Project Details	
Project Code	MRCNMH25Ex Dempster
Title	Cell-type-specific epigenetic regulation of gene expression in Motor Neuron Disease
Research Theme	Neuroscience & Mental Health
Summary	This project aims to uncover new molecular insights into Motor Neuron Disease (MND) using advanced molecular technologies. It involves studying human post-mortem brain tissue and cells derived from patient stem cells to examine gene regulation in different brain cell types. Specifically, the focus will be on different cells from patients with and without a large expansion in the C9ORF72 gene, the most common genetic cause of MND. Recent research indicates that C9ORF72 is modified in patient neurons and linked to patient survival. This project could lead to the discovery of new treatment targets for this incurable disease.
Description	Motor Neuron Disease (MND) is a fatal incurable neurodegenerative condition characterised by loss of motor neurons(MN) which leads to progressive muscle paralysis with average survival 2–5 years after diagnosis. A repeat expansion in the C9ORF72 gene (C9RE) is MND's most common genetic cause. Healthy individuals usually display around 2-3 repeats whereas >1000 repeats are commonly reported in MND patients. Downstream molecular consequences of this repeat expansion include lower C9ORF72 gene expression, and the formation of toxic nuclear RNA foci and protein aggregates, together contributing to motor neuron dysfunction and cell death. Epigenetic processes mediate the reversible regulation of gene and orchestrate a diverse range of important neurobiological processes in the brain and CNS. DNA methylation is the most stable epigenetic modification and has been strongly implicated in the aetiology and progression of MND 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 modifiable. **Purpose of the proposed project** The main aim of this project is to understand the relationship between DNA modifications in neural cell types in different subgroups of MND and how these modifications mediate gene regulation through alternative spicing. Aim 1) To characterise the DNA modification profile of the C9RE locus in motor neurons and other cell types isolated from C9RE MND carriers. We have developed a robust method to isolate nuclei from specific neural cell populations from post-mortem brain tissue using Fluorescence Activated Nuclei Sorting (FANS). We can isolate different neural cell -types encompassing a range of distinct neuronal and glial cell types including motor neurons. The first stage of this project will profile DNA modifications and simultaneously confirm repeat length in motor

	neurons and other neural cell types isolated from the brain and spinal cord from C9RE carriers and a subset of controls. Oxford Nanopore Technologies (ONT) targeted sequencing will distinguish several base modifications such as 5'methylcytosine while generating reads >10Kb. Using this approach, you will produce a detailed assessment of the relationship between DNA modifications and repeat length in discrete neural cell populations and how they compare to several C9RE iPSC model systems. 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. Aim 2) To identify neural cell-type-specific genome-wide DNA methylation signatures of C9RE MND and sporadic ALS. Using our FANS protocol, you will isolate a range of distinct neural cell types including motor neurons from postmortem brain and spinal cord from C9RE MND (as above), sporadic MND and control samples. Following nuclei isolation, you will measure DNA methylation across the genome using Illumina DNA methylation EPICv2 arrays in these distinct neural cell populations from different groups of MND patients and controls. Aim 3) To investigate the cell-type specific transcriptome using long read-ONT sequencing in MND with and without the C9RE, matched controls and induced pluripotent stem cells (iPSC)-derived motor neurons Using long-read RNA sequencing you will explore the transcriptomic diversity in discrete cell types in the two distinct patient groups (C9RE carriers and sporadic MND) along with control samples (isolated in Aims 1&2) and different iPSC models of MND. This approach will identify and characterise novel splice variants associated with disease processes. The project has scope to enable the student to direct research for example to prioritise different iPSC models of MND (e.g. TDP43 mislocalisation) and to include samples with other genetic causes of MND for example SOD1 mutations The information generated from incorporating the different "omic" approaches from the three different aims in distinct brain cell types promises to provide critical insights into the deviations in gene regulation occurring within specific cell types of the brain and central nervous system in MND. Furthermore, this research will serve to elucidate both commonalities and disparities between MND cases with the C9RE repeat expansion and those that are sporadic. Such knowledge will be instrumental in advancing our understanding of this devastating disorder and may guide the development of more effective diagnostic and therapeutic strategies

Supervisory Team	
Lead Supervisor	
Name	Dr Emma Dempster
Affiliation	Exeter
College/Faculty	Faculty of Health and Life Sciences
Department/School	Clinical and Biomedical Sciences
Email Address	e.l.dempster@exeter.ac.uk

Co-Supervisor 1	
Name	Dr Akshay Bhinge
Affiliation	Exeter
College/Faculty	Faculty of Health and Life Sciences
Department/School	Living Systems Institute
Co-Supervisor 2	
Name	Dr Owen Peters
Affiliation	Cardiff
College/Faculty	
Department/School	School of Biosciences
Co-Supervisor 3	
Name	
Affiliation	
College/Faculty	
Department/School	