Autism Spectrum Disorder (ASD): Interaction Between Sex and Epigenetic Factors on the Neuronal Transcriptome

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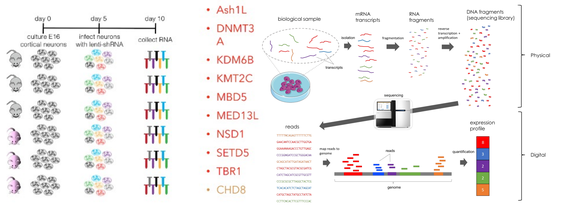
Introduction

Autism Spectrum Disorder (ASD) affect millions worldwide [1]. The cause of autism has been mainly attributed to genetic factors. Interestingly, males are more prone to ASD diagnosis than females, with a ratio of around 4.5:1. The cellular basis for this differential susceptibility remains unclear [2]. A recent study revealed many new chromatin-modifying ASD-risk genes [3]. Chromatin modifiers control the accessibility of the genome to transcription and fine-tune the expression of up to thousands of genes [4]. This study aims to investigate the interactions between chromatin modifications and the neuronal sex-specific transcriptome.

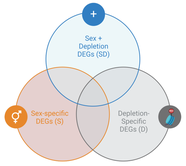
Methods

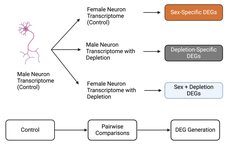
RNA-seq



E16 cortical neurons were cultured, infected with lentiviral shRNA targeting key chromatin-modifying genes. RNA-seq was then performed on the extracted transcriptome [5].

Additive & Suppressive Analysis





$$AdditiveDEGs = \{\forall g \mid g \in SD \wedge g \notin S \wedge g \notin D\}$$
$$SuppressiveDEGs = \{\forall g \mid g \in D \wedge g \notin S \wedge g \notin SD\}$$

Additive DEGs are only DEGs in a female + depletion combination but not male or depletion alone, and Suppressive DEGs are DEGs in male and depletions combinations but lost in a female background.

Results

The transcriptomic differences between female and male mice neurons in differentiation and maturation

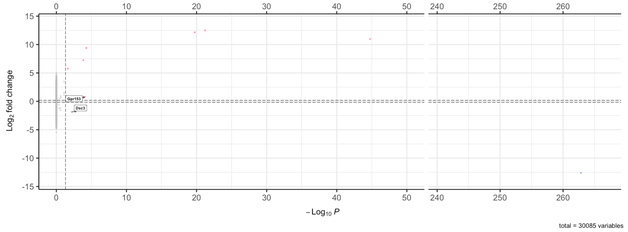


Figure 1. Volcano plot of pairwise RNA-seq comparison between male and female Luciferase-KD libraries show limited amount of DEGs. Most DEGs in this comparison are sex-linked, except for several genes including Gpr153 and Dsc3.

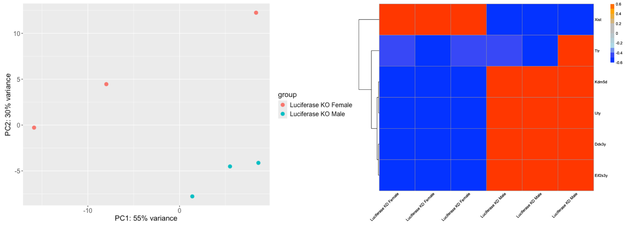


Figure 2 & 3. PCA plot and heatmap shows robust quality control of the libraries involved in the Luciferase samples comparison.

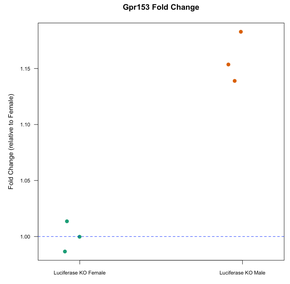


Figure 4. Gpr153 transcript is expressed 15% higher in the male Luciferase samples than in the female samples.
Gpr153 is highly expressed in the brain and has been linked to autism spectrum disorder (ASD) [6]. Knockdown of Gpr153 in mouse models leads to cognitive defects [7]. Additionally, Gpr153's upregulation post immune challenge through cerebral LPS injection in mice hippocampus is sex-specific—it is only upregulated in males, but not females [8].

Interaction between ASD-linked chromatin modifiers and the neuronal sex-specific transcriptome

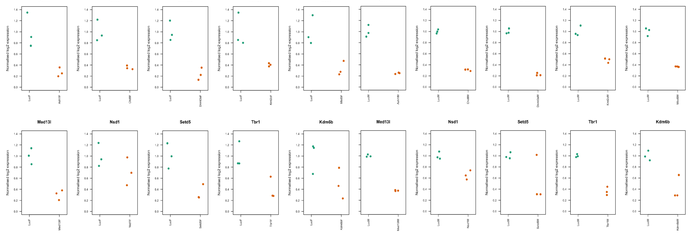


Figure 5. Strip charts showing robust and consistent target depletion efficiency across all libraries in the dataset.

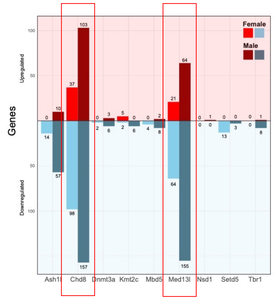


Figure 6. Male and female samples generate the highest number of DEGs upon loss of Chd8 and Med13l.

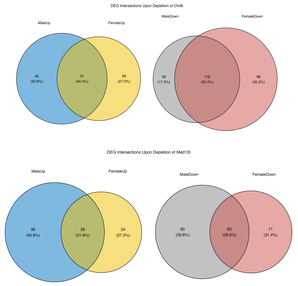


Figure 7. Male and females generate considerable, distinct subset of DEGs upon Chd8 and Med13l depletion

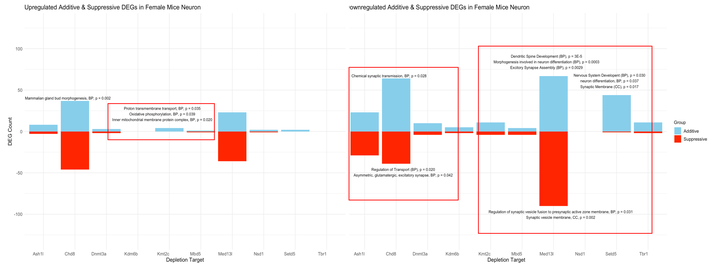


Figure 8 & 9. Count of additive & suppressive upregulated and downregulated DEG generated upon target depletion. Chd8 and Med13l have the largest number of Additive and Suppressive DEGs, and for these two targets, we see enriched additive and suppressive effects around downregulated DEGs involved in neuronal biology upon depletion.

Conclusions

Sex-specific differential expression of Gpr153 in the brain is neuron-specific and is not reliant on hormonal differences in animals. The loss of Med13l and Chd8 results in a sex-specific neuronal transcriptomic response, with evidence suggesting that the interaction between Med13l and Chd8 loss and the sex-specific transcriptome impacts critical neurodevelopmental and cognition-related biological pathways.

References

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