FUTURENEURO UNDERGRADUATE PILOT
RESEARCH SUMMER STUDENTSHIP AWARD 2018

PROJECT LIST

Please indicate preferred Project No. and Title
on application form.

Project 1

Project Title: Characterisation of the RNA editing pathway in human epilepsy

PI: Prof David Henshall, RCSI.

Project Outline:
Epilepsy is a common, chronic brain disease characterized by recurrent seizures. The development of epilepsy following a brain injury is associated with select changes in gene expression. Identification of affected pathways may lead to novel treatments. Studies exploring the molecular mechanisms regulating gene expression have identified widespread editing of RNA in the brain, mediated by a family of enzymes (ADARs). Editing of noncoding RNAs, including microRNAs has also been reported in the brain which may influence targeting and direct certain RNAs into exosomes for extracellular release.

Objectives:
The objective of this summer project is to test the hypothesis that RNA editing is a novel patho-mechanism in epilepsy. Experiments will aim to identify cell type-specific changes in expression of enzymes in the RNA editing pathway in the brain.

Methodology:
The student will work within the RCSI-based research group working on cell and molecular mechanisms of epilepsy. They will be trained and perform techniques including handling and processing of brain tissue, extraction of proteins and RNA, histology and microscopy.

Expected Outcomes:
The results from this research project will establish evidence for the RNA editing pathway in epilepsy. The student will gain expertise in a variety of useful research techniques and gain a deep understanding of patho-mechanisms of brain disease.

Potential to form basis of PhD application:
RNA editing is an emerging area of major significance in our understanding of how gene expression is controlled. This project offers an opportunity to develop preliminary data that could support a major investigation of how this process influences the molecular landscape of the epileptic brain. The project would lead to more extensive studies employing techniques to characterize at fine molecular level the edited transcripts and use genetic models to selectively interfere with editing and examine impacts on brain excitability that could lead to novel treatment approaches for epilepsy.

Project 2

Project Title: Using next generation sequencing to characterise somatic mutations as a cause of epilepsy.

PI: Prof Gianpiero Cavalleri, RCSI

Outline: A molecular diagnosis provides the platform for precision therapeutics in epilepsy and other neurological disorders. Next generation sequencing (NGS) is revolutionising our ability to identify disease-causing mutations, and a molecular diagnosis is now achievable in a significant proportion of people with epilepsy. However, for the majority of people with epilepsy, a state-of-the-art genetic test conducted on blood extracted DNA does not point to a clearly identifiable, pathogenic mutation. In such cases, it could be that the epilepsy is multifactorial and complex in aetiology, or it could be that the genetic cause is somatic, and specific to epileptogenic area of the brain.

Objectives: The objective of this project is to identify tissue specific, somatic mutations in epileptogenic tissue resulting from the surgical treatment of epilepsy.

Methodology: The student will learn how to conduct NGS library preparations, they will gain experience with Illumina sequencing technology, and they will run bioinformatic pipelines to identify and annotate somatic mutations.

Potential to form basis of PhD application: The expected outcome of the project is to generate results and outline research questions that could be expanded to a PhD. Long term, the aim is to develop genomic testing as a tool for the pathological assessment of epilepsy.

Project 3

Project Title: Cerebrovascular contributions to seizure development and epilepsy

PI: Prof Matthew Campbell, TCD

Outline: The blood-brain barrier (BBB) positioned along blood vessels of the central nervous system is one of the most selective and tightly regulated barriers, reflecting the brain's critical roles in cognitive function, maintaining homeostasis and strictly coordinating the functions of peripheral organs. The BBB is important in regulating the exchange of ions and nutrients between the blood and brain but also to protect delicate neural tissue from potentially damaging blood-borne agents such as pathogens, immune cells and anaphylatoxins [1]. At the BBB, claudin-5 is the most enriched tight junction protein that is critical to the barrier function of microvascular endothelial cells [2]. Recently, we have generated a doxycycline-inducible claudin-5 "knockdown" mouse and shown that chronic suppression of claudin-5 induces behavioural changes in mice with impairments in learning and memory, anxiety-like behaviour and sensorimotor gating. Additionally, these mice develop tonic-clonic and absence-like seizures and die within 4-5 weeks of persistent claudin-5 suppression [3], reinforcing the crucial role of claudin-5 in maintaining normal neurological function. BBB disruption has been associated with seizures in both congenital disorders such as GLUT-1 deficiency and in acquired disorders such as those resulting from traumatic brain injury (TBI) [4, 5]. However, there is much debate over whether BBB breakdown occurs before, during or after seizure or whether a compromised BBB is a component of the aetiology of epilepsy or a consequence of seizure activity. Therefore, this project will investigate the role of the BBB and specifically tight junction components in the development of seizure activity in mice.

Objectives: Characterisation of changes in tight junction protein and mRNA expression following status epilepticus in C57BL/6 mice. Assess changes in BBB permeability to systemically injected tracer molecules (600 Da, 1, 3, 10 kDa) following status epilepticus.

Methodology: Successful applicants will be trained in various techniques well established in the lab including: tissue dissection, capillary isolation, western blot and qRT-PCR analysis, immunohistochemistry and confocal microscopy.

Expected outcomes: Successful completion of this project will provide valuable information on the dynamic changes in the expression and localisation of tight junction components and BBB integrity in the development of seizures in rodent models.

Potential for PhD application: Following completion of the project, the individual will have obtained numerous skills and techniques essential for a PhD in molecular biology. In addition, results obtained from the project will form the focus of a PhD investigating the therapeutic potential of drugs that modulate the BBB to restore normal neural homeostasis in epilepsy but also in other neurological disorders such as Alzheimer's disease, schizophrenia and TBI.

References:

1. Abbott, N.J., et al., *Structure and function of the blood-brain barrier.* Neurobiol Dis, 2010. **37**(1): p. 13-25.
2. Daneman, R., et al., *The mouse blood-brain barrier transcriptome: a new resource for understanding the development and function of brain endothelial cells.* PLoS One, 2010. **5**(10): p. e13741.
3. Greene, C., et al., *Dose-dependent expression of claudin-5 is a modifying factor in schizophrenia.* Mol Psychiatry, 2017.
4. De Giorgis, V. and P. Veggiotti, *GLUT1 deficiency syndrome 2013: current state of the art.* Seizure, 2013. **22**(10): p. 803-11.
5. Friedman, A., *Blood-brain barrier dysfunction, status epilepticus, seizures and epilepsy: a puzzle of a chicken and egg?* Epilepsia, 2011. **52**(Suppl 8): p. 19-20.

Project 4

Project title: Technology enabled medication adherence improvement in patients with epilepsy

PI: Prof Colin Doherty

Outline: Epilepsy is a chronic disease characterized by unpredictable, sometimes lifelong, often dangerous seizures which result in involuntary alterations in behaviour and consciousness. The condition affects about one in every 100 people and is second only to stroke as the commonest chronic neurological disorder in Europe. Of the 40,000 sufferers in Ireland, only about 70% are well controlled on medication, leaving about 12-15,000 people who have breakthrough seizures, and are in regular contact with secondary and tertiary hospital services. Many patients with epilepsy suffer from mental health problems, and the condition has significant implications for social, vocational and occupational aspirations.

The treatment of chronic illnesses like epilepsy commonly includes the long-term use of pharmacotherapy. Although these medications are often effective in combating disease, their full benefits may not be realized because approximately 50% of patients do not take their medications as prescribed. Factors contributing to poor medication adherence are myriad and include those that are related to patients (e.g., suboptimal health literacy and lack of involvement in the treatment decision–making process), those that are related to physicians (e.g., prescription of complex drug regimens, communication barriers, ineffective communication of information about adverse effects, and provision of care by multiple physicians), and those that are related to health care systems (e.g., clinic visit time limitations, limited access to care, and lack of health information technology). Because barriers to medication adherence are complex and varied, solutions to improve adherence must be multifactorial. This project aims to try and understand the key elements of medication adherence difficulties in an Irish cohort of chronic disease patients and address them with technology enabled solutions in mind.

Objectives: To determine the role of medication adherence in suboptimal outcomes in patients with epilepsy and then use ethnographic and co-design methodology to determine how mobile and other technologies may help to improve both adherence and outcomes.

Methodology: Building on a clinical audit in St James's department of neurology, we have identified key characteristics of patients who are at risk of medication adherence problems. We intend to continue to increase the size of the sample studies in the audit over the summer and invite participants to be part of a participatory action research groups to develop the co-design workshops. We will use ethnographic methodologies to do in-depth interviews with at risk groups and indeed those who have high adherence rates to see if we can determine granular data on this subject.

Expected Outcomes: Complete the audit of patient adherence in James's and set up the PARs and co-design groups generate for the audit data.

Potential to form basis of PhD application:
This project aligns with FutureNeuro plans for the development of an eHealth platform for patients with epilepsy. We feel that the creation of the PARs and the expansion of the audit data will be a substantial start to a PhD in this area.

Project 5

Project Title: High Sensitivity Companion Diagnostic Device

PI: Prof Robert Forster, DCU

Outline: Our strategy to maintaining the optimum drug release profile is to ***measure the concentration of disease biomarkers*** that correlate with treatment efficacy using a microfluidic device with revolutionary sensitivity. This information can then be used to optimise the drug delivery profile in terms of concentration and time. To inform the full translation highway, the team includes world leading materials scientists, theoreticians and clinicians.

Objectives: To develop **High Sensitivity Assays for Drug Efficacy Biomarkers** consisting of a simple, self-contained microfluidic device capable of detecting biomarkers of disease and drug efficacy in a small drop of patient's blood at low concentrations.

Methodology: We will work closely with Prof. Henshall to create a microfluidic device for the ultrasensitive detection of *markers of drug efficacy in epilepsy.* The biomarker panel will focus on miRNA that will be detected using wireless electrochemiluminescence (ECL, light emission from an excited state created by electrogenerated reactants) within a novel displacement assay format implemented in a microfluidic device. Specifically, electronically conducting particles, loaded with custom synthesised electrochemiluminescent complexes and functionalised with nucleic acid probe strands will be produced. The particles are initially bound to a capture surface, but are displaced by the biomarker due to its higher affinity (fully complementary to capture strand) for the capture surface. Light is then generated from the reaction of the oxidised electrochemiluminescent dye *within* the particle reacting with a co-reactant, e.g., tripropyl amine or oxalate, in the carrier buffer. This detection strategy represents a significant step beyond the current state-of-the-art in electrochemiluminescent assays we and others have reported previously.[i,ii,iii]

Expected Outcomes: The **companion diagnostic** will allow the drug delivery rate and dosage to be optimised resulting in enhanced drug efficacy and reduced side effects. Unlike traditional assays where light is generated only at the electrode solution interface, ***light can be generated from particles anywhere in the channels of the microfluidic device dramatically enhancing detection sensitivity***. Moreover, ***different biomarkers can be detected simultaneously***. We expect that digital assays for nucleic acid biomarkers will bring about a similar paradigm shift to that of digital PCR in DNA sequencing.

Potential to form basis of PhD application: The project will generate the preliminary data to support an IRC application and would position the student very strongly to participate in an EU Training Network. This technology oriented project would also open up the possibility of joint IRC-industry co-fund applications.

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[i] *Highly Sensitive Detection of NADH Using Electrochemiluminescent Metallopolymer-Gold Nanocomposites*, Devadoss A., Dickinson C., Keyes T. E., Forster R. J., *Electrochem. Comm*. 2012, 19, 43-45.

[ii] *High Sensitivity Carbon Nanotube Based Electrochemiluminescence Sensor Array*, Venkatanarayanan A., Crowley K., Lestini E., Keyes T. E., Rusling J. F., Forster R.J. , *Biosens. and Bioelectron*. 2012, 31(1), 233–239.

[iii] *Nanostructured Materials for Electrochemiluminescence (ECL)-based Detection Methods: Recent Advances and Future Perspectives*, Bertoncello P., Forster R. J., *Biosens. and Bioelectron.*, **2010**, Vol. 24(11), 3191-3200