Role of a Preconception Maternal Nutrition Supplement and Pre-pregnancy BMI on Amnion DNA Methylation at Birth in Guatemalan Mother-Infant Dyads: The Women First Trial

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Objectives: Maternal nutrition can alter the offspring epigenome at birth. We sought to examine epigenome-wide DNA methylation (DNAme) from a subset of Guatemalan mother-infant dyads from the Women First Preconception Maternal Nutrition Trial (WF). Women were randomized to either: Arm 1) women consumed a daily maternal nutrition supplement (MNS) $\geq$ 3 months prior to conception until delivery; Arm 2) women consumed the same MNS starting at 12 weeks gestation until delivery; or Arm 3) no MNS. We tested if infant DNAme from amnion tissue at birth (N = 99) was associated with: 1) timing of exposure to maternal MNS; 2) pre-pregnancy body mass index (ppBMI); and 3) the interaction of maternal MNS and ppBMI.

Methods: Bisulfite-converted DNAme libraries were constructed using Roche NimbleGen SeqCap Epi CpGiant probes and were sequenced via 2 $\times$ 150 paired end reads. We assessed the relationship between Arm, ppBMI, and Arm x ppBMI interaction on CpG methylation. All statistical models adjusted for multiple testing using false discovery rate (FDR) and controlled for maternal age, infant sex, exposure to smoke, infant genetics, and cellular heterogeneity. Gene set enrichment analyses were performed via Enrichr.

Results: We identified 480 CpGs associated with Arm, 4 CpGs associated with ppBMI, and 22 CpGs associated with the interaction of Arm x ppBMI (FDR $<$ 0.05). Further, we found that DNAme was changed between Arms (1 vs 2, 1 vs 3). There were 300 CpGs that were different between Arms 1 and 2 and 159 CpGs that were different between Arms 1 and 3 that annotated to genes and passed FDR $<$ 0.05. These results suggest preconception consumption of maternal MNS elicits different epigenetic responses as compared to MNS commencing during gestation or not at all. In addition, CpGs that annotated to genes were enriched in pathways associated with growth, development, and metabolism that included circadian rhythm, TCA cycle, Wnt signaling, and melatonin metabolism.

Conclusions: Our findings indicate that maternal MNS was robustly associated with amnion DNAme at birth. More specifically, preconception MNS resulted in DNAme changes that differed from the other Arms in biologically relevant pathways suggesting timing of maternal nutrition impacts the fetal epigenome. Future studies will examine DNAme associated with birth outcomes.

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