Project Details	
Project Code	MRCNMH25Ex Flynn
Title	Epigenetic mechanisms in stem cell models of Rett syndrome
Research Theme	Neuroscience & Mental Health
Summary	Rett syndrome (RTT) is a neurodevelopmental disorder that causes physical and mental disability from early childhood. It is characterised by impaired motor and cognitive function and autistic-like behaviours. We have begun to appreciate that gene regulatory elements called enhancers are perturbed in RTT, but the precise mechanisms involved remain unclear. This project will investigate how mutations in the epigenetic regulator MeCP2 that are associated with RTT lead to changes in enhancer activity in human stem cell models. Using state-of-the-art sequencing technologies, the student will profile chromatin structure and epigenetic marks during neural differentiation and identify regulatory mechanisms that drive RTT.
Description	The human genome encodes hundreds of thousands of gene regulatory elements called enhancers. Enhancers are activated cell type-specifically to ensure that different cell types express a unique subset of active genes, thereby playing a central role in gene expression in human development and disease. Their dysregulation is increasingly implicated in neurodevelopmental disorders, but the mechanisms involved are poorly understood. The overall goal of this project is to address this gap. Rett syndrome (RTT) is a neurodevelopmental disorder characterised by impaired motor and cognitive function and autistic-like behaviours. It is caused by mutations in the gene encoding MeCP2, which is expressed at very high levels in neurons where it binds to epigenetic DNA modifications present on enhancers. Recent models propose that MeCP2 mutations cause RTT by disrupting enhancer activity, highlighting the importance of studying the underlying mechanisms involved. Human-induced pluripotent stem cell (hiPSC) lines carrying specific mutations in MeCP2 provide a powerful model to study RTT, as they can be differentiated into neurons and compared to control cell lines with the same genetic background. The aim of this project is to use hiPSC models to understand how MeCP2 mutations disrupt enhancer activity during neural differentiation. The objectives in Year 1 will be to: 1) identify the enhancers that are affected by MeCP2 mutations and 2) map changes in epigenetic modifications and chromatin structure at those enhancers. To do this the student will use cutting-edge genome sequencing technologies to identify enhancer-promoter pairs (with Dr. Sean Flynn) and to map epigenetic DNA modifications (with Dr. Jack Hardwick and Prof. Jonathan Mill) during differentiation. The student will be trained in methods for profiling: (i) Accessible chromatin. Activation of enhancers is associated with an accessible, nucleosome-depleted chromatin state. (ii) Epigenetic marks. Changes in enhancer activity are associated with changes in histone and DNA modifications including H3K27ac, 5-methylcytosine and 5-hydroxymethylcytosine. (iii) DNA secondary structure. Although DNA usually exists as a double helix, enhancer elements often adopt a four-stranded DNA structure called a G-quadruplex.

	(iv) Enhancer-promoter contacts. Enhancers are often located long distances away from their target genes and are thought to be brought into contact by the 3D structure of the genome. The objective of Year 2 will be to integrate these genomic datasets to understand the order of events that occur during enhancer activation in normal differentiation, and how these are disrupted upon MeCP2 mutation. With support from each of the supervisors, the student will be trained in bioinformatics and data science, allowing them to interrogate and integrate the datasets generated during Year 1. This will allow the student to characterise the interplay between epigenetic features that regulate enhancers involved in RTT. The objective of Year 3 is to test whether chromatin and epigenetic changes identified by these analyses play a causal role in MeCP2-mediated disruption of enhancer function. The three supervisors will provide complementary expertise and support in functional genomics approaches providing the student with an optimal environment to develop as an independent researcher – from developing a specific research question that interests them to devising and executing the experiments to address it. Depending on their interests, this may include using CRISPR methods for genome and epigenome editing and the use of reporter assays to measure enhancer function. Alternatively, the approach may employ brain organoids or the analysis of clinical data via collaboration with the NIHR Exeter BRC to test specific hypotheses in stem cell models derived from patients. Overall, this project will reveal important insights into neurodevelopment and provide the student with the freedom to develop into an independent scientist.
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