M40. EFFECT OF CHRONIC TREATMENT WITH PSYCHIATRIC MEDICATIONS ARIPIPRAZOLE AND RILUZOLE ON DNA METHYLATION PROFILES IN THE RAT STRIATUM AND PREFRONTAL CORTEX

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Background One of the main epigenetic mechanisms is DNA methylation. It mostly occurs in CpG islands in the promoters by addition of a methyl group to a cytosine that may inhibit gene expression. It is known that many developmental disorders are characterized by alterations in epigenetic mechanisms. Moreover, studies in animal models of neuropsychiatric disorders have shown that epigenetics may play an important role in pathogenesis and therapy. The aim of this study is to investigate the effect of drugs used in different psychiatric diseases including Tourette Syndrome (TS) on DNA methylation to find epigenetic targets that may influence the efficacy of treatment.

Methods Treatment: Wistar Kyoto rats were chronically treated from post-natal day (PND) 35 to PND50 by intraperitoneal injection of either aripiprazole or riluzole or vehicle. Metabolite changes during the treatment were quantified by magnetic resonance spectroscopy in the striatum and prefrontal cortex.

Samples: DNA isolated from striatum and prefrontal cortex was used for methylation studies. Reduced Representation Bisulfite Sequencing (RRBS): RRBS is a high-throughput technique used to analyze genome-wide methylation profiles at the single nucleotide level. It provides information of the most relevant genomic regions which are likely to influence gene expression. We analyzed striatum and prefrontal cortex of 2 rats in each group treated with aripiprazole (a dopaminergic neuroleptic) or riluzole (a glutamatergic anxiolytic) or vehicle in order to identify DNA methylation changes due to these drug treatments.

Mass-Spectrometry: The analysis was performed by LC-MS/MS on striatum and prefrontal cortex in order to quantify the amount of 5-methyl-Cytosine (mC) and 5-HydroxyMethylCytosine (5hmC) after drug treatment.

Results Mass-Spectrometry: LC-MS/MS showed significant differences in 5hmC levels in prefrontal cortex after treatment with aripiprazole and riluzole. The same trend was also observed for mC levels. We found no differences in methylation or hydroxy-methylation levels in striatal samples.

RRBS: we observed differences in overall methylation levels between treatment using the two drugs and the vehicle. We identified differentially methylated CpG sites (DMS) and characterized their distribution across the genome (eg. promoter, intron, exon, UTR, etc.).

Treatment with riluzole led to identification of 156 DMS in striatum and 346 DMS in prefrontal cortex. Treatment with aripiprazole yielded 117 DMS in striatum and 466
DMS in prefrontal cortex. The identified CpG sites were annotated, a candidate gene list was created and pathway analysis was also performed on these genes. Interestingly, most of the changes occurred in intergenic regions far from TSS suggesting that enhancers are the regions most prone to undergo methylation changes. Moreover, several genes (eg. OXTR, DRD2, etc.) have been associated with brain disorders or functions.

**Discussion** The known functions of implicated genes suggest that some of the observed epigenetic changes might underlie the amelioration of symptoms by these drugs and some may account for certain adverse effects. Data on differential DNA methylation will be compared with imaging data on the respective brain regions. The results give insights into the mechanism of action of aripiprazole and riluzole as well as the side effects, not just in TS, but also in a broader context regarding these psychiatric medications.

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