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Basic Science

Abstract Title
Investigating the Phenotypic and Epigenomic Impact of MeCP2 Loss-of-Function Missense and Nonsense Mutations in Transgenic Mice

Background
The methyl-CpG-binding protein 2 (MeCP2) plays a critical role in regulating gene expression and maintaining epigenetic stability, particularly in the brain. Mutations in the MECP2 gene are known to disrupt normal brain development and function, leading to a range of neurobiological and phenotypic abnormalities.

Objective
In this study, we investigated the phenotypic and epigenomic impacts of MeCP2 nonsense and missense mutations, T158M and R255X, in transgenic mice. Phenotypic evaluation criteria included activity, gait, hindlimb-clasping, tremor, breathing, and general condition in male and female wild-type and mutated mice.

Methods
Transgenic mouse models carrying T158M and R255X mutations in the MeCP2 gene were studied. Phenotypic assessments evaluated six criteria: activity, gait, hindlimb-clasping, tremor, breathing, and general condition. Male and female wild-type and mutant mice were assessed to capture sex differences and symptom progression. Brain tissue samples from distinct regions underwent histone extraction using acid-based protocols. Histones were separated via SDS-PAGE to quantify core (H2A, H2B, H3, H4) and linker (H1) histone ratios. Acid-urea-Triton (AUT) gel electrophoresis resolved histone variants based on charge, size, and hydrophobicity. Histone H1 levels were quantified using Western blotting and immunohistochemistry.

Results
Our preliminary results showed severe phenotypic effects across all criteria in both sexes, indicating significant disruption of neurological and physiological functions. Molecular analyses revealed changes in histone H1 levels in specific parts of the murine brain, with elevated levels in mutant brain region 1 but reduced levels in brain region 2 compared to wild-type. This suggests widespread epigenetic dysregulation due to MeCP2 mutation. To further investigate these findings, we employed SDS-PAGE and acid-urea-Triton (AUT) gel electrophoresis to analyze core and linker histones. Our results corroborated the region-specific alterations in histone levels, particularly the H1-to-H4 ratios.

Conclusion
Through this research, we aim to deepen our understanding of the functional regulatory role of MeCP2 in maintaining epigenetic stability in the brain and its implications for global gene regulation. By exploring the interplay between MeCP2 and H1, we seek to elucidate the epigenetic mechanisms underlying the phenotypic effects of MeCP2 mutations, contributing to a more comprehensive understanding of MeCP2's influence on the epigenome structure of brain cells.

Investigating the Phenotypic and Epigenomic Impact of MeCP2 Loss-of-Function Mutations in Transgenic Mice

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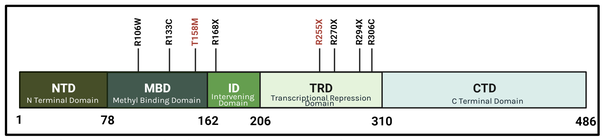
Background

The **MeCP2** (Methyl CpG binding protein-2) is a critical regulator of gene expression and epigenetic stability, particularly in the brain. It encodes a protein that binds to methylated DNA and modulates chromatin structure, thereby influencing transcriptional activity. Mutations in MeCP2 are known to disrupt these regulatory functions, leading to widespread epigenetic dysregulation and severe neurological and phenotypic abnormalities. While MeCP2 is widely studied in the context of specific neurological disorders, its broader role in maintaining the global epigenome and its impact on brain function remains not fully understood. This study focuses on two common MeCP2 mutations, **T158M** (missense) and **R255X** (nonsense), to investigate their phenotypic and epigenomic consequences in transgenic mouse models. By examining these mutations, we aim to uncover the mechanisms by which MeCP2 dysfunction alters the **epigenome** and contributes to **neurological** and **physiological** impairments.

Introduction

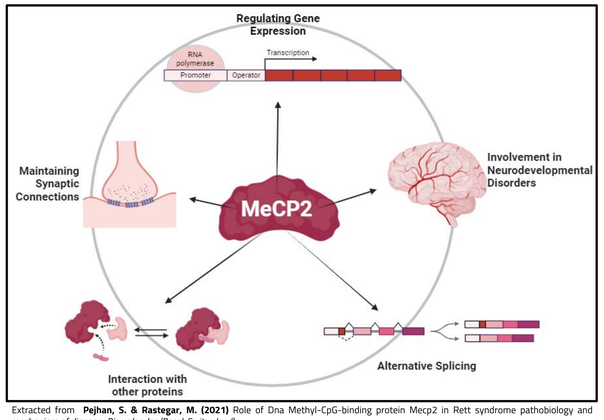
The two mutations that I will be focusing on in my project are,

T158M – Threonine to Methionine (Mis-sense mutation)
R255X – Truncated protein (Non-sense mutation)



Adapted from **Bin Akhtar, G. et al. (2022)** MeCP2 and transcriptional control of eukaryotic gene expression. *European journal of cell biology*

***Figure 1*: MeCP2 Domains and Mutations** – *This figure represents the five different domains of MeCP2 protein (E2 isoform), along with the occurrence of eight most common mutations causing Rett syndrome. The two mutations that are red in color are the ones that I will be focusing on.*



Extracted from **Pejhan, S. & Rastegar, M. (2021)** Role of Dna Methyl-CpG-binding protein Mecp2 in Rett syndrome pathobiology and mechanism of disease. *Biomolecules (Basel, Switzerland)*

***Figure 2*: Functions of MeCP2** – *This figure shows the diverse functional effects of MeCP2, amongst which "Involvement in Neurodevelopmental Disorders" & "Regulation of Gene Expression" is of primary importance.*

Results

Males ♂

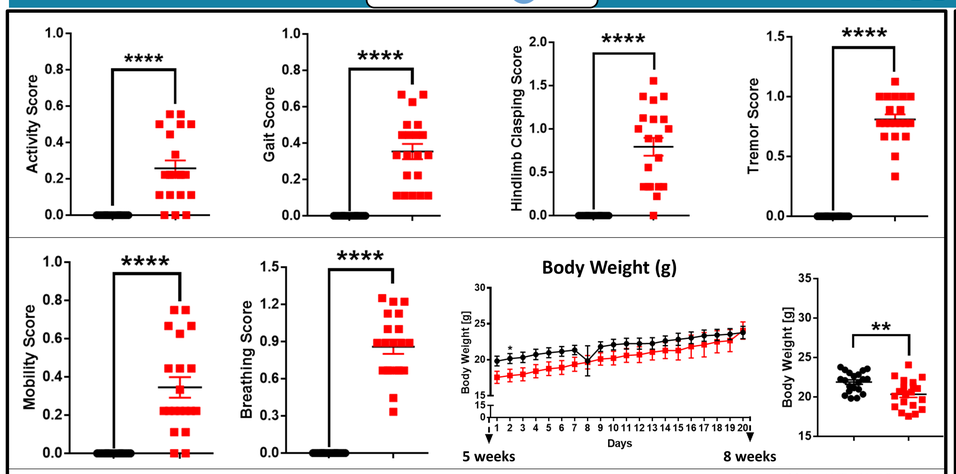


***Figure 4*: Phenotypic Assessment of Male T158M mutant Sham Mice compared to Wild-Type counterparts**

Females ♀

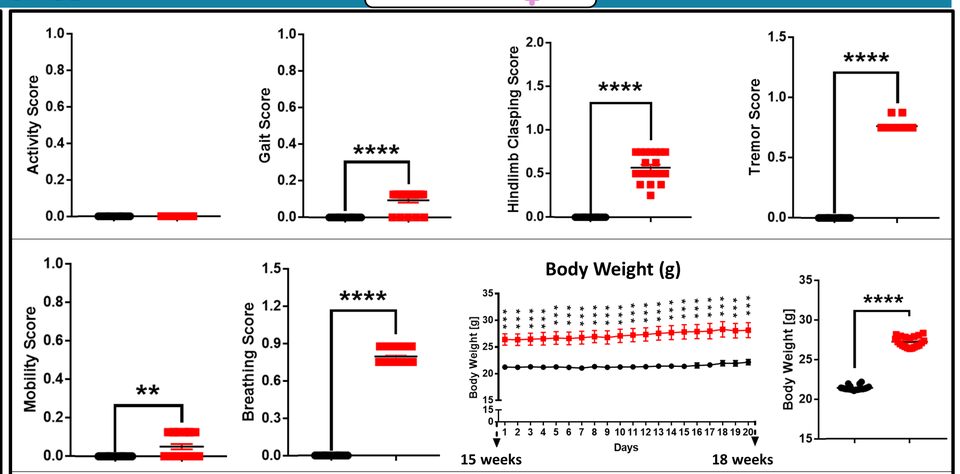


***Figure 5*: Phenotypic Assessment of Female T158M mutant Sham Mice compared to Wild-Type counterparts**

 # Figures 4 and 5 are results obtained from Rastegar's Lab, and in preparation for a publication.

Experimental Methods and Animal Model

1. Mouse Monitoring -> **Scoring Tests** based on Six Criteria
2. Histone Extraction -> **SDS PAGE** and **AUT based separation**
3. Protein Extraction -> **Western Blotting**

		FEMALE CARRIERS	HEMIZYGOUS MALES
T158M MICE	B6.129P2(Cg)-*Mecp2*$^{tm4.1Bird}$/J	viable, fertile, and develop a mild progressive phenotype from 4 months of age	viable but not fertile, can become phenotypic from 4 weeks of age and have a average survival of 12 weeks
R255X MICE	*Mecp2*$^{tm1.1Irsf}$/J	viable, fertile, mild phenotype including some obesity, reduced coordination, and lethargy	reduced life span, severe neurological phenotype, hindlimb clasping, excessive weight gain, behavior defects

***Figure 3*: T158M & R255X Mice Models,** *along with their genotypes and phenotypes*

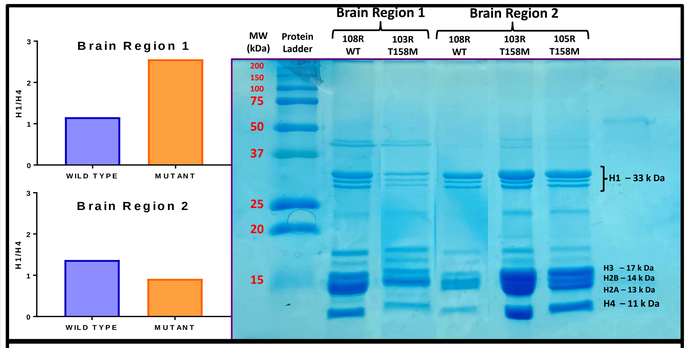


***Figure 6*: Extraction of histones followed by separation using SDS – PAGE in Wild-type and T158M Mutant Mice Brain Tissues (Brain Region 1 and 2)**

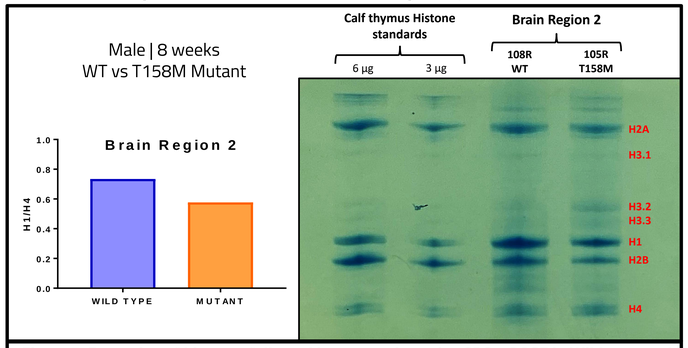
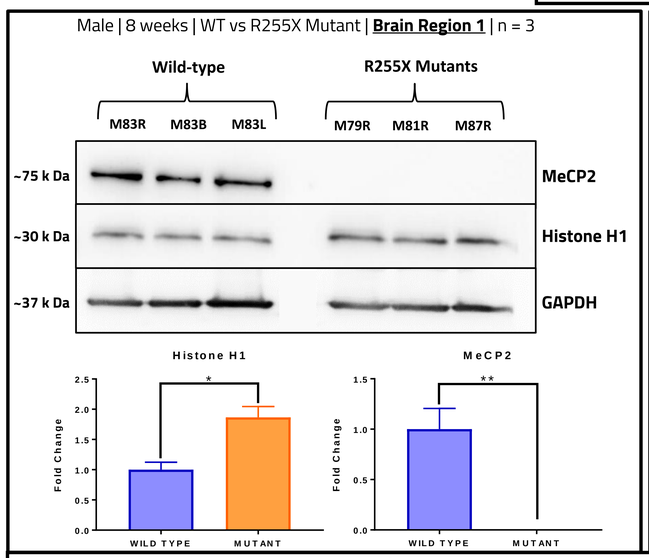


***Figure 7*: Extraction of histones followed by separation using SDS – PAGE in Wild-type and T158M Mutant Mice Brain Tissues (Brain Region 1 and 2)**



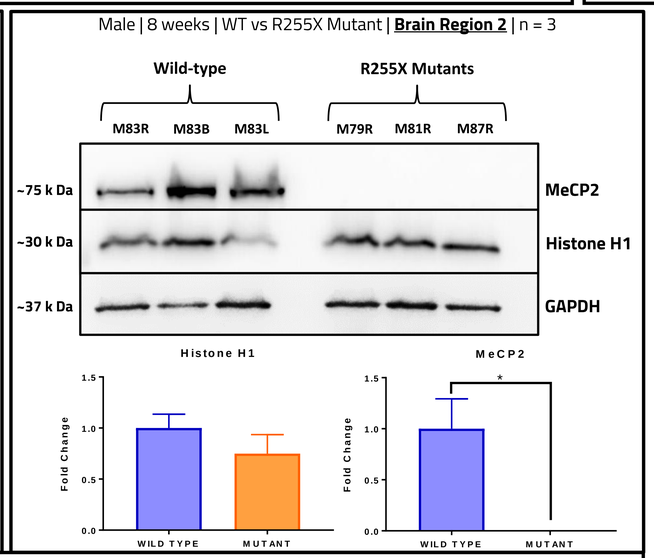


***Figure 8 and 9*: Western Blot Analysis of Histone H1 and MeCP2 in Wildtype and R255X Mutant Mice Brain Tissues (Brain Region 1 and Brain Region 2)**

Conclusions

- Phenotypic assessment results from the **T158M** mice show **severe effects** across all six criteria in both sexes, indicating **significant disruption** of **neurological** and **physiological** functions. These mice exhibited **reduced activity**, **abnormal gait**, **hindlimb-clasping**, **tremors**, **irregular breathing patterns**, and **poor general condition**, indicating significant **neurological** and **physiological** dysfunction.
- We also observed a **decreased body weight** in **males**, while it **increased** in **females**.
- **SDS-PAGE** results revealed a striking region-specific imbalance in histone H1/H4 ratios. Mutant brain region 1 showed **elevated H1 levels** relative to H4, while brain region 2 displayed a **reduction in H1/H4 ratios**. **AUT gel electrophoresis** confirmed these findings, highlighting reduced H1 variants in mutant brain region 2 and elevated H1 in brain region 1. These results align with MeCP2's proposed role in competing with histone H1 for DNA binding, **where loss of functional MeCP2 disrupts chromatin compaction**.
- Molecular analyses with **Western blotting** show changes in **histone H1 levels** in specific brain regions, suggesting **widespread epigenetic dysregulation** due to MeCP2 mutations. Notably, we found a **significant two-fold increase** in histone H1 in one brain region but not another, highlighting the **region-specific nature** of these epigenetic changes.

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CIHR IRSC Canadian Institutes of Health Research / Instituts de recherche en santé du Canada

GETS

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