QMB AWCBR Rare Genetic Diseases of the Brain Abstracts

R1 From support to research – or are they intertwined?

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The story behind Motor Neurone Disease New Zealand's decision to become a recognised supporter of New Zealand research in to Motor Neurone Disease.
On average the life expectancy of people with Motor Neurone Disease from diagnosis is 2-5 years. And during that time those people are likely to be faced with relentless and progressive functional loss. Few have time or energy to raise awareness or funds. Most families, after having lived with the heartbreak of watching their loved one slowly robbed of life, do not want involvement with the Association afterwards.
MND New Zealand is a not-for-profit organisation with limited financial resource. Its contract for service with the Government only provides around 7% of its income. MND New Zealand has always struggled to have sufficient funding to maintain its core service delivery to people living with motor neurone disease, their families and carers, and information to the health professionals who support them.
What then convinced us that contributing to research would be worthwhile?
How could we ensure that the modest funds we had available would make a meaningful contribution?
And how are we ensuring our policy remains relevant?

R2 Kearns Sayre syndrome – a Dad's Perspective

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I'm a physician, a senior lecturer, public speaker, GP, Harley-rider, but above all, a Dad.
Poppy is my little girl. She has Kearns Sayre syndrome (and yes ... I had to look it up too)
This is Poppy's story ... she can't tell it herself anymore, and I struggle too. Her story is one of lessons for us all - as carers, doctors, nurses, therapists, but crucially as parents of children who need our advocacy, support, protection, and most importantly our love.

R3 Patients, Research and NZORD

Dr Collette Bromhead, Chief Executive NZ Organisation for Rare Disorders

The NZ Organisation for Rare Disorders (NZORD) was established in September 2000 and is the only national organisation supporting all New Zealanders who live with a rare condition, and the people who care for them. Our enquiries line, website (www.nzord.org.nz) and 100+ support group networks allow patients to access knowledge quickly. NZORD works with the Government, researchers, clinicians and industry to promote research, diagnosis, treatment and services. In this brief talk I wish to highlight the value of biomedical research to rare disease patients who may not currently have access to anything other than symptomatic treatments. I will talk about the need for a national registry for all rare disorders as well as NZORDs upcoming initiatives to improve connections and collaborations between local researchers.

R4 Funding medicines for rare disorders: PHARMAC's revised policy settings and processes

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[1]PHARMAC (Pharmaceutical Management Agency)

In 2014 PHARMAC trialled a contestable funding pilot for medicines for rare disorders. The pilot demonstrated that the PHARMAC model was successfully able to promote competition amongst suppliers of these medicines and, as a result, 10 new medicines for treatments of rare disorders have been approved for listing on the Pharmaceutical Schedule in New Zealand.

Following the evaluation of the pilot, PHARMAC has now introduced of a set of dedicated features for considering funding of medicines for the treatment of rare disorders. This includes the establishment of a rare disorders clinical advisory subcommittee, a regular call for funding applications for medicines for rare disorders and the adoption of adjusted policy settings.

PHARMAC's adoption of policy settings for considering funding of medicines for rare disorders acknowledges that there can be limitations for these medicines entering the New Zealand market. These changes will better enable rare disorders medicines to be considered for funding within PHARMAC's decision-making process.

R5 Protein aggregation and templating

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Protein intracellular inclusions within the central nervous system are hallmarks of several progressive neurodegenerative disorders in humans. The protein constituents of those deposits and the affected regions within the brain differ from one neurodegenerative disorder to another. Until recently, the vicious circle consisting of spread, seeded assembly and accumulation over time within the central nervous system of misfolded proteins aggregates was thought to be restricted to the prion protein PrP which aggregation causes in humans Creutzfeldt-Jacob disease. Recent reports suggest that other protein aggregates spread and amplify within the central nervous system leading to distinct diseases (e.g. Alzheimer, Parkinson, Huntington diseases).

I will present data illustrating the propagation propensities of alpha-synuclein assemblies and compare these properties to that of Huntingtin Exon 1 aggregates. I will describe how pathogenic protein assemblies bind to the neuronal cell membranes, what they bind to, how they evolve with time after their transition from a 3D space, the extracellular space to a 2D space, the membrane. I will also show the cellular consequences of their binding to the plasma membrane. I will present a quantitative assessment of fibrillar alpha-synuclein and Huntingtin Exon1 uptake, transport and export by neurons grown in microfluidic devices that mimic an extremely basic brain on a chip. I will show data demonstrating that exogenous pathogenic protein assemblies localize to the endo-lysosomal cellular compartment and that minute amounts reach the cytosol where they amplify by seeding the aggregation of their soluble counterpart.

The aggregation of alpha-synuclein leads to different diseases. How the aggregation of one given protein leads to distinct diseases is a fascinating and mysterious topic. I will show that this is the consequence of assembly into distinct fibrillar polymorphs that have different intrinsic architectures, stability, seeding propensity and surfaces.

R6 DNA and histone methylation changes in Huntington's disease

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Huntington's disease (HD) is a neurodegenerative disorder caused by a CAG repeat in the *huntingtin* gene. Despite the genetic basis of the disease, genetically identical twins with HD exhibit variation in the age of onset, extent of cortical cell loss and symptom profiles, demonstrating a role for epigenetic factors altering disease-associated pathogenesis and clinical presentation[1-3]. This is supported by studies of transgenic HD animal models showing that environmental factors have a substantial influence on the extent of neurochemical degeneration in the basal ganglia and on symptom progression[4]. To investigate epigenetic changes in post-mortem HD brain, we utilised Illumina HM450K beadchips, to measure methylation at CpG sites across the entire Human Genome. We identified 33 differentially methylated loci in HD cases (n = 12) compared to controls (n = 12). Of particular interest, we found methylation at an intronic locus of SMYD2 to be significantly reduced in Huntington's disease. This gene encodes a histone methyltransferase, which has previously been shown to be increased in Huntington's disease and found to interact with mutant huntingtin[5]. We also found global di-methyl histone H3 levels to be significantly increased in HD cases. These findings provide novel targets for future experiments and therapeutic interventions in the context of HD.

References

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Immune and gene-based therapeutic approaches for ALS and SMA

Haidar, M.[1], Perera, N.D.[1], Sheean, R.K.[1], Smith, K.S.[2], Jones, L.[2], Talbot, K.[3], Rogers, M.L.[2], Turner, B.J.[1]

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Widespread survival motor neuron (SMN) deficiency causes selective loss of spinal motor neurons and infant death in spinal muscular atrophy (SMA). Given that low levels of SMN confer vulnerability of spinal motor neurons, we determined whether SMN expression is also abnormal in ALS. Our evidence from spinal cords of ALS patients and genetic mouse models demonstrates SMN depletion occurs in motor neurons and may contribute to ALS pathogenesis. Neuronal SMN overexpression in mutant SOD1 and TDP-43 mice significantly delayed symptom onset, extended lifespan and improved spinal motor neuron survival. Our results therefore suggest that restoring SMN levels may be beneficial for ALS.

In an effort to build a non-viral gene therapy for ALS, we have employed a novel approach using "immunogenes" as vectors for targeted motor neuron delivery of SMN from the periphery. Bicistronic plasmids encoding human SMN and GFP were conjugated to antibodies targeting neurotrophic receptors expressed by motor neurons. Systemic injection of immunogenes into neonatal mice demonstrated: (1) broad targeting of cervical, thoracic and lumbar motor neuron populations, (2) efficient immunogene transduction of ~50% motor neurons and (3) sustained immunogene expression lasting 7 days in spinal cords of mice. The functional impact of SMN immunogenes on disease onset, progression, neurodegeneration, and SMN levels and function will be determined in mouse models of ALS and SMA.

Our data demonstrating widespread, efficient and prolonged immunogene targeting of motor neurons in the spinal cord highlight the promise of immunogenes to correct inadequate SMN expression levels for ALS and SMA.

R7 Developing Regenerative Therapies for Friedreich's Ataxia using Sensory Neurons derived from induced Pluripotent Stem Cells

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Friedreich ataxia (FRDA) is a disease characterised by neurodegeneration and cardiomyopathy. FRDA is due to insufficiency of the mitochondrial protein, FRATAXIN, which leads to mitochondrial dysfunction, cell toxicity and cell death, particularly within the nervous system and cardiac tissue. The sensory dorsal root ganglia (DRG) is one of the primary and most significant sites of degeneration occurring in FRDA. Sensory neurons derived from human pluripotent stem cells (hPSC) are a valuable resource to develop regenerative therapies to treat FRDA either for drug discovery platforms and/or cell replacement transplants. We have developed an efficient system for deriving DRG sensory neurons from hPSC [1]. Using Q-PCR, immunostaining and multi-electrode array analyses, we demonstrate that hPSC-derived sensory neuronal cultures consist of heterogeneous population of DRG subtypes, including proprioceptors, mechanoreceptors and nociceptors. Furthermore, hPSC-derived sensory neurons were transplanted in the adult rat DRG regions to examine their capacity to mature and functionally integrate in vivo. In vitro and in vivo characterization of sensory neurons have also been performed using FRDA induced pluripotent stem cells (iPSC) showing potential clinical applications of using iPSCs to treat FRDA neurodegeneration.

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R8 The power of the yeast model of Niemann-Pick type C disease from fundamental sterol transport to rare disease clinical trials

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Niemann-Pick type C (NPC) disease is a fatal, pediatric neurodegenerative disease due primarily to mutations in the lysosomal membrane-spanning NPC1 that result in the lysosomal accumulation of cholesterol and sphingolipids. The molecular bases by which NPC1 transports these lipids is not known, which underlies there currently being no effective therapy to treat NPC disease. We hypothesize identifying genetic and physical interactions with NCR1, the functional yeast orthologue of NPC1, will provide insight into the function of NPC1 and identify novel therapeutic strategies. Using genome-wide analyses of the yeast model of NPC disease (*ncr1Δ*) in the specific condition of sterol auxotrophy, we identified histone deacetylase gene deletions exacerbated disease severity in yeast and used histone deacetylase inhibitors to reverse cholesterol defects in human patient cells and a mouse model of NPC disease, results that led to this drug now being tested in clinical trial as well as the development of our exacerbate-reverse methodology. Using myriocin to mimic the disruption of sphingolipid metabolism in human patients, genome-wide analyses identified defective ubiquitin-dependent endocytic pathways in NPC disease, which notably identified compounds that regulate sphingolipid as well as cholesterol metabolism in mammalian cells. Since these genetic interactions are not necessarily physical interactions, we also conducted genome-wide analyses that identified protein-protein interactions involving NCR1. These genetic and physical interactions in yeast are currently being translated with experiments in cultured cell lines, mouse models, and human patients.

R9 Occupation and Motor Neurone Disease: A New Zealand Case-Control Study

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Background: The aetiology of MND is largely unknown, although some occupational exposures are believed to carry increased risk. This NZ population based case-control study has been conducted to investigate associations between occupations and MND.

Methods: Cases were recruited through the New Zealand Motor Neurone Disease Association and New Zealand hospital discharges, and population controls from the New Zealand Electoral Roll in the period 2013 -2016. A standardized questionnaire was used to obtain information on personal and demographic details, lifestyle factors and a full occupational history. We estimated odds ratios by occupation and industry, with all analyses adjusted for age, gender, ethnicity, SES and smoking using logistic regression.

Results: We recruited 321 cases and 605 controls. Two-thirds of the cases were male and aged over 60. Significantly elevated risks for MND were observed for field crop and vegetable growers (OR= 2.93, 95%CI 1.10-7.77); fruit growers (OR= 2.03, 95%CI 1.09-3.78); gardeners and nursery growers (OR= 1.96, 95%CI 1.01-3.83); crop and livestock producers (OR = 3.61, 95%CI 1.44-9.02); fishery workers, hunters and trappers (OR= 5.62, 95%CI 1.27-24.97); builders (OR= 2.90, 95% CI 1.41-5.96); electricians (OR= 3.61, 95% CI 1.34-9.74); caregivers (OR= 2.65, 95% CI 1.04-6.79), forecourt attendants (OR= 8.31, 95% CI 1.79-38.54); plant and machine operators and assemblers (OR= 1.42, 95% CI 1.01-2.01); telecommunications technicians (OR=4.20, 95% CI 1.20-14.64) and draughting technicians (OR= 3.02, 95% CI 1.07-8.53). Analyses by industry also showed significantly elevated risks in agricultural (in particular horticulture and fruit growing), construction, non-residential care services, motor vehicle retailing, mining and sport and recreation industry.

Conclusions: This study has shown strong associations between agricultural occupations and MND risk, as well as significantly increased risk for building trades workers, forecourt attendants, electricians and telecommunications technicians, and has generated a range of hypotheses for specific occupational risk factors.

R10 Ocular gene therapy in ovine models of CLN5 and CLN6 Batten disease

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A common feature across most forms of Batten disease is the progressive loss of vision. Visual deficits are initially due to a loss of neurons in the primary visual cortex, however photoreceptor cells in the retina also die subsequent to neuronal loss in the visual cortex. Gene therapy delivered to the brain in sheep models of CLN5 and CLN6 Batten disease can halt disease progression, however the animals still lose their sight, albeit later than if no treatment was given.

In the current study we performed intravitreal injections of self-complementary AAV9 vectors packaged with either CLN5, CLN6, or in combination, into 3-month-old *CLN5-/-* or *CLN6-/-* animals. The vector was delivered to the posterior of the left eye, as close to the retina as possible without disturbing the retinal surface. Electroretinography (ERG) was performed every month following treatment, and funduscopy was performed bi-monthly.

Qualitative comparisons between the treated (left) and untreated eye were made of fundus images at 8 months of age (5 months post injection). At this age there was no vascular attenuation evident, however in untreated animals marked thinning of retinal blood vessels does not present until 11 months of age. Analysis of ERG b-wave amplitude at 8 months also showed no significant difference between the treated and untreated eyes. In untreated animals b-wave amplitude begins to decline at approximately 9-11 months and is extinguished by 15-17 months of age.

The above results indicate the need for ongoing monitoring of these animals as their symptomology and neuropathology progress. We expect to know if the treatment is effective by September 2018 when these animals reach 10 – 12 months, an age at which they would typically show visual deficits.

R11 Gene hunting in Meier-Gorlin syndrome

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Meier-Gorlin syndrome (MGS) is a rare, autosomal recessive disorder characterised by proportionate growth reduction of the brain (microcephaly) and height, as well specific features of small ears and small/absent kneecaps and other skeletal anomalies. Our previous genetic analysis has revealed MGS is a disorder of the earliest steps of DNA replication. To establish DNA replication, genomic origins of replication are bound in G1 phase by the pre-replication complex (preRC), which is formed by the interaction of subunits 1-6 origin recognition complex (ORC), CDC6 and CDT1, to recruit two inactive MCM2-7 heterohexameric helicases. We have previously discovered that mutations in many components of the preRC underlie MGS. As cells enter S phase, the MCM helicases are activated by CDC45 and accessory proteins (pre-initiation complex), to unwind the DNA helix and allow access for DNA synthesis by DNA polymerase. We have also found multiple mutations in CDC45 cause the defining features of MGS along with craniosynostosis (premature fusion of the skull plates). More recently, we have harnessed the latest adaptions of genome sequencing and identified further replication components not previously associated with disease, harbouring mutations in MGS patients. Intriguingly, we have also identified biallelic variants in the gene *DONSON*. Separately, we have characterised DONSON as a DNA replication stress surveillance protein causing microcephaly and skeletal abnormalities in humans (*Nat Genet* 2017), however the MGS findings are novel and challenge our understanding of DONSON in replication. We hypothesise that these mutations negatively impact DNA replication speed, causing an overall slowing of the cell cycle with consequent reduced proliferation during periods of rapid development, reducing stem cell pools and thereby reducing total cell number and overall body size.

R12 Genetic counseling in the genomic era

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Next-generation sequencing, whole exome and whole genome sequencing are becoming the standard of care in both clinical and research settings. Testing of multiple genes increases the chance of variants of uncertain significance, incidental findings, and uncertain prognoses. As clinicians and researchers it is our duty to ensure that our patients and participants receive concise information and that their expectations are managed appropriately. This talk will discuss the use of fundamental concepts of genetic counseling to ensure patients and participants are able to make informed decisions, and are supported to deal with the uncertainties of the results.

R13 The Ice Bucket Challenge Sporadic ALS Australia Systems Genomics Consortium: SALSA-SGC

Henders, A[1] , Henderson, R., Lokeshappa, M., Ngo S., Garton F., Beyamin, B., Al-Chalabi A, Edis R, Kiernan M, Laing N, Lamont P, Mathers S, Needham M, Nicholson G, Pamphlett R, Rowe D, Schultz D, Talman P, Veldink J, van den Berg L, Visscher P.M., Vucic S, Williams K, Zhao Q, McCombe P, Blair I.P., Wray N.R

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Background: The biological basis of sporadic ALS remains poorly understood. There has been progress in familial ALS through genetic discoveries, but this has not yet translated into understanding of the biological pathways underlying the aetiology of sporadic disease. Discovery of new genomic risk factors through a systems genomics approach will accelerate understanding of ALS disease onset and progression and contribute to progress towards new diagnostics and treatments.

Objectives: Experience from studies of other common complex genetic disorders have demonstrated the importance of large, deeply phenotyped cohorts of data that are collected under consistent protocols. W established the **Sporadic ALS Australia Systems Genomics Consortium: SALSA-SGC** to provide specific infrastructure to enable increased participation. The short-term objectives to share and harmonise protocols for optimised collection of phenotypic data and biological samples, analyse genetic and genomic data to identify DNA variants and modifications associated with ALS and disease progression.

Methods: In consultation with ALS studies internationally and clinicians in Australia we developed a secure on-line data collection tool to record demographic information, clinical variables and collection of biological samples at all clinic visits. This was built concurrently with research governance submissions to gain approvals for recruitment, collection of biological samples and generation and sharing of genomic data.

Results: The on-line data collection platform has been rolled out across Australia and central training of clinical research nurses continues, particularly on the importance of maintaining consistent methodologies. The centralised training for the research nurses has provided an important sense of national identity that conveys the importance of contributing to a genomics research program for new participants.

Discussion and conclusions: The consortium hopes to ensure that the majority of people attending ALS clinics in Australia have the opportunity to participate in research to identify genetic and genomic risk factors for ALS. The implementation of consistent protocols in clinical variables and biological sample collection has begun to support a broad range of clinical research activities at the individual sites. Importantly, each site retains primary guardianship of the data it has collected. Research projects conducted on the collected data requires additional agreements. We are willing to share our protocols and online data collection platform, and we seek collaborations for future genomics studies.

R14 Hugh Green Biobank

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Diseases of the human brain including rare genetic diseases are proving resistant to therapy. In particular, there is a failure to translate disease-modifying treatments developed in animal models of these diseases to patients. Over the 10-15 years my lab has focused on developing methods to grow and study human brain cells to identify disease mechanisms and drug targets, and to use these cells for drug screening, to promote the translation of therapies to the clinic. With generous funding from the Hugh Green Foundation we formalised this platform and established the Hugh Green Biobank (HGB) – a facility focused on developing and using human brain cell culture for neuroscience research and CNS drug and drug target discovery and testing.

Using defined cell culture conditions we are now able in the HGB to routinely grow human neurons, astrocytes, microglia, pericytes, neural stem cells, glioma stem cells and endothelial cells from donors. Most importantly, using these techniques we are studying the basic biology of human brain cells, which has never previously been described, and testing novel treatments using these cell systems. In this talk I will describe these methods and studies.

R15 The New Zealand Motor Neurone Disease Registry; One Year On

Walker, K.[1], Rodrigues, M.[1], Chancellor, A.[2], Watson, B.[3], Reilly,C.[3], Scotter, E.[4], Brunton, H.[5], Turnbull, J.[6], Roxburgh, R. [1,4]

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Motor neurone disease is a rare, terminal, neuromuscular disease for which there are very few treatments currently available, each with only modest benefits. However, there is a large amount of research and drug discovery currently underway worldwide. The New Zealand Motor Neurone Disease Registry was established in 2017 in order to facilitate participation in research and clinical trials for people in New Zealand with motor neurone disease, and to aid researchers in planning and recruitment for studies and clinical trials.

The NZ MND Registry is an opt-in patient registry which collects demographic, contact and clinical data for those who choose to enrol, in order to be trial ready. We report anonymised amalgamated data from the first year's enrolment.

As at July 2018, there are 142 participants enrolled on the NZ MND Registry. Participant sex distribution reflects the demographics reported worldwide, but ethnicity is divergent from what is seen in New Zealand overall, with an over-representation of people who identify as New Zealand European. 85.5% of participants are diagnosed with sporadic MND and 6.1% with familial MND. The remainder are participants who have not been diagnosed but have a family history, or positive genetic test for a MND-causing genetic mutation. Levels of disability are reported with the most recent ALSFRS-R scores for each participant, and show that the majority of participants are within the higher range of the scale. The registry has facilitated entry of patients into three studies to date.

The establishment of the NZ MND Registry illustrates a swift and efficient step-up and launch of a rare disease patient registry. The role of patient registries is an ever changing one, but with clear utility at every point of along the pathway in support of research and drug discovery.

R16 New Zealand Motor Neuron Disease Research Network

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The large networks that exist internationally for Motor Neuron Disease (MND) research are not present in New Zealand. Overseas these networks assist researchers to maintain contact and awareness of what work others are doing and what future studies they are looking towards. They also allow researchers access to data or samples they may not otherwise be aware of.

The New Zealand Motor Neuron Disease Research Network has been created to fill this gap by profiling current New Zealand MND research, highlighting national resources and research opportunities available, and creating connections between researchers and research groups. This has been achieved by contacting known MND researchers and profiling individual and group research on our website, as well as growing the network by reaching out to new contacts.

The website was launched on 1st November 2017 and to date (10 July 2018) has been viewed by 446 unique visitors with an average session duration of 3:00 minutes.

Researchers profiled by the network have identified common needs which are currently lacking in our NZ research community - namely the need for richer clinical information to be linked with the biological or statistical information. The network has already raised the profile of MND research within the wider research community and will be involved in addressing some of the MND specific issues and solutions at the MND round table session as part of Queenstown Research Week 2018. www.mndresearch.auckland.ac.nz

R17 Getting the Balance Right: Targeting Excitatory Dysfunction in the ALS Cortex

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In amyotrophic lateral sclerosis (ALS), hyperexcitability of the motor cortex is a prominent event, often preceding motor neuron degeneration. While many factors may be attributed to this excitatory pathophysiology, a possible candidate, the interneuron, has largely been overlooked. Our previous research has identified that degeneration in the transgenic G93A SOD1 mouse model of ALS is marked by progressive and dynamic interneuron involvement. Changes in the number of both calretinin- (CR) and neuropeptide Y-expressing (NPY) interneurons in the motor cortex of this familial model were identified, suggesting their potential involvement in motor neuron circuitry defects. However, it remains unclear if these changes represent a primary or secondary disease mechanism. To further explore the underlying mechanisms we utilised a primary culture approach. We found that mutant G93A SOD1 significantly altered the intrinsic firing properties and neuronal morphology of cortical interneurons. We also found a differential vulnerability of bipolar versus multipolar interneurons to disease. In addition, the neurite morphology of bipolar interneurons was unaltered while multipolar interneurons had significantly increased neurite complexity observed as increased branch number and neurite tree path length. Our results have shown for the first time that cortical interneurons are innately vulnerable to the human G93A SOD1 mutation and suggest that differential priming of interneurons may be an early step in the initiation of disease. Collectively our work indicates that inhibitory involvement in ALS may not be a static phenomenon, but instead involves dynamic changes throughout disease, which determine the susceptibility and vulnerability of MNs to disease. Therefore, the inhibitory system may represent a viable early target for the prevention and treatment of ALS.

R18 Generation of a sheep model of CLN7 Batten disease using the CRISPR/Cas9 genome editing system – preliminary results and ethical reflections

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The neuronal ceroid lipofuscinoses (Batten disease) are a group of fatal neurodegenerative inherited diseases in humans and animals[1]. Animal models have been instrumental to further the understanding of genetics, of the underlying disease mechanism, and most importantly are crucial for safety and proof of concept studies for therapeutic interventions[2]. Considerable progress has come from studying three naturally occurring ovine models[3]. There is no cure, but studies in animal models and clinical trials suggest that enzyme replacement therapy and gene therapy can be beneficial for variants that are caused by mutations in genes coding for soluble proteins. Variants that are caused by mutations in genes coding for membrane proteins are likely to require other therapeutic interventions and large animal models are essential to develop and validate such therapies. We thus proposed using CRISPR/Cas9 technology to generate an ovine CLN7 research flock. After evaluation of different transfection methods in sheep fibroblasts, we are currently optimising electroporation as a method of transfection in the *in vitro* produced sheep embryos. In addition to technical constraints relating to the use of genome editing in large animals, regulatory and ethical issues need to be considered.

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R19 Brain urea increase is an early pathogenic event in Huntington's disease

Handley, R.R.[1], Reid, S.R.[1], Brauning, R.[2], Maclean, P.[2], Mears, E.R.[1], Fourie, I.[1], Patassini, S.[1,3], Cooper, G.J.S.[1,3], Rudiger, S.R.[4], McLaughlan, C.J.[4], Verma, P.J., Gusella, J.F., MacDonald, M.E.[5], Waldvogel, H.J.[1], Bawden, C.S., Faull, R.L.M.[1], Snell, R.G.[1]

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The neurodegenerative disorder Huntington's disease (HD) is characterized by extensive loss of striatal neurons and the midlife onset of debilitating and progressive chorea, dementia, and psychological disturbance. HD is caused by the expansion of a poly-glutamine coding CAG repeat in the Huntingtin (HTT) gene. The pathogenic mechanism resulting in cell dysfunction and death beyond the causative mutation is not well defined and currently there is no therapy that can prevent or slow the disease. To further delineate the early molecular events in HD, we performed RNA-sequencing (RNA-seq) on striatal tissue from a cohort of 5-y-old OVT73-line sheep (n = 6 OVT73, 6 control) expressing a human CAG-expansion HTT cDNA transgene. Our HD OVT73 sheep are a prodromal model and exhibit minimal pathology and no detectable neuronal loss. We identified significantly increased levels of the urea transporter *SLC14A1* in the OVT73 striatum, along with other important osmotic regulators. Further investigation using a biochemical assay revealed elevated levels of the metabolite urea in the brain (striatum and cerebellum) of the same OVT73 sheep. In parallel, we discovered that levels of urea are also increased in post-mortem human brain from HD cases, including those with low-level neuropathology (Vonsattel grade 0/1). This elevation in urea indicates increased protein catabolism, possibly as an alternate energy source, given the generalized metabolic defect in HD. Increased urea and ammonia levels due to dysregulation of the urea cycle are known to cause neurologic impairment. Taken together, our findings indicate that aberrant urea metabolism could be the primary biochemical disruption initiating neuropathogenesis in HD.

R20 Genomics Aotearoa: Aiming to improve the use of genomics in New Zealand

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Genomics Aotearoa is an MBIE funded partnership between 3 Universities and 4 CRIs tasked to improve the use and uptake of genomics in New Zealand. Genomics Aotearoa aims to build capacity and capability in genomics and bioinformatics through exemplar projects that develop tools and technology to solve problems of importance to New Zealand. Genomics Aotearoa is developing both bioinformatics infrastructure, and skilled personnel to develop the use of genomics in health, environment and primary production. Many of the issues to be addressed are of importance to Māori, so Genomics Aotearoa will work in partnership with Māori to ensure benefit sharing and the development of trust.
This talk will present the principles of Genomics Aotearoa, introduce its current suite of funded activities and indicate upcoming opportunities for New Zealand researchers.

R21 A step towards unifying health genomic activity in NZ introducing Genomic Health Alliance New Zealand (GHANZ)

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The GHANZ vision is to improve the health of all New Zealanders by integrating genomics into routine healthcare. In the talk I will discuss the key objectives and current outcomes of GHANZ.

R22 Efficacy of gene therapy in a sheep model of CLN5 Batten disease

Mitchell, N.L.[1,2], Russell, K.N.[1], Wellby, M.P.[1], Wicky, H.E.[3], Schoderboeck, L.[3], Barrell, G.K.[1], Melzer, T.R.[4], Gray, S.J.[5], Hughes, S.M.[3], Palmer, D.N.[1]

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The neuronal ceroid lipofuscinoses (NCLs; Batten disease) are neurodegenerative lysosomal storage diseases, predominantly affecting children. The disease is fatal and few treatments exist. Sheep with naturally occurring CLN5 Batten disease are ideal candidates for testing gene therapies. Their gyrencephalic brain is similar in physical organisation to human brains and in size to non-human primates, thus providing a good approximation of dose requirements and vector distribution in humans. Additionally, CLN5 affected (*CLN5*$^{-/-}$) sheep share the main neuropathological features of the human disease.

The single intracranial administration of either lentiviral or recombinant adeno-associated viral (AAV9) vectors encoding ovine *CLN5* into 3-month-old pre-symptomatic *CLN5*$^{-/-}$ sheep provided long term protection against stereotypical disease, the only clinical sign being a much-delayed loss of vision. *In vivo* monitoring methods, similar to those used in human medicine, showed retention of neurological and cognitive function in treated sheep. Longitudinal neuroimaging confirmed preservation of brain structure and volume. Lifespan was extended and one AAV9-treated sheep remains alive today, at 58 months, almost triple the life expectancy of untreated *CLN5*$^{-/-}$ sheep.

Whilst therapeutic intervention at the earliest time is desirable, the diagnosis of NCL in humans can be prolonged and typically follows clinical presentation. Hence, in a more clinically relevant setting, seven-month-old *CLN5*$^{-/-}$ sheep with established disease symptoms and neurodegenerative changes also received gene therapy. The treatment halted any further decline in motor, neurological or behavioural capability. There has been little further post-injection brain atrophy and the treated sheep remain healthy in the field at 33 months of age. In contrast, untreated *CLN5*$^{-/-}$ sheep developed advanced disease symptoms, with manifest seizure activity, and did not survive beyond 21 months.

Together, these data in both pre- and post-symptomatic sheep provide a strong rationale for clinical translation to CLN5 affected human patients.

R23 Treating the cause

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Spinal muscular atrophy is a rare genetic neurodegenerative disorder that affects around 100 New Zealanders currently. It's the leading genetic killer of infants throughout the world and the disease is also widely variable in its presentation. SMA ranges from onset at or before birth in severely affected 'Type 0' babies through to Type 1 babies presenting in the first few months of life, through to mildly affected adults. The story of how an antisense oligonucleotide was developed for the effective treatment of spinal muscular atrophy is a remarkable one relevant for many genetic disorders.

R24 Moving from Gene Discovery to Gene Therapy for Motor Neuron Disease and Frontotemporal Dementia

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Twenty-five years of genetic research has provided a multitude of genetic clues as to the pathogenic mechanisms driving neurodegeneration in Motor Neuron Disease (MND) and Frontotemporal Dementia (FTD). The cytoplasmic accumulation of the RNA-binding protein TDP-43 is the molecular nexus for known genetic mutations and apparently sporadic disease in 95% of MND and 60% of FTD cases. This talk will give an overview of the major mechanistic pathways and the opportunities for intervention through gene therapy.

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Summary of Abstracts for the Poster Session

No.	Title	Presenter	Institutions
R25	Preliminary investigations on the functionality of INGX, a pseudogene found in the XDP (X-linked Dystonia Parkinsonism) locus	Marie Viola	National Institute of Molecular Biology and Biotechnology, University of the Philippines Diliman
R26	Presence of genetic disease-specific aggregates in the anterior olfactory nucleus of the human olfactory bulb	Blake Highet	Centre for Brain Research, The University of Auckland, Auckland, NZ
R26	Identification and characterisation of protein-protein interactions mediating cholesterol transport in Niemann-Pick type C disease.	Natalie Hammond	School of Biological Sciences, Victoria University of Wellington
R27	The Establishment of the New Zealand Motor Neuron Disease Research Network	Jayne McLean	Centre for Brain Research, Department of Pharmacology, University of Auckland
R28	Loss of Blood-Spinal Cord Barrier Integrity Displays Regional Patterning in Amyotrophic Lateral Sclerosis	Emma Scotter	Department of Pharmacology and Clinical Pharmacology, Centre for Brain Research, University of Auckland
R29	ATP13A2: Characterization of Novel Human iPS Cell Models of Parkinson's and Batten's Disease.	Oluwatobi Eboda	Department of Biochemistry, University of Otago, Brain Health Research Centre, and Brain Research New Zealand

R29 Preliminary investigations on the functionality of INGX, a pseudogene found in the XDP (X-linked Dystonia Parkinsonism) locus

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X-linked Dystonia Parkinsonism (XDP; Lubag) is a rare middle-onset neurodegenerative disorder almost exclusive to males of Filipino descent, with 505 documented cases, only 5 of whom are women. Dystonia manifests during the second or third decade of life and eventually progresses to Parkinsonism within approximately the next decade. Neuropathology of XDP patient brains exhibits severe neuronal loss and astrogliosis. The XDP disease locus has been mapped to Xq13.1 spanning a TAF1/DYT3 multiple transcript system with a disease haplotype of 5 disease-specific mutations, an SVA insertion, and a 48-bp deletion. TATA-box binding protein 1 (TAF1), a crucial initiator of transcription, is believed to be the main causative gene due to the observed downregulation of its neuronal specific isoform, N-TAF1, in XDP patient brains. However, investigation of other genetic lesions that may cause the severe progressive biphasic nature of XDP is warranted. Often overlooked is INGX, an annotated pseudogene antisense to the region of the TAF1/DYT3 transcript. Its function is unknown. ING1, its functional counterpart, is an extensively studied tumour suppressor, with vast regulatory functions in cellular proliferation, apoptosis and senescence - hallmarks common to both cancer and neurodegeneration. It was thus imperative to establish that INGX is a functional pseudogene as a prelude to understanding its possible roles in cancer and neuronal cells. Gene expression profiling of INGX across cancer and normal cell lines via quantitative PCR revealed tissue specific expression - characteristic of a functional pseudogene. Preliminary data on knockdown of INGX in human colorectal cells (HCT116) showed a significant increase in proliferation, and a decrease in apoptotic capability, implying tumour suppressive capabilities. In a neuronal context, INGX exhibited a dynamic gene expression profile across stages of neuroblastoma differentiation, as shown by qRT-PCR. Functional assays on INGX knockdown in a neuronal context are underway.

R30 Presence of genetic disease-specific aggregates in the anterior olfactory nucleus of the human olfactory bulb

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Olfactory impairments are frequently observed pre-clinically in many neurodegenerative diseases. It has been hypothesised in Alzheimer's and Parkinson's disease that olfactory dysfunction results from deposition of disease-specific protein aggregates in the olfactory bulb (OFB). However, disease-specific aggregates in the OFB have not been described in genetic diseases such as Huntington's disease (HD) and familial forms of Motor Neuron Disease (MND) which also present with olfactory deficits. To determine whether pathological protein aggregates are present in HD and MND OFBs, fluorescent immunohistochemistry was performed on post-mortem human OFB sections from eleven HD, five MND and five normal cases. Sections were stained with antibodies against disease specific aggregates and cell specific markers with previously reported expression within the OFB. We found mutant huntingtin aggregates in all HD OFBs almost exclusively within the anterior olfactory nucleus (AON). Furthermore, in two familial cases of MND (C9ORF72 mutants) we found dipeptide repeat aggregates within the AON. Both aggregate types were found to be co-localised within calbindin, calretinin, somatostatin or tyrosine hydroxylase immunoreactive cells. No staining for alternative pathological proteins including β-amyloid, tau and α-synuclein was seen in HD OFBs, however positive tau staining was seen in 3 out of 5 MND OFBs. Therefore, olfactory deficits seen across the spectrum of neurodegenerative disorders, whether sporadic or genetic, share similar aggregate pathologies in the OFB concentrated to the AON. This suggests that aggregate pathology within the AON could play a role in the common symptom of olfactory dysfunction seen across neurodegenerative diseases.

R31 Identification and characterisation of protein-protein interactions mediating cholesterol transport in Niemann-Pick type C disease.

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Niemann Pick type -C (NP-C) disease, is a rare neuro-visceral disorder, whereby cholesterol and sphingolipids accumulate to toxic levels in the lysosome of the cell. NP-C disease is aggressive, consuming the lives of sufferers between 10-25 years of age. Sadly, no therapy yet exists. NP-C disease is caused by a monogenic mutation in either *NPC1* or *NPC2* (95% of cases). The NPC1 and NPC2 proteins function together in cholesterol egress, shuttling exogenous cholesterol through the lysosome where upon exit from the lysosome, it is then passed to an unidentified protein and transported to the endoplasmic reticulum and plasma membrane. We hypothesize that physical protein-protein interactions with NPC1 are critical for normal cholesterol transport and defects in that interaction are the cause for 5% of NP-C disease patients without mutations in *NPC1* or *NPC2*. Previously, the yeast model of NP-C disease (*ncr1*Δ) identified genetic interactions conserved from yeast to mammal; here we will utilize this model to identify protein-protein interactions with Ncr1, the functional orthologue of human *NPC1*. A membrane yeast two hybrid (MYTH) assay identified 11 candidate interactors with Ncr1. Validation and further characterisation of these candidate PPIs are ongoing and will be presented herein. If orthologous in humans, our results will identify interacting protein(s) that hold therapeutic promise to treat the rare NP-C disease as well as identify proteins critical to intracellular cholesterol transport in healthy persons.

R32 The Establishment of the New Zealand Motor Neuron Disease Research Network

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The large networks that exist internationally for Motor Neuron Disease (MND) research are not present in New Zealand. Overseas these networks assist researchers to maintain contact and awareness of what work others are doing and what future studies they are looking towards. They also allow researchers access to data or samples they may not otherwise be aware of. The New Zealand Motor Neuron Disease Research Network has been created to fill this gap by profiling current New Zealand MND research, highlighting national resources and research opportunities available, and creating connections between researchers and research groups. This has been achieved by contacting known MND researchers and profiling individual and group research on our newly built website, as well as growing the network by reaching out to new contacts. The website was launched on 1st November 2017 and up to the date this poster was created (15th December 2017) had been viewed by 182 unique visitors with an average session duration of 3:33 minutes. Researchers profiled by the network have identified common needs which are currently lacking in our NZ research community- namely the need for richer clinical information to be linked with the biological or statistical information. The network has already raised the profile of MND research within the wider research community and an important next step is the kanohi ki te kanohi / face-to-face opportunity and the MND Round Table event at Queenstown Research Week, August 2018.

R33 Loss of Blood-Spinal Cord Barrier Integrity Displays Regional Patterning in Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease marked by TDP-43 protein inclusions in the brain and spinal cord. TDP-43 deposition displays regional patterning across the spinal cord, with cervical and lumbar regions showing greatest deposition, and this corresponds with heavier motor neuron loss at these levels (1). The blood-spinal cord barrier (BSCB) is a specialised interface that restricts the entry of potentially neurotoxic blood components into the cord parenchyma, but the BSCB is compromised in ALS patients (2,3). Given the regional patterning of TDP-43 pathology and motor neuron loss in the ALS spinal cord, we examined their spatial correlation with BSCB leakage. Human spinal cord sections from cervical, thoracic and lumbar cord, from control (n = 5) and ALS (n = 13) cases, were immunostained for hemoglobin (vessel leakage), lectin (vessels), SMI-32 (motor neurons) and phosphorylated TDP-43. Spinal cord sections were imaged and analysed using a semi-automated pipeline designed in-house. pTDP-43 pathology and motor neuron loss were observed across the spinal cord, with heaviest motor neuron loss at the cervical level. Hemoglobin extravasation was striking in most ALS spinal cords, being seen in both grey and white matter, and most severe at thoracic levels T7-T9. We propose that BSCB leakage is transient; evolving and resolving first in the cervical and lumbar regions. Extensive hemoglobin leakage in the thoracic cord together with significant motor neuron loss suggests that BSCB compromise is either promoted by, or contributes to, neurotoxicity in ALS.

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R34 ATP13A2: Characterization of Novel Human iPS Cell Models of Parkinson's and Batten's Disease.

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Mutations in *ATP13A2* lead to the development of two distinct neurological disorders, Kufor-Rakeb Syndrome, a juvenile parkinsonism and CLN12 Batten disease, a lysosomal storage disease. The underlying pathology of Parkinson's Disease (PD) is currently unknown, though the accumulation of misfolded proteins suggests that improper disposal of aggregate-prone proteins has an important role in the pathogenesis of neurodegenerative disorders. Accordingly, 18 genes linked to familial forms of PD are caused by mutations in PARK genes which encode proteins that are involved with the autophagosome-lysosomal pathway (ALP).
Conversely, Batten disease is a more well characterized lysosomal storage disorder. PD associated mutations, including ATP13A2, have been modelled in animal and cell models, though none fully recapitulate the pathology seen in human disease. Therefore, it is imperative to develop better models for disease study and high throughput drug screening.
I have established a novel line of iPSC cultures that provides modelling of human neuronal cell biology. These isogenic human iPSC lines contain a doxycycline-inducible neurogenin2 transgene allowing differentiation into functionally mature glutamatergic cortical neurons in a timely and uniform manner.
CRISPRi will be used to inhibit the endogenous ATP13A2 locus, transduced with vectors carrying one mutation corresponding to PD, and one to Batten disease, as a control for lysosomal dysfunction, then differentiated into neurons.
The cells will then be characterized to assess whether they recapitulate human disease etiology and to compare lysosomal dysfunction in Parkinson's disease against Batten disease. The aim is to establish new models of Batten's and Parkinson's disease.