73  **Prenatal maternal stress and Shank3ex4-9 mutation alter hippocampal stratum radiatum white matter (WM) in male mice offspring**

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**OBJECTIVE:** To address whether prenatal maternal stress exposure interacts with an autism predisposition allele, we examined if maternal stress influences WM fractional anisotropy (FA) by diffusion tensor imaging (DTI), comparing exposed, cardially perfused with paraformaldehyde (PFA). The skull was removed in PFA and stored in 5mM gadopentate dimeglumine until imaging. Brains were imaged for DTI using 20 gradient directions and data processed using DTI Studio to yield FA volume maps. All brains were masked, upsampled and aligned to template mouse brain FA map. Brains were then segmented into left and right components for 29 regions and the mean FA values for the voxels computed. Two-way ANOVA was applied to a whole brain voxel-voxel and to the average FA values in regions of interest (ROI).

**RESULTS:** We analyzed WM tractography for genotype (G) (WT vs. KO) and environment (E) effect (NST vs. ST) and gene-environment interaction (GxE). In all, 15 ROIs had significant differences in FA between the tested groups as summarized below:

<table>
<thead>
<tr>
<th>Region of interest (ROI)</th>
<th>Genotype effect</th>
<th>Environment effect</th>
<th>Gene-environment interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stratum radiatum</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hippocampus-Right</td>
<td>0.048 ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Hippocampus-Left</td>
<td>0.043 ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Neocortex-Left</td>
<td>0.0919</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Neocortex-Right</td>
<td>0.0019</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Hypothalamus-Left</td>
<td>0.0011</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Hypothalamus-Right</td>
<td>0.0019</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Amygdala-Lobe</td>
<td>0.0235</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Amygdala-Right</td>
<td>0.0019</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Piriform Cortex-Right</td>
<td>0.0030</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Piriform Cortex-Left</td>
<td>0.0816</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Septum-Right</td>
<td>0.0012</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Septum-Left</td>
<td>0.0075</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Acumbens-nucleus-right</td>
<td>0.0451</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Acumbens-nucleus-left</td>
<td>0.00277</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

The stratum radiatum showed significant change in FA in WT vs. KO NST offspring and in WT NST vs. WT ST offspring. WT ST and KO NST offspring had similar changes, but stress did not have an additive effect on stratum radiatum FA in KO offspring.

**CONCLUSION:** We have identified a contiguous region (stratum radiatum) within the hippocampus whose FA is significantly altered by genotype and environmental stress interaction. This supports the hypothesis that prenatal maternal stress can influence the phenotype of autism-predisposing mutations in offspring.

74  **Epigenetic programming by maternal lactation status**

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**OBJECTIVE:** Maternal benefits of lactation include risk reduction of breast cancer, ovarian cancer, metabolic syndrome, hypertension, type 2 diabetes mellitus and cardiovascular disease. Our goal is to investigate epigenetic influences on pathways involved in diseases for which lactation is protective.

**STUDY DESIGN:** This study is nested from a prospective cohort study of healthy women over the first six months postpartum. Blood samples, demographic information, and detailed lactation history were obtained. DNA was extracted from preserved peripheral blood mononuclear cells of women who breastfed versus women who did not breastfeed. Illumina Human methylation 450K BeadChip was used to interrogate over 485,000 methylation sites. Quality control and differential methylation analyses were performed. The two groups were compared using t-test and Mann-Whitney U test with False Discovery Correction (FDR) in order to adjust for the multiple testing. To ensure that the observed change was biologically meaningful, a probe was considered significant if the difference in mean or median β-value between the two groups was larger than 0.2.

**RESULTS:** DNA methylation was performed on 24 samples in the fourth to sixth postpartum month comparing those who breastfed (n = 15) to those who did not breastfeed (n = 9). There was a low degree of missing values and all samples had a bimodal distribution indicating high quality data. Eight genes were significantly differentially methylated: AP2A2, GDF7, LDHC, MYT1L, PM20D1, PWP1, TNFRSF6B, TNNT1. AP2A2, MYT1L and GDF7 methylation modification occurred in the body of the gene and likely does not impact the gene expression. PM20D1 was significantly methylated but was race dependent. Examples of differentially methylated genes are shown in figure 1. The specific genes methylated in this study have previously been linked to conditions for which lactation is protective: PM20D1 - obesity, LDHC - breast and ovarian cancer, TNFRSF6B - breast and ovarian cancer, and TNNT1 - CVD prevention.

**CONCLUSION:** This is the first study to show an association between maternal lactation status and DNA methylation in humans. These results provide a potential mechanism explaining the relationship between lactation and prevention of chronic diseases.
75 Whole exome sequencing identifies rare variants implicated in preterm birth

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OBJECTIVE: To identify rare variants contained within or near the coding regions of genes that explain a significant portion of the risk for PTB.

STUDY DESIGN: Women of European ancestry who had previously experienced at least one PTB less than 36 weeks were recruited from Denmark. Whole exome sequencing was performed using the Complete Genomics platform (BGI, Shenzhen, China) on 192 samples representing 93 sister pairs and 2 sister triads. The majority (83%) of sister sets were comprised of all sisters experiencing a previous spontaneous PTB. Genes containing variants found to appear in all sisters in the same family were considered for further analyses. Variants with low quality or low coverage depth were excluded as were those with a minor allele frequency >0.5% in publically available genomic data sets including the 1,000 genomes project. Genes are prioritized for further follow-up based on statistical and/or biological significance.

RESULTS: We identified 2,353 genes that contained rare variants shared by the sister pair in at least 10 families. Combining pipelines and filtering for genes relevant to pregnancy and/or PTB we identified 15 promising candidate genes for further analysis. Of note were rare mutations in the HSPG2 gene. We found that 34 of the 95 families (35.8%) had one or more rare (<0.5% in 1,000 genomes) variants in the HSPG2 gene shared by all sisters in a particular family. Of the 38 distinct single nucleotide variants and 1 deletion identified in this gene, 2 are located in the 3′UTR, 15 are in the coding region of the gene and 22 are outside the coding region. Of the 15 variants within the coding region, one is a novel mutation, 1 is a synonymous mutation and the remainder are missense mutations.

CONCLUSION: Rare mutations in the HSPG2 gene may explain up to 36% of the preterm birth in our population, with rare potentially deleterious missense mutations explaining up to 11%. HSPG2 encodes the perlecan protein that is expressed in the placenta and has been identified as a potential amniotic fluid biomarker of premature rupture of the membranes. Further studies evaluating the frequency of these mutations in women with and without preterm birth are warranted.

76 Maternal obesity and ovarian gene expression in offspring

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OBJECTIVE: The role of maternal nutrition in fetal programming of obesity is well established and obesity is associated with polycystic ovary syndrome (PCOS). Our objective was to explore the effect of pre-pregnancy obesity on genes involved in ovarian function in offspring later in life.

STUDY DESIGN: CD1 mice were fed either high fat diet (34.9% fat, HF) or regular chow (5.8% fat, SF) for 3 months before breeding. After weaning, all pups were placed on a regular chow. Offspring were sacrificed at 1 month (mo), 3 mo, and 6 mo of age. Quantitative real time RT-PCR was used to measure expression of the following ovarian genes involved in cell metabolism, folliculogenesis, cell signaling, and ovulation: insulin receptor (CYP17IR), facilitated glucose transporter or GLUT1 (Slc2a1), low density lipoprotein receptor (LDLR), interleukin1 receptor (IL1R1), insulin like growth factor1 receptor (IGF1R), growth and differentiation factor 9 (GDF9), and luteinizing hormone receptor (LhcgR). Student t-test and one-way ANOVA were used for statistical analysis as appropriate (significance: P<0.05).

RESULTS: Offspring in the HF group were heavier at 6 mo of age, but not at 1 or 3 mo, compared with SF. At 3 mo, IGF1R (P=0.02) mRNA levels were higher and IL1R1 (P=0.02) lower in HF mice. 6mo old HF mice showed significantly lower expression of IGF1R (P<0.02, Figure), LDLR (P<0.01), and Slc2a1 (P<0.04). IL1R1 gene expression decreased significantly with age in HF ovaries, while it increased at 3mo and then decreased by 6mo in the SF group. LDLR expression increased significantly by 3mo and then decreased significantly by 6mo in both groups.

CONCLUSION: Maternal pre-pregnancy obesity alters ovarian gene expression in offspring long term. Our findings support a role for fetal programming in ovarian function and PCOS.