Lysosomal Diseases Research Unit

The Lysosomal Diseases Research Unit is a large, well-established, multidisciplinary team of scientists, research assistants and students, and is a world leader in lysosomal disorder research. The Unit has been extremely successful at both basic science and translating this to successful clinical and commercial outcomes.

We are investigating a naturally occurring, neonatally lethal, neuronopathic sheep model of Gaucher disease (1). This is the most common lysosomal storage disorder, arising from mutations in the β-glucocerebrosidase gene that lead to the functional loss of the lysosomal enzyme, β-glucocerebrosidase, and the cellular accumulation of glucocerebroside. Our research is directed towards understanding the fundamental biological processes that influence and drive the process of neurodegeneration in Gaucher disease and other neurodegenerative disorders, such as Parkinson’s disease.

Honours Project

Understanding the pathogenic role of ganglioside metabolism in Gaucher disease

Cellular membranes are made up of lipids and proteins. The three major classes of membrane lipids are phospholipids, glycolipids, and cholesterol. Gangliosides are sialic acid-containing glycosphingolipids that are mainly localised in the outer leaflets of plasma membranes and are integral components of cell surface microdomains (lipid rafts); together with sphingomyelin and cholesterol they participate in cell-cell recognition, adhesion, and signal transduction. Ganglioside metabolism is altered in several neurodegenerative diseases, with most changes occurring in cellular membrane microdomains or lipid rafts (2-4). Functional studies on alpha-synuclein show a strong association between gangliosides and α-synuclein, the aggregation of which is implicated in many neurodegenerative disorders, including Parkinson disease (5-6).

This project focuses on examining the role that gangliosides play in the pathogenic mechanisms that underpin neurodegeneration, and will utilise samples from the sheep model.

Students can expect to gain experience in techniques such as sample preparation, lipid extractions, percoll gradients, western blot analysis and mass spectrometry.

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Honours Project

**Cloning, expression, purification and characterisation of the most common glucocerebrosidase mutations using the sheep model**

The most common GCase mutations that give rise to Gaucher disease are N370S and L444P(2). The aim of this project is to recombinantly express, purify, and characterise these mutations, and introduce them into the sheep \(\text{GBA}1\) gene, followed by expression of the sheep GCase mutants in mammalian cells. Purification, followed by enzyme characterisation of each mutant will be performed and compared to the wild-type enzyme.

Students can expect to gain experience in techniques such as site-directed mutagenesis, cloning, cell culture techniques, protein purification, and protein characterisation.

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Honours Project

microRNA Biomarkers for Parkinson’s disease

Mutations in the GBA1 gene are recognised as the leading genetic risk factor for Parkinson’s disease (PD) and related Lewy body disorders. PD frequency is increased amongst heterozygous GBA1 mutation carriers, with an approximate 5-fold increase in mutation prevalence amongst PD population cohorts(2,3).

MicroRNAs (miRNAs) are short RNA molecules that regulate gene expression at the post-transcriptional level by either suppressing translation or inducing messenger RNA (mRNA) degradation. Expression of microRNAs (miRNAs) is altered in many disease states, including neurodegeneration, and their detection in biofluids has made them ideal candidates as potential biomarkers for neurodegenerative diseases. Many brain-associated miRNAs have been implicated in PD(4).

This project aims to investigate disease progression in GBA1 heterozygous Gaucher sheep by examining miRNA expression in different brain regions, plasma and CSF as the animal ages.

Students can expect to gain experience in techniques such as RNA extraction and real-time PCR.

References
CNS Therapeutics Section
Lysosomal Diseases Research Unit

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The CNS Therapeutics Section primarily utilises a mouse model of a neurodegenerative lysosomal storage disorder known as mucopolysaccharidosis (MPS) type IIIA(1,2). Other disease models are also being established. MPS IIIA is presently untreatable and so we are examining the cascade of neuropathological changes that occur within the MPS IIIA brain as a result of the accumulation of substrate, and their response to experimental treatments(3). Gaining a better understanding of how the disorder manifests will permit effective application of therapies.

Honours Project

Elucidating the basis of cognitive dysfunction in a murine model of a paediatric neurodegenerative disorder.

This project will examine the anatomical and physiological basis for the development of cognitive disease in the MPS IIIA mouse model. Both structural and neurochemical changes occurring in discrete brain regions during development will be evaluated in order to determine their contribution to cognitive dysfunction.

Students can expect to gain experience in the handling and use of a viral (gene therapy) vector, stereotaxic surgery, mouse handing, post-surgical monitoring and post-mortems, immunohistochemistry using epifluorescence, and confocal microscopy-based imaging and analysis. Molecular and biochemical assays may also be utilised.

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The Mass Spectrometry Facility offers core analytical chemistry services within SAHMRI. The Facility also has an active role in the Lysosomal Diseases Research Unit’s research program using high end lab equipment to study lysosomal disorders.

Honours Project

MALDI-MS imaging to aid the understanding of pathological changes in lysosomal storage disorders.

MALDI (Matrix-Assisted Laser Desorption/Ionisation) mass spectrometry imaging is a powerful technique for determining the spatial distribution of molecules in tissues. A particular advantage of this technique is the ability to map the distribution of specific small molecules, such as lipids, in tissue.

This project focuses on the development of a reproducible MALDI-MS imaging method for the analysis of both endogenous and exogenous small molecules in tissue sections from multiple models of lysosomal storage diseases, predominantly focusing on Gaucher disease. The project includes optimisation of sample preparation techniques, both manual and robotic methods; imaging data will be used in conjunction with analytical techniques, including liquid chromatography-mass spectrometry (LC-MS) and histology to further the understanding of pathological changes associated with disease.

Students can expect to gain experience with sample preparation methodologies, cryosectioning, histological staining, MALDI-MS imaging, ion mobility mass spectrometry, liquid chromatography, qualitative and quantitative mass spectrometry and statistical data analysis. Knowledge of these techniques will lead to a solid understanding of applied biological mass spectrometry and will be an excellent grounding in good experimental design and procedure.

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Honours Project

Biomarker discovery in neuronopathic Gaucher disease

Biomarkers are compounds that show disease-specific changes in patient samples, i.e. being more or less concentrated than in healthy specimens. These markers can be used in a number of different ways, e.g. in diagnosis and prognosis, as well as monitoring treatment efficacy. The discovery of changes in molecules hitherto unknown to be involved in the disease cascade can also lend new insights into the fundamental biochemical processes underlying the disease.

This project will utilise samples from a naturally occurring, neuronopathic, neonatally lethal, sheep model of Gaucher disease. An untargeted metabolomics approach (liquid chromatography coupled to mass spectrometry (LC-MS)) will be used to study serum and cerebrospinal fluid samples. In this type of experiment, full chemical fingerprints from different samples are compared statistically. As this tactic is, in theory, un-biased, previously unknown chemical differences between groups may be unearthed, providing the opportunity to gain new insights into the biochemical mechanisms involved in disease progression and identification of potential new biomarkers.

Students can expect to develop laboratory skills using high end laboratory equipment, sample handling and preparation. There is also a strong focus on good experimental design and careful evaluation of experimental data.
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Honours Project

Identification of biomarkers for mucopolysaccharidosis type IIIA (MPS IIIA)

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An untargeted metabolomics approach (liquid chromatography coupled to mass spectrometry (LC-MS)) will be used to study serum and cerebrospinal fluid samples. In this type of experiment, full chemical fingerprints from different samples are compared statistically. As this tactic is, in theory, un-biased, previously unknown chemical differences between groups may be unearthed, providing the opportunity to gain new insights into the biochemical mechanisms involved in disease progression and identification of potential new biomarkers.

Cerebrospinal fluid and serum samples from MPS IIIA animal models will be subjected to appropriate pre-treatment. Data will be generated by LC-MS. Rigorous statistical data analysis will be employed to determine differences between sample sets.

Students can expect to develop laboratory skills using high end laboratory equipment, sample handling and preparation. There is also a strong focus on good experimental design and careful evaluation of experimental data.

References
Lysosomal Diseases Research Unit

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We are investigating novel treatment mechanisms for the neurodegenerative lysosomal storage disorder, mucopolysaccharidosis (MPS) type IIIC. The disorder arises from a deficiency in the integral lysosomal membrane enzyme, heparan acetyl CoA: α-glucosaminide N-acetyltransferase (HGSNAT), which ultimately leads to the incomplete degradation of its substrate heparan sulphate. As a multipass transmembrane protein, current treatment approaches for other lysosomal storage disorders are not amenable to MPS IIIC. Furthermore, although a ubiquitous enzymatic deficiency, MPS IIIC is a neurological disorder, so any systemically-administered therapy will also need to cross the blood brain barrier, thus providing a further complication to effective treatment.

Honours Project

Development of a therapy for MPS IIIC
This project will investigate novel approaches by which to restore HGSNAT function in MPS IIIC cells. The efficacy of these techniques will be determined primarily via measuring the presence of lysosomal HGSNAT activity and protein within the recipient cells, and ultimately a reduction in the molecular phenotype of MPS IIIC, as exemplified by a decrease in the levels of cellular heparan sulphate.

Students can expect to gain experience in molecular and cellular biology such as DNA cloning and manipulation, tissue culture, generation of stable cell lines to express exogenous proteins, immunoblotting, in vitro activity assays, immunofluorescence, confocal microscopy, and mass spectrometry.

This project has relevance to treatment for other disorders that result from mutations in integral membrane proteins, such as cystic fibrosis, and are thus equally difficult to effectively treat.

References