Maternal Obesity During Pregnancy Impacts Pancreatic DNA Methylation and Protein Expression in the Offspring

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It has been postulated that DNA methylation is one of the mechanisms by which developmental programming causes long term, multi-generational impacts on the developing offspring. However, the role this molecular mechanism has in mediating the effect of maternal obesity on the offspring's pancreas is limited. We hypothesize that maternal obesity during pregnancy will result in changes to offspring pancreas DNA methylation that would be reflected as corresponding changes in protein expression. C57BL/6J (N= 6) female mice were were fed a high fat diet (HFD; n = 3; TD.07011 Pellet Envigo, Somerset, NJ) to induce obesity or a control diet (CON; n = 3) for fourteen weeks prior to breeding. These animals also remained on their treatment diets for the duration of pregnancy and lactation. Pups were euthanized at 8 wks of age and pancreas tissue was collected. Pancreas tissue DNA was isolated and whole genome bisulfite sequencing was performed at Genewiz (Illumina Hi-Seq; Azenta, Cambridge, MA). Data were analyzed in conjunction with CD genomics (New York, NY)**.** Read alignment to the *Mus musculus* reference genome (mm39) were performed using Bismark (v0.24.2; Babraham Bioinformatics). Differentially methylated regions (DMR) and the associated locations were determined using methylKit v3.18 (Bioconductor; $q \leq 0.05$). Proteomics analyses were performed via ultra-performance liquid chromatography-tandem mass spectrometry (Thermofisher Scientific Q Exactive HF mass spectrometer; University of Connecticut Proteomics and Metabolomics Facility, Storrs, CT). The number and identity of differentially expressed proteins were determined using the average precursor intensity in Scaffold v5.3.2 (Proteome Software, Inc; Portland OR)**.** From our analyses we were able to identify 41,515 and 44,958 DMR for CON vs HFD males and females respectively ($q \leq 0.05$). 30.23% of the top 25% of DMR were hypomethylated whereas 69.76% were hypermethylated in CON vs HFD females. Likewise, 39.61% of the DMR identified were hypomethylated and 60.39% of DMR were hypermethylated in CON vs HFD males. We were able to identify a subset of proteins in our treatment comparisons whos change in expression match the change in methylation status for that DMR. 3 targets (heparan sulfate proteoglycan 2, collagen type VI alpha 2 chain, and collagen type IV alpha 2) exhibited a reduction in DNA methylation that corresponded with an increase in protein expression in CON vs HFD males. 31 targets in the CON vs HFD males exhibited a reduction in protein expression that corresponded with increased DNA methylation of the DMR. For CON vs HFD females, Cathepsin A expression was reduced while there was increased DNA methylation at an associated DMR. Three proteins (immunoglobulin heavy constant gamma 1, isopentenyl-diphosphate delta isomerase 1, and glutamic-oxaloacetic transaminase 2) exhibited increased expression while having reduced DNA methylation at an associated DMR. In conclusion, we have demonstrated that there is a relationship between the

DMR identified and changes in the pancreatic protein expression of the offspring from HFD-fed dams. This multi-omic approach has provided valuable insight into the interplay between epigenetics, maternal diet, and the pancreas tissue of the offspring.