Project Details	
Project Code	NMH27Ba Licchesi
Title	Decoding Neurodevelopmental Disorders: From Patient Variants to Disease Mechanisms
Research Theme	Neuroscience & Mental Health
Project Type	Wet lab
Summary	Rare genetic variants are a major cause of neurodevelopmental disorders, including autism, epilepsy and intellectual disability, yet the underlying disease mechanisms remain poorly understood. This multidisciplinary project will investigate how disease-associated mutations disrupt the function of an essential E3 ubiquitin ligase involved in protein quality control and cellular proteostasis during brain development. Using structural biology, quantitative proteomics, human iPSC-derived neurons and zebrafish disease models, the student will uncover how genetic variation leads to neurological dysfunction. Functional consequences will also be assessed using advanced imaging and multielectrode array technology, providing new insights into rare neurological disorders and variant interpretation.
Description	Brief Background Neurodevelopmental disorders (NDDs), including autism spectrum disorder, epilepsy, intellectual disability and developmental delay, affect millions of children worldwide. Although genomic sequencing has identified many disease-associated variants, the mechanisms linking genetic variation to altered brain development remain poorly understood. HECTD1 is an E3 ubiquitin ligase required for embryonic development, neural tube closure and postnatal brain development. E3 ligases regulate proteostasis by controlling the stability and activity of cellular proteins, thereby coordinating signalling pathways essential for normal development. Variants in HECTD1 have been identified in individuals with neural tube defects, autism spectrum disorder, epilepsy and severe developmental conditions, although the clinical significance and functional consequences of many variants remain unknown. Computational analysis suggests that some variants destabilise HECTD1, while others may impair ubiquitin ligase activity, substrate recognition or the regulation of downstream signalling pathways. However, experimental evidence supporting these mechanisms is currently lacking. Understanding how HECTD1 variants affect protein function and neurodevelopment will provide insights into disease mechanisms and establish broadly applicable approaches for studying other NDD-associated genes. Research Question Determine whether and how HECTD1 variants alter cellular signalling networks during neurodevelopment. Objective 1: Define how disease-associated HECTD1 variants alter protein structure and function The student will investigate how disease-associated HECTD1 variants affect protein structure, stability and catalytic activity. Computational modelling will be integrated with biochemical and structural studies to determine whether variants act through protein destabilisation, altered conformational dynamics or impaired ubiquitin ligase function. Functional consequences will be assessed using established assays

	developed in the Licchesi laboratory, including reconstituted in vitro ubiquitination assays and cell-based protein degradation experiments. Variant stability and subcellular localisation will be quantified using microscopy, flow cytometry and immunoblotting. Objective 2: Investigate variant-specific mechanisms in human stem cell models CRISPR-Cas9 genome editing will be used to generate isogenic induced pluripotent stem cell (iPSC) lines carrying selected patient-derived HECTD1 variants. Particular emphasis will be placed on variants that do not exhibit substantial protein destabilisation, as these may reveal pathogenic mechanisms involving altered substrate recognition, signalling outputs or ubiquitin ligase activity. The project will benefit from expertise and methodologies established in the Ngo laboratory at Cardiff University for investigating neurodevelopmental disorder-associated genetic variation. These models will enable mechanistic studies in a physiologically relevant human system while establishing experimental pipelines applicable to a broad range of genetically defined neurodevelopmental disorders. Objective 3: Identify HECTD1 function in neural progenitor cells The identity of HECTD1 substrates remains largely unknown, particularly in neural cell types relevant to neurodevelopmental disorders. Human neural progenitor cells (NPCs) provide an attractive model because HECTD1 plays important roles during early neurodevelopment and neural tube formation. Using quantitative proteomics, interactome mapping and substratome analyses, the student will identify HECTD1-interacting proteins and ubiquitination substrates in wild-type NPCs and selected variant lines generated from the iPSC models established in Objective 2. Candidate substrates will be validated through interaction studies, ubiquitination assays and protein degradation analysis. These studies will define the molecular pathways regulated by HECTD1 and establish how variants may perturb proteostasis networks during neurodevelopment. Objective 4: Determine how early molecular defects influence neuronal network function To understand how molecular defects arising during development translate into neuronal dysfunction, selected isogenic HECTD1 iPSC lines will be differentiated into Glutamatergic neurons. Neuronal maturation, excitability and network activity will be assessed using multielectrode array (MEA) technology through an established collaboration with Dr Rob Williams (University of Bath). These studies will determine whether HECTD1 variants alter neuronal connectivity and synchronisation, thereby establishing a direct link between disrupted cell signalling and protein quality control pathways, impaired neurodevelopmental processes, and clinically relevant neurophysiological phenotypes. Objective 5: Validate disease mechanisms in zebrafish HECTD1 knock-in models Selected HECTD1 variants identified in patients with neurodevelopmental disorders will be introduced into zebrafish using genome-editing approaches. The predominantly heterozygous nature of these variants makes them particularly well suited for knock-in modelling. Zebrafish studies will assess neural tube development,

	embryonic brain formation and behavioural phenotypes, while providing an in vivo platform to test molecular pathways identified through the structural, proteomic and cellular studies. These experiments will establish mechanistic links between patient-derived genetic variation, disrupted developmental pathways and neurodevelopmental outcomes. Student Ownership and Opportunities to Steer the Project: This ambitious multidisciplinary project spans structural biology, proteomics, stem cell biology, electrophysiology and animal models. The student will play a central role in shaping the project by prioritising work packages, selecting variants for detailed investigation and adapting research directions as findings emerge. There will be flexibility to pursue the most promising mechanistic hypotheses and align aspects of the project with the student's developing interests, within an interdisciplinary and supportive supervisory environment that will ensure delivery within the PhD timeframe. The project will evolve alongside newly identified HECTD1 variants from clinical and public databases, and the student will have opportunities to engage with the HECTD1 patient community and disseminate findings.
Can be completed part time?	No
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