Project Details	
Project Code	MRCNMH25Ca Smith
Title	How does DNA damage and mitochondrial dysfunction contribute to Huntington's disease?
Research Theme	Neuroscience & Mental Health
Summary	Huntington's disease (HD) is an inherited disorder that causes specific neurons in the brain to die. HD patients develop uncontrollable movements as well as cognitive and sleep problems, and there is currently no effective treatment. In order to develop therapies, we need to understand the mechanism of how these neurons are lost. To help answer this, the PhD student will be trained in: Drosophila (fruit fly) genetics, human iPSC-derived neuron cultures and big data driven Metabolomic analysis. The PhD student will also test whether several drugs can alleviate hallmark disease features in models of HD.
Description	Neuron death is a hallmark feature of neurodegenerative conditions such as Huntington's disease (HD). The molecular events that drive neuron death in HD are unclear, leaving a substantial knowledge gap in our ability to design effective therapeutics. HD is hereditary and caused by a mutation in the huntingtin gene that causes an expansion of a (CAG) tract, many more times than is normal. This causes a toxic buildup of the protein within a cell that causes a wide range of cell signaling problems. Excessive DNA damage throughout the genome and dysfunction of mitochondria are established features of HD, but to what extend they ultimately cause neurons to die is unknown. Supervisor labs have recently found that a processes called poly-ADP-ribosylation (PARylation) may link DNA damage and mitochondrial dysfunction, regulated by an enzyme called PARP1. Our leading hypothesis is that increased DNA damage, causes hyper PARylation and depletes the cells of key metabolites leading to mitochondrial damage. The student will probe the mechanism by which the dysregulated PARP1 contributes to mitochondrial dysfunction and test whether PARP1 inhibition could be a therapeutic target. Aim 1: Determine the impact and mechanism of PARP inhibition on longevity, locomotion, protein aggregation and neurodegeneration in a Drosophila model of HD in vivo. Pathways relating to PARP1 are highly conserved between animal systems. HD Drosophila models that express the human mHTT transgene have been used extensively to gain new insights into HD pathogenesis and recapitulate hallmark features. By exploiting the genetic tractability of Drosophila we aim to dissect the genetic pathway downstream of the mutated HTT-induced DNA damage in vivo. Drosophila carrying human transgenic HTT with a pathological mutation have deficits in motor function, show increased inclusion formation and neurodegeneration, and have reduced lifespan compared to control. We will quantify markers of DNA damage and mitochondrial dysfunction in the brain over the lifetime, and measure any PARP upregulation. We will then determine the protective effects of genetic and pharmacological PARP inhibition in our fly models of HD. *Student input – The student will design inhibition experiments, choose most effect compounds, best doses and in vivo dosing strategies.

	Aim 2: Utilize human induced pluripotent stem cell (iPSC)-derived neurons to define relations between DNA damage, PARylation, neurodegeneration and test efficacy of PARP inhibition in vitro. The student will be trained to explore underlying mechanisms by assaying DNA damage (gH2AX, 8-oxodG staining; comet assays) in these human iPSC lines though immunohistochemistry and Western blotting. We will assess the efficacy of PARP1 inhibition, using the range of inhibitors used in Aim 1 to ameliorate iPSC phenotypes. Dynamic mitochondrial changes will be assessed by immunohistochemistry (TOMM20) and live cell imaging using the OperaPhenix system (MitoTracker). TMRM and mitoSOX live dye assays over a 72hr time course will determine mitochondrial membrane potential and oxidative stress. Respiration capacity will be measured by assessment of OXPHOS and glycolysis (SeahorseXF analyser), and by quantification of NAD+ and ATP levels. *student input -i) Choosing the most relevant cellular phenotypes to probe based off Drosophila in vivo findings ii) There are plenty of analysis methods and tools available for in vitro work and the student will be encouraged to develop different techniques and methodologies to measure a range of cell specific changes. Aim 3) To decipher energetic changes in HD models and PARP1 inhibition using Metabolomics. Depletion of NAD+ by DNA damage-induced PARP activation may hinder cellular metabolism. PARP1 may also contribute to ATP depletion, having been previously reported to inhibit hexokinase glycolysis enzyme. We therefore predict that PARylation and NAD+ consumption contribute to the inhibition of glycolysis in HD. Specific changes in NAD+/NADPH and ATP will first be measured through sensitive plate reader kits (Sigma). Both whole adult fly brains and human iPSC neuron pellets collected for in-depth analysis of OXPHOS/glycolytic metabolic changes through untargeted MassSpec. Dysregulated metabolites will be mapped onto pathways through MetPA, visualized through KEGG. Conserved enzymes related to key metabolite changes can be investigated in Drosophila, using RNAi knockdown approaches to see if they are necessary and sufficient to induce phenotypes. *Student input – relative metabolite changes will need to be verified through the use of enzymatic/metabolite imaging reporters or ELISA and the student will steer the project to find, narrow down and optimise specific assays for the most promising candidates.
Supervisory Team	
Lead Supervisor	
Name	Dr Gaynor Ann Smith
Affiliation	Cardiff
College/Faculty	College of Biomedical and Life Sciences
Department/School	School of Medicine
Email Address	Smithga@cf.ac.uk
Co-Supervisor 1	
Name	Dr Thomas Massey
Affiliation	Cardiff
College/Faculty	College of Biomedical and Life Sciences
Department/School	School of Medicine

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