Project Details	
Project Code	MRCNMH25Br Hodge
Title	Towards a better understanding and treatments for the rare genetic brain disorder called CASK
Research Theme	Neuroscience & Mental Health
Summary	CASK is a protein found at synapses between neurons in the brain. Human mutations cause brain and behavioural problems including severe epilepsy, sleep, movement and learning disabilities. You will be trained to generate fly and cell-based models of CASK disease characterising them using cutting-edge molecular genetics, behaviour, electrophysiology, imaging, computational modelling and pharmacology. You will work with our clinical team and patient groups generating human CASK molecular and clinical data identifying new mutations and symptoms which you will then study using your fly and cell models allowing you to test novel genetic and drug treatments, prior to follow-on clinical trials.
Description	CASK (Ca2+/CaM-dependent serine protein kinase, a type of enzyme that switches on/off proteins and their signalling) is a synaptic scaffolding molecule (interacts with multiple proteins and coordinates synaptic signalling). Human CASK loss of function (LOF) or knock-out (KO or null) gene mutations (e.g. R639X, a stop mutation at arginine residue-639 of CASK) cause intellectual developmental disorder with microcephaly (small brain) and pontine and cerebellar hypoplasia (decreased growth of these brain regions) (MICPCH) and epilepsy. We have shown human CASK displays very high identity (74% amino acids are identical) to Drosophila CASK with expression of human CASK in CASK null (the gene is completed knocked-out) flies returning their memory loss and synaptic defects to normal. This indicates that fly and human CASK are orthologous (closely related gene with conserved function) having conserved neuronal function, validating their use as a model of CASK function in the healthy and diseased brain. Objective 1: To determine the contribution of different CASK mutations to disease relevant behavioural problems Hypothesis 1: CASK mutants will cause disease relevant phenotypes (similar symptoms as you see in human patients) in flies Based on the student steer they can help our team's CASK patient genomic (changes in DNA), methylomic (epigenetic changes in DNA) and transcriptomic (changes in gene expression) analysis led by Dr Doretta Caramaschi (Exeter), identifying novel molecular changes that can then be characterised using our proposed disease modelling approach (putting disease causing genes into cells and flies and looking at their effect). They will use our CASK null, RNAi loss-(gene knockdown, less gene made) and gain-of-function (overexpression) mutants and CRISPR (genome editing) mediated incorporation of T2A-Gal4-polyA (a DNA sequence which targets a specific gene and degrades it leaving a promoter or DNA sequence that allows you to express any gene of your choice) into the CASK gene of flies. The latter will generate a null allowing Gal4 responsive expression of different human CASK transgenes e.g. MICPCH null (R639X), allow the student to study the effect of the human mutant

	proteins in CASK expressing cells of flies without their own copy of fly CASK. We will test the effect of overexpression of human CASK, different domain constructs (mutant CASK lacking each protein interacting sequence or kinase) and mutants in different parts of the brain at different developmental times phenotypically characterising (seeing the effect of the mutation on the function, appearance and behaviour of) the flies related to symptoms seen in CASK patients: 1) Development, lethality and lifespan 2) Learning and memory difficulties 3) Movement 4) Seizures 5) Circadian rhythms and sleep Objective 2: To determine the CASK signalling and cellular pathways underlying behavioural deficits (what is the function of CASK in the neuron) Hypothesis 2: Disease causing CASK mutants will cause common cellular pathology Based on the T2A-Gal4 CASK expression and Gal4 mapping of CASK mutant phenotypes (you remove CASK from different parts of the brain to see their function), they will characterise the physiological changes of the relevant CASK neurons by: 1) Ca2+ signalling with GCaMP6f measuring Ca2+ signalling/neural activity using mushroom body memory neuron (part of the fly brain controlling memory) optogenetic imaging (using lasers to activate and then record brain activity). 2) Cell viability (cell health), proliferation (cell division) and neurodegeneration (cell death) assays. CASK affects proliferation causing cerebellar (part of the brain that controls movement and memory) hypoplasia with mutations also proposed to cause cerebellar neurodegeneration. We will use neurogenesis (neuron birth and growth) and neurodegeneration assays to look for changes in cell number of defined sets of neurons labelled by a given Gal4 promoter line expressing the different LOF and GOF CASK mutants e.g. central complex EB1 neurons part of the fly brain that controls movement, sleep and motor/place memory. Based on results and student's motivation they could characterize any neurogenesis or neurodegeneration further using live cell imaging including of mitochondria (cell energy factories), etc in Dr Gaynor Smith's lab (DRI, Cardiff) Objective 3: To determine therapeutic approaches to reverse CASK pathophysiology (correct what goes wrong in the disease) Hypothesis 3: Drosophila is an effective model to identify novel therapeutic approaches (flies can be used to discover new drugs for CASK disease) Use fly and human cells to identify if CASK and its interacting genes are good targets to screen for new drugs and genetic treatments. Prof Ben Housden (Exeter) will show the student how to use his novel screening experiments to identify (or screen) for genes, the proteins they make and drugs that correct the function of disease-causing CASK mutations. The student will generate fly and human CASK mutant cell lines and look

	at the effect on cell viability, proliferation/degeneration and Ca2+ signalling. They will test CASK as a potential drug target by screening drugs that switch on/off kinases like CASK seeing if they make CASK mutant cells and flies normal and healthy.
Supervisory Team	
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