Project Details	
Project Code	MRC23NMHEx Dempster
Title	Investigating the role of DNA methylation in C9ORF72 Amyotrophic Lateral Sclerosis
Research Theme	Neuroscience and Mental Health
Summary	During this PhD you will use innovative molecular techniques to investigate a repeat expansion in the C9ORF72 gene, the most common genetic cause of Amyotrophic lateral sclerosis (ALS). Recent evidence indicates C9ORF72 is epigenetically modified in patient neurons and correlated with patient survival. Using CRISPR-cas9 gene editing and motor neuron cultures obtained from patient stem cells you will investigate the role epigenetic factors play in ALS progression.
Description	Amyotrophic lateral sclerosis (ALS) is a fatal incurable neurodegenerative condition characterized by loss of motor neurons (MNs) which leads to progressive muscle paralysis with average survival 2–5 years after diagnosis. A repeat expansion in the C9ORF72 gene (C9RE) is the most common genetic cause of ALS. Healthy individuals usually display around 2-3 repeats whereas >1000 repeats are commonly reported in ALS patients. Downstream molecular consequences of this repeat expansion include lower C9ORF72 gene expression, formation of toxic nuclear RNA foci and protein aggregates, together contributing to MN dysfunction and cell death. Epigenetic processes mediate the reversible regulation of genes and orchestrate a diverse range of critical neurobiological processes in the brain. DNA methylation is the most stable epigenetic modification and has been strongly implicated in the aetiology and progression of ALS neuropathology. In particular, DNA methylation is altered at C9RE and is associated with both repeat length and disease progression. Importantly, increased DNA methylation at C9RE is associated with, later age at death and decreased disease duration. It has also been correlated with transcriptional silencing of the C9ORF72 gene and decreased accumulation of toxic RNA foci suggesting that this DNA modification may have protective effects in repeat carriers and could be potentially modifiable. The aim of this PhD project is to decipher the relationship between C9RE DNA methylation, repeat length and motor neuron function. These investigations will provide deeper mechanistic insights into how C9RE methylation status contributes to MN function and will provide potential therapeutic avenues based on targeted epigenetic manipulations. ED and NA have independently optimised a novel approach that uses a catalytically dead Cas9 fused to methylation machinery, TET to perform targeted demethylation of C9RE and two effector domains KRAB and DNMT3A-3L for targeted methylation. The student will use these approaches to evaluate the functional and molecular changes that occur in C9RE patient iPSC-derived motor neurons after targeted epigenetic manipulation. The main aims of the PhD project will be: 1, To perform a thorough review of the literature on the C9RE region and to use bioinformatics tools to mine our existing DNA methylation data sets on motor neurons and ALS patient samples to identify optimal targets within the locus. For example, targets could include the repeat itself and/or the proximal CpG Island. 2, Using the latest epigenetic editing tools to target the C9RE region in differing repeat length carriers and isogenic control derived

	iPSCs and differentiated motor neurons, this aspect of the project will be guided by ED and NA. 3, Investigate how manipulating C9RE methylation changes the transcriptional landscape of C9RE and isogenic control iPSC-derived motor neurons under the guidance of AB and ED. The DNA methylation status of the C9RE locus in response to the targeted editing will be evaluated using Oxford Nanopore Technologies (ONT) long-read sequencing employing adaptive sampling. This approach allows accurate detection of all base modifications including DNA methylation and generates reads >10Kb, enabling accurate repeat molecular characterization other methods cannot achieve. ONT RNA sequencing is quantifiable and allows the direct assessment of alternatively spliced transcripts. 4. MNs differentiated from different length C9RE ALS patient-derived iPSCs will be used to test the phenotypic effects of manipulation of the C9RE locus. Phenotypic assays will be performed on electrically active day 30 MNs. A wide range of assays can be chosen to evaluate the cellular consequences of altering the C9RE methylation status depending on the direction of the project. Including neuronal survival, morphological analysis, synapse formation and neuronal activity using Ca2+-signalling (guided by AB and NA).
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