Choline supplementation in an adolescent nicotine exposure model- a whole genome epigenetic perspective.

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Background: Chronic nicotine exposure during adolescence produced persistent changes in neuronal function leading to learning deficits in adulthood. Choline supplementation reversed these deficits, but the mechanism involved is still unclear. We examined whether the action of choline as a methyl donor in DNA methylation may be involved; thus leading to changes in DNA methylation and subsequently changing gene expression.

Methods: Adolescent C57BL/6J mice were implanted with pumps delivering saline or nicotine. Then animals received either standard or choline diet and underwent fear conditioning testing. Dorsal hippocampi were extracted and DNA/RNA isolated. Methylated DNA Immunoprecipitation Sequencing was performed using ABI Solid™. The MEDIPS Bioconductor™ package was used for identification of Differentially Methylated Regions (DMRs). Enrichment for gene pathways was performed using PANTHER. Expression changes in selected genes were assessed by RT-PCR.

Results: Whole genome comparison of dorsal hippocampi revealed DMRs that were altered in nicotine exposed animals compared to controls and were reversed by choline supplementation. 453 of these DMRs were located in proximity (5kb of TSS) to brain expressed genes. Gene enrichment analysis showed the greatest enrichment for chromatin remodeling genes. qRT-PCR analysis in a subset of the chromatin remodeling gene category revealed, significant expression changes which were in ersely correlated with promoter methylation changes in a number of chromatin remodeling genes. Of which to note is *Smarca2,* a member of the neuronal specific SWI/SNF ATP dependent chromatin remodeling complex - BAF, which has been shown to participate and regulate differentiation of neuronal precursor cells to mature neurons both in vitro and in vivo. Genetic polymorphisms in SMARCA2 and other SMARC family members have been shown to be associated with Schizophrenia, Autism spectrum disorders and a possible association with alcohol use disorders.

Conclusions: To our knowledge this is the first study to examine whole genome epigenetic effects of choline supplementation after chronic nicotine exposure in adolescence. The findings that the main gene targets involved in choline's restorative function are chromatin remodeling genes, which by themselves serve as epigenetic regulators, points to a complex multi-layered mechanism of action. The elucidation and understanding of the interplay between these epigenetic systems and the identification of downstream targeted effectors can assist in better understanding of the mechanisms by which choline reverses deficits in adult cognition due to adolescent nicotine exposure.