**FUTURENEURO UNDERGRADUATE**

**RESEARCH SUMMER STUDENTSHIP AWARD 2019**

**PROJECT LIST**

Please indicate preferred Project No. and Title

on application form.

**Project 1**

**Project Title:** **Characterisation of astrocyte dysfunction in epilepsy**

**PI:** [**Prof David Henshall, RCSI**](https://www.futureneurocentre.ie/team/david-henshall/) and [**Dr Janosch Heller, RCSI**](https://www.futureneurocentre.ie/team/janosch-heller/)**.**

**Project Outline:**

Epilepsy is a common, chronic brain disease characterized by recurrent seizures. A universal hallmark of epileptic tissue is astrocyte dysfunction which has not received much attention as a therapeutic target. In the healthy brain, astrocytes adopt a sponge-like morphology, with thousands of fine protrusions that contact blood vessels (astrocytic endfeet) and enwrap ~60% of excitatory synapses across the brain (tripartite synapses). Three key roles of astroglia: rapid uptake of neurotransmitters such as glutamate, extracellular potassium buffering and release of gliotransmitters are disturbed in disease, leading to increased extracellular glutamate and potassium concentrations, which in turn mediate further neuronal depolarisation and seizures. It was shown recently that astrocytes locally translate mRNA transcripts of receptor and transporter genes in their peripheral processes. However, it is not known whether the translation of these transcripts is regulated by locally-acting miRNAs and whether this process is deregulated in disease.

**Objectives:**

The objective of this summer project is to visualise astroglia in epileptic tissue. Moreover, experiments will aim to identify miRNAs in astrocyte processes and their potential deregulation in disease.

**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, including super-resolution techniques.

**Expected Outcomes:**

The results from this research project will establish evidence for miRNA control of local translation in astrocyte processes. 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:**

Astrocytes are essential players in the healthy brain and their dysfunction is a hallmark of many brain diseases. Hardly anything is known about control of local translation in astrocyte processes. This project offers an opportunity to develop preliminary data that could support a major investigation of how local translation in astrocytes is altered in the epileptic brain. The project would lead to more extensive studies employing techniques to characterize miRNA-targeted transcripts and use molecular probes to selectively interfere with locally acting miRNAs that could lead to novel treatment approaches for epilepsy.

**Project 2**

**Project Title: Integrating polygenic risk scores with exome data to help decipher the genetic basis of the epilepsies.**

**PI:** [Prof Gianpiero Cavalleri, RCSI](https://www.futureneurocentre.ie/team/gianpiero-cavalleri/)

**Outline:** The epileptic encephalopathies represent a rare and devastating forms of epilepsy that begin in infancy and are accompanied by cognitive dysfunction and higher rates of treatment-resistant seizures. They typically present without any family history of epilepsy. A significant proportion of EEs are known to be monogenic, caused by *de-novo* mutations of large effect, not seen in the general population, or by rare, highly pathogenic recessive-acting variants. Exome sequencing is currently able to provide a molecular diagnosis of a single, causative mutation in 30-40% of EE patients. The highly deleterious nature of the confirmed pathogenic mutations, coupled with their low population frequencies, suggests that they are disease-causing on any genetic background, independent of any common genetic burden for epilepsy or other neurological diseases. However, similar mutations are often seen in EE cases which have quite different co-morbidities (e.g. autism spectrum disorder or ADHD). This has led us to hypothesise that background polygenic burden can influence the nature of comorbidities that present with some monogenic forms of epilepsy.

**Objectives:** The objective of this project is to quantify, using a technique known as polygenic risk scoring, genetic burden at the individual level for a variety of epilepsy-related co-morbidities, and then test whether polygenic burden correlates with patient phenotypes.

**Methodology:** The student will learn a range of analytical techniques required to handle and annotate large datasets of next-generation sequence data, as well as how to calculate polygenic risk scores from GWAS data, and related statistical analysis. The student will have the opportunity to work with large genomic datasets, in the strong and supportive environment provided by the FutureNeuro Research Centre.

**Potential to form basis of PhD application:** We are seeking a student with background and/or strong interest in analytics and data science. 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 the range of applications of genomic testing in the neurology clinic.

**Project 3**

**Project Title: Investigation of tRNA fragment complexes in ALS and Epilepsy**

**Supervisor:** [Dr Marion Hogg, RCSI.](https://www.futureneurocentre.ie/team/marion-hogg/)

**Affiliated PIs:** Prof’s Henshall & Prehn, RCSI.

**Outline:** Transfer RNAs (tRNAs) function during protein translation to coordinate amino acid addition to nascent polypeptides according to the codon specified by the mRNA. However during stress conditions tRNAs can be cleaved to generate tRNA fragments. We have recently identified specific tRNA fragments that are elevated in pre-seizure plasma samples from epilepsy patients. In a separate study we have also identified a different set of tRNA fragments that are elevated in ALS mouse models and ALS patients with a slow progressing form of the disease. We currently investigate these novel non-coding RNA fragments as blood-based biomarkers of neuronal health. However, we know little about the proteins that interact with these fragments, and specifically how they are protected from degradation in blood, which is known to contain high levels of RNases.

**Objectives:** Here we aim to define the protein complexes that protect tRNA fragments from degradation in biofluid samples from epilepsy and ALS models.

**Methodology:** We will investigate secretion pathways in cell culture model systems using pharmacological blocking agents. Additionally we will fractionate media samples and monitor tRNA fragment levels across the gradient. We will then purify total protein from tRNA fragment containing fractions for LC-MS analysis of protein content. Candidate protein interactions will be validated by targeted immunoprecipitation and qPCR. Once established *in vitro,* interactions will be validated with *in vivo* samples from epilepsy and ALS models.

**Expected outcome:** By the end of the project, we expect to fully define extracellular protein complexes responsible for trafficking tRNA fragments and gain information on secretion pathways. This will increase our understanding of this novel class of RNAs and inform tests currently under development for quantifying tRNA fragments as biomarkers in ALS and epilepsy.

**Potential to form basis of PhD application:** The molecular biology skills utilised in this project will provide a student with a valuable basis to continue on to PhD training. The area of non-coding RNA biology is rapidly expanding and forms the basis of several research groups within FutureNeuro. Research into tRNA fragments as biomarkers of neurological disorders is a priority area within FutureNeuro and we hope to find an engaged and motivated student to help us investigate this exciting new research field.

**Project 4**

**Project Title: Molecular mechanisms of blood-brain barrier dysfunction and repair in Epilepsy**

**PI/supervisor:** [Dr Cristina R Reschke, RCSI.](https://www.futureneurocentre.ie/team/marion-hogg/)

**Affiliated PIs:** Prof’s Henshall, RCSI and Campbell, TCD.

**Project Outline:**

Epilepsy affects up to 65 million people worldwide and about one-third of patients do not respond to current treatments. A crucial step towards developing better treatment options is to improve our understanding of the pathogenic mechanisms underlying the disease. Acute injuries to the brain and certain chronic neurological diseases, such as epilepsy, are associated with blood-brain barrier (BBB) disruption. The impairment of the BBB, which is a complex and specialised structure critical for maintaining brain homeostasis, allows passage of molecules and cells normally excluded into brain tissue. This is thought to cause inflammation and promote neuronal dysfunction. Moreover, when seizures occur they may directly open the BBB and further promote molecular changes that contribute to an enduring state of hyper-excitability. The pathogenic mechanisms underlying BBB dysfunction are not yet fully understood, however, recent findings have highlighted a role for a group of small non-coding RNAs called microRNAs (miRNAs). miRNAs are known to regulate gene expression in epilepsy and some epilepsy-associated miRNAs are predicted to target tight junction (TJ) genes in the BBB. TJ proteins are key regulators of BBB integrity and miRNA-mediated downregulation of these genes could promote BBB dysfunction.

**Objectives:**

The aim of this project is to investigate the role of epilepsy-relevant miRNAs in mediating BBB impairment.

**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 cell culture model system, handling and processing of brain tissue, extraction of proteins and RNA, Western Blotting, immunohistochemistry and microscopy.

**Expected Outcomes:**

The results from this research project will establish evidence for the role of miRNAs in BBB disruption during epileptogenesis. The student will gain expertise in a variety of useful research techniques and gain a deep understanding of patho-mechanisms of epilepsy.

**Potential to form basis of PhD application:**

The pathogenic mechanisms underlying BBB dysfunction in epilepsy are poorly understood, and a number of key questions remain unanswered. This project offers an opportunity to develop preliminary data that could support a major investigation. The PhD training will focus on the central hypothesis that microRNAs contribute to the altered expression of tight junction genes that promotes epilepsy via BBB dysfunction. Accordingly, we will investigate whether restoring expression of these genes or interference in the microRNAs could repair the BBB and produce disease-modifying effects in epilepsy.

**Project 5**

**Project Title:** **Examination of screen time during clinician-patient encounters.**

**PI: Dr Colin Doherty**

**Project Outline:**

‘eHealth’ is an umbrella term incorporating any area that combines healthcare and technology to improve efficiencies and reduce costs. The proliferation of internet and mobile technologies globally have resulted in major changes to how healthcare is delivered. A notable eHealth innovation are electronic health records (EHR). With the rising use of EHR, concerns have emerged about increased computer use during clinician-patient encounters. The primary worry is that increased computer use will negatively affect the clinician-patient relationship and decrease patient and clinician satisfaction. The eHealth Research Team of FutureNeuro and clinicians in the Dublin Mid Leinster Epilepsy Service wish to examine how the Epilepsy Electronic Patient Record (EEPR) is shaping clinical encounters with patients. The successful applicant to this position will examine how long clinicians are utilising the EEPR during patient encounters and if the use of EEPR and IT affects clinician and patient satisfaction.

**Objectives:**

This project aims to:

* Assess the feasibility of collecting screen time data in real time.
* Explore how the use of the Epilepsy Electronic Patient Record (EEPR) during encounters affects the amount of time clinicians spend looking at their computer screen rather than at the patient.
* Determine if the amount of computer use affects clinician and patient satisfaction.

**Methodology:**

Data will be collected from clinics in St. James’s and Beaumont Hospitals. The researcher will record various timing aspects of epilepsy clinical encounters and subsequently perform data analysis. A questionnaire will be designed and implemented to measure patient and clinician satisfaction as it relates to screen time.

**Expected Outcomes:**

This research will produce valuable information about numerous elements of clinical encounters from the perspective of clinicians and patients. The results obtained will inform if screen time and the rising use of EHR’s are impacting various aspects of the clinician-patient relationship.

**Potential to form basis of PhD application:**

This project offers the opportunity to generate preliminary data in a largely unexplored aspect of epilepsy care. The impact digital technology has on the numerous elements of epilepsy healthcare delivery is a priority research area of FutureNeuro. We hope to find a motivated and hard-working student to help us investigate the rapidly expanding field of eHealth.