

Project Details	
Project Code	NMH27Ca Ngo
Title	Guarding the Brain's Blueprint: Investigating UPF3B in DNA Repair, Autism, and ADHD
Research Theme	Neuroscience & Mental Health
Project Type	Wet lab
Summary	Neurodevelopmental disorders (NDDs) like autism and ADHD affect millions, yet the underlying causes remain unclear. This PhD project investigates how mutations in the gene UPF3B, linked to NDDs, impair DNA repair in neurons. Using advanced techniques including CRISPR gene-editing, biochemical assay, nanopore DNA sequencing, and stem cell-derived neurons, the student will explore a novel disorder mechanism at the intersection of genome stability and brain development. The project offers multidisciplinary training in genetics, biochemistry, genomics, neuroscience, and bioinformatics. This work could contribute to improved diagnosis and understanding of NDDs, making a real-world impact on one of today's most pressing health challenges.
Description	Background Neurodevelopmental disorders (NDDs), including autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD), intellectual disability (ID), and schizophrenia (SCZ), affect over 5% of the global population. ASD alone is estimated to impact approximately 78 million people worldwide, with the UK economic cost of ASD reaching £32 billion annually as of 2014 (Buescher et al., 2014). ADHD diagnoses have increased twentyfold in recent years (McKechnie et al., 2023), with both children and adults facing prolonged delays in receiving a diagnosis. Despite the high prevalence and socioeconomic burden, the root causes of most NDDs remain poorly understood, making diagnosis, mitigation, and treatment highly challenging. NDDs are typically characterised by impairments in social, cognitive, and emotional functioning. Different NDD subtypes frequently co-occur in the same individual, suggesting shared underlying mechanisms. Many NDDs exist on a spectrum, with presentations ranging from severe disability to exceptional ability, yet the factors driving this variability are largely unknown. Advances in genomics have identified numerous genes, referred to as NDD risk genes, which are implicated in these disorders. One key emerging concept is that of 'mutational load,' which proposes that the severity of an individual's condition may be influenced by the cumulative number of mutations in these risk genes. However, most NDD cases remain genetically unexplained, with known risk factors accounting for only a minority (23.7%) of clinical diagnoses (Stefanski et al., 2021). UPF3B syndrome is a rare genetic disorder associated with an increased risk of developing NDDs, with variants linked to ASD, ADHD, ID, and SCZ (Deka et al., 2021). However, the molecular mechanisms driving these neurodevelopmental disorders remain poorly understood. Recent work from our group has shown that UPF3B protein, together with its interacting partner UPF1, promotes DNA repair by stimulating the formation of R-loops - novel three-stranded nucleic acid structures containing DNA-RNA hybrids (Ngo et al., 2021). We hypothesise that

	reduced R-loop-dependent DNA repair exacerbates accumulation of mutations that may disrupt NDD risk genes. This hypothesis offers a new and potentially transformative explanation for the increased risk of NDDs. This project aims to investigate how dysfunction of the UPF3B gene contributes to DNA repair defect and NDDs, using a multidisciplinary approach that integrates genetics, genomics, biochemistry, bioinformatics, and neuroscience. We will focus on characterising pathogenic UPF3B missense variants through advanced biochemical reconstitution assays, CRISPR-based gene editing, nanopore DNA sequencing, bioinformatic analyses, and induced pluripotent stem cell (iPSC)-derived neurons. By uncovering how UPF3B dysfunction leads to NDDs, this project will provide insight into a novel mechanism of NDD risk and contribute to improving the diagnosis and potential mitigation of these disorders. Objectives The first objective is to investigate the effect of UPF3B missense variants on R-loop-dependent DNA repair in vitro. The student will receive training in the following techniques: i) purification of UPF3B protein and UPF3B missense variants associated with ID, ASD, ADHD and SCZ using an insect cell system (Berger-Schaffitzel Lab, University of Bristol) and ii) assessing the activities of these variants using biochemical assays (co-supervised by Dr Ashley Parkes, Ngo Lab, Cardiff University). The second objective is to characterise neurodifferentiation in iPSCs carrying UPF3B missense variants (Allen Lab, Cardiff University). The student will receive training in the following techniques: i) generating knock-in mutants with pathogenic UPF3B missense variants using CRISPR-based gene editing, ii) inducing differentiation of iPSCs into neurons, and iii) characterising both young and mature neuronal populations. The third objective is to examine R-loop-dependent DNA repair and mutation load in iPSCs carrying UPF3B missense variants. The student will receive training in the following techniques: i) mapping R-loops in the genome (Ngo Lab, Cardiff University), ii) detecting chromatin and epigenetic changes associated with R-loops (Flynn Lab, Exeter University), iii) identifying mutation using nanopore sequencing (Ngo Lab, Cardiff University) and iv) analysing large genomic dataset using bioinformatic pipeline. Throughout the project, the student will be supported in developing into an independent researcher. In Years 1 and 2, they will work closely with more experienced team members to design and execute experiments. By Years 3 and 4, they will have increasing autonomy to plan, prioritise, and conduct their own experiments. They will also have the opportunity to extend their analyses by integrating their data with relevant clinical datasets in the final year. Additionally, the student will be encouraged to present their findings at both national and international conferences of their choice. Overall, this project offers a comprehensive, multidisciplinary training experience that will prepare the student for a successful research career. References
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	Buescher AVS et al. JAMA Pediatrics. (2014) Aug 1;168(8):721–8. doi: 10.1001/jamapediatrics.2014.210. McKechnie DGJ, et al. BJPsych Open. (2023) Jul;9(4):e121. doi: 10.1192/bjo.2023.512. Stefanski, A. et al. Epilepsia (2021) 62, 143-151. doi: 10.1111/epi.16755. Deka B, et al Biochimie. (2021) Jan:180:10-22. doi: 10.1016/j.biochi.2020.10.011. Ngo GHP, et.al. Nat Commun. (2021) Jun 22;12(1):3849. doi: 10.1038/s41467-021-24201-w.
Can be completed part time?	No
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