Project Details	
Project Code	MRCNMH26Ca Smith
Title	When DNA Breaks and Mitochondria Fail: Investigating Drivers of Huntington's Disease
Research Theme	NMH
Project Type	Wet lab
Summary	Huntington's disease (HD) is a fatal inherited brain disorder with no effective treatment. It causes specific neurons in the brain to degenerate, leading to involuntary movements, cognitive decline and sleep disturbances. This PhD project aims to uncover how these neurons die by combining advanced tools in Drosophila genetics, human iPSC-derived neurons and metabolomics. The student will also screen potential therapeutic compounds in HD models. This is a unique opportunity to work across model systems and technologies to understand disease mechanisms and explore new treatment strategies.
Description	Neuron death is a hallmark of neurodegenerative diseases such as Huntington's disease (HD). However, the molecular events that lead to neuronal death in HD remain unclear, limiting our ability to develop effective therapeutics. HD is a hereditary disorder caused by a CAG trinucleotide repeat expansion in the huntingtin (HTT) gene, leading to the production of a mutant HTT (mHTT) protein. This mutant protein accumulates and disrupts various cellular processes, including cell signaling, transcription, and metabolism. Excessive DNA damage and mitochondrial dysfunction are well-established features of HD, but their causal roles in neuron loss remain incompletely understood. Recent work in the supervisor's laboratories has identified a possible mechanistic link between DNA damage and mitochondrial dysfunction via poly-ADP-ribosylation (PARylation), a post-translational modification catalyzed by the enzyme PARP1. We hypothesize that increased DNA damage in HD leads to hyperactivation of PARP1, resulting in excessive PARylation, depletion of cellular NAD+ and ATP, and subsequent mitochondrial dysfunction. This project will explore the mechanistic basis of PARP1-mediated metabolic dysfunction and assess whether PARP1 inhibition is a viable therapeutic strategy in HD. Aim 1: Determine the impact and mechanism of PARP1 inhibition on neurodegeneration, protein aggregation, locomotion, and lifespan in a Drosophila model of HD. PARP1-related pathways are highly conserved, and Drosophila models expressing human mHTT transgenes replicate key features of HD, including motor deficits, inclusion formation, neurodegeneration, and reduced lifespan. Taking advantage of the genetic tractability of Drosophila, we will dissect the downstream pathways of mHTT-induced DNA damage in vivo. We will quantify markers of DNA damage and mitochondrial dysfunction over the Drosophila lifespan and assess whether PARP1 expression or activity is altered. The protective effects of both genetic and pharmacological PARP1 inhibition will then be evaluated. Student input: The student will design inhibition experiments, select the most effective compounds and doses, and optimize in vivo dosing strategies.

	Aim 2: Use human iPSC-derived neurons to define the relationship between DNA damage, PARylation, mitochondrial dysfunction, and test the therapeutic potential of PARP1 inhibition in vitro. To probe human-relevant mechanisms, the student will assess DNA damage (via γH2AX, 8-oxodG staining, and comet assays) and PARylation in iPSC-derived neurons using immunohistochemistry and Western blotting. The efficacy of PARP1 inhibitors (from Aim 1) will be tested for their ability to rescue HD-relevant cellular phenotypes. Mitochondrial health will be assessed using TOMM20 staining and live-cell imaging (Opera Phenix system with MitoTracker). Mitochondrial membrane potential and oxidative stress will be quantified using TMRM and MitoSOX dyes over a 72-hour time course. Bioenergetic capacity will be evaluated via Seahorse XF Analyzer (measuring OXPHOS and glycolysis), along with NAD+ and ATP quantification. Student input: The student will select the most relevant cellular phenotypes to prioritize based on Drosophila findings and will explore and optimize in vitro methodologies to assess cell-specific changes. Aim 3: Characterize metabolic changes in HD models and after PARP1 inhibition using metabolomics. PARP1 activation can deplete NAD+ and ATP, potentially disrupting cellular energy metabolism. It may also inhibit glycolysis by suppressing hexokinase activity. We hypothesize that excessive PARylation and NAD+ consumption impair glycolytic and oxidative metabolism in HD. Initial assessments of NAD+/NADPH and ATP levels will be performed using sensitive plate-reader assays. For more detailed insights, untargeted mass spectrometry will be used to profile metabolic changes in fly brains and human iPSC-derived neurons. Dysregulated metabolites will be mapped onto biochemical pathways using MetPA and visualized via KEGG. Enzymes implicated in key metabolite alterations will be studied using Drosophila RNAi knockdown to test necessity and sufficiency for phenotypes. Student input: The student will verify key metabolite changes using enzyme activity assays, metabolite imaging reporters, or ELISAs, and will help steer the project toward the most promising targets by optimizing relevant assays. This interdisciplinary project integrates fly genetics, stem cell biology, imaging, metabolomics, and bioenergetics to address a fundamental and unresolved question in HD research. The student will gain training in advanced experimental techniques across multiple model systems and have opportunities to develop hypotheses, drive experimental design, and contribute novel insights into the mechanisms of neurodegeneration.

Supervisory Team	
Lead Supervisor	
Name	Dr Gaynor Smith
Affiliation	Cardiff
College/Faculty	The College of Biomedical and Life Sciences
Department/School	School of Medicine
Email Address	smithga@cf.ac.uk

Co-Supervisor 1	
Name	Professor James Hodge
Affiliation	Bristol
College/Faculty	Faculty of Life Sciences
Department/School	School of Physiology, Pharmacology & Neuroscience
Co-Supervisor 2	
Name	Dr Thomas Massey
Affiliation	Cardiff
College/Faculty	College of Biomedical and Life Sciences
Department/School	School of Medicine
Co-Supervisor 3	
Name	Dr Mariah Lelos
Affiliation	Cardiff
College/Faculty	The College of Biomedical and Life Sciences
Department/School	School of Biosciences