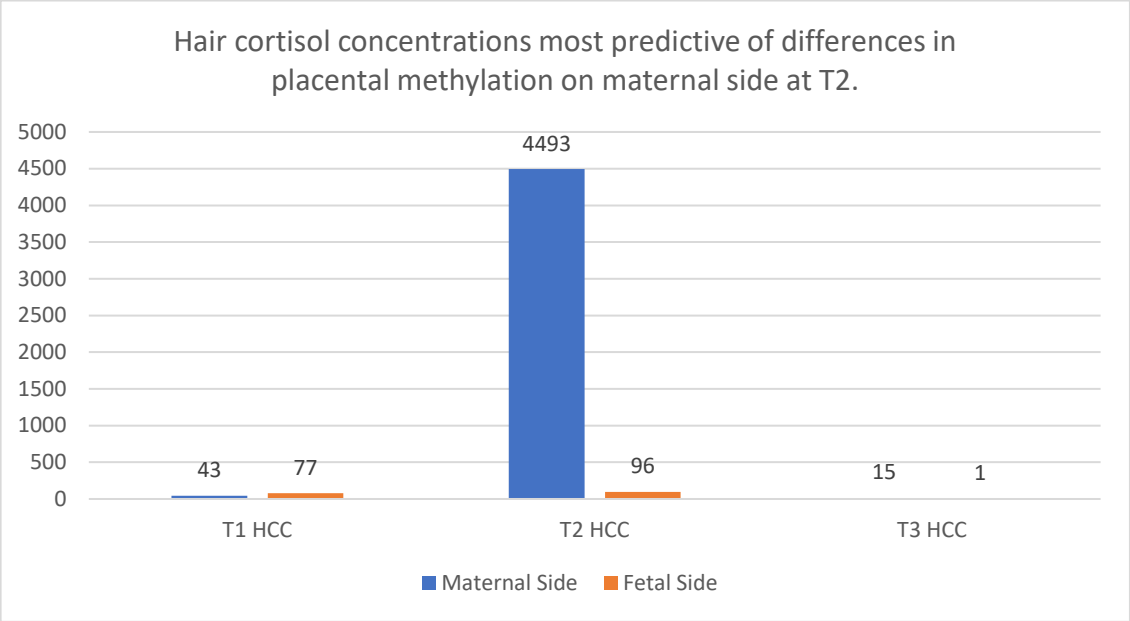


Fig 2. Number of CpG site hits for psychological distress across trimesters.