**Background:**
Motor neurone disease (MND) is a devastating neurodegenerative condition caused by the selective loss of motor neurones in the central nervous system. The disease is characterised by progressive muscle weakness and wasting typically leading to death within 2-5 years of symptom onset [1]. There are no diagnostic markers for the disease and diagnosis currently relies on clinical assessment to exclude diseases that mimic MND, which can take several months [2].

MicroRNAs (miRNAs) are a recently discovered class of small, non-coding RNA molecules that are predicted to regulate at least one third of all human genes [3]. miRNAs modulate protein expression by binding short sequences within the 3’ untranslated region (3’UTR) of specific mRNAs, thereby targeting them for degradation or translational repression. miRNAs play key regulatory roles in a diverse range of pathways and there is accumulating evidence to suggest their dysfunction could contribute to neurodegenerative disease [4].

The recent discovery of stable miRNAs in the blood, which reflect the pathophysiology of different disease states, has led us to investigate circulating miRNAs that could serve as biomarkers for MND [5]. We have used profiling technology to identify potential biomarkers by comparing miRNA expression profiles from MND cases to those of patients diagnosed with conditions that mimic MND. One of the disease mimic groups is myopathy, a group of conditions affecting the muscle to cause weakness, which has to be ruled out during the MND diagnostic process. This project will validate potential miRNA biomarkers that distinguish MND cases from those diagnosed with myopathy.

There is currently no published literature reporting which miRNAs are expressed in motor neurones, this project will therefore investigate whether the miRNAs identified as biomarkers for MND are expressed within motor neurones using laser capture technology to isolate these cells from post mortem tissue.

**Hypothesis/Aims:**
Circulating miRNAs will serve as informative biomarkers useful in distinguishing MND patients from individuals with myopathy. The aim of this project is to validate miRNA markers that differentiate MND from myopathy patients in additional serum samples and investigate their expression in motor neurones.

**Research Plan/Methods:**

*QPCR validation*
miRNA has been extracted from human serum samples and stored at -80°C. The expression of potential miRNA biomarkers identified via profiling experiments will be validated by quantitative PCR (QPCR) using individual TaqMan assays (Applied Biosystems) in 10 MND cases and 10 myopathy patient samples. QPCR experiments will be performed on an MX3000 real time PCR system (Stratagene) to measure the TaqMan probe signals and the relative expression calculated using the 2-ΔΔCT method.

*Laser capture microdissection of motor neurones*
The expression of validated miRNA targets will be investigated in post mortem neuronal tissue using laser capture microdissection (LCM) to isolate motor neurones from spinal cord samples. Frozen spinal cord samples from 3 control individuals and 3 MND patients will be obtained from the Sheffield Brain Tissue Bank. These samples will be sectioned, fixed and stained with toluidine blue to highlight cells for microscopy. For each post mortem sample 200-500 motor neurones will be isolated using the Veritas laser capture microdissection system (Applied Biosystems).
QPCR of miRNAs from LCM material
Total RNA will then be extracted using the RNA purification micro kit (Norgen) according to manufacturer’s instructions. The expression of target miRNAs within the motor neurones of control individuals and MND patients will then be determined using TaqMan QPCR assays (Applied Biosystems) as detailed above.

Statistical Analysis
Statistical analysis will be performed using the GraphPad Prism V5 (GraphPad Software, San Diego, USA). The observed expression levels obtained via QPCR will be compared between MND and myopathy or control individuals using T-tests.

Time scale:
Week 1: Lab safety induction and introduction to good laboratory practice procedures
Week 2: QPCR of selected miRNA targets in serum miRNA samples
Week 3/4: Training in the use of LCM methods and isolation of motor neurones from spinal cord tissue
Week 5: Extraction of miRNA from LCM material and QPCR to test for presence of miRNA targets
Week 6: Project write up and oral presentation

References: