14th MND Australia Research Conference

Investing in Innovation, Partnering for Progress

Friday 9 November 2018
The Florey Institute of Neuroscience and Mental Health
Ian Potter Auditorium, Kenneth Myer Building
University of Melbourne

#MNDmeeting18
About MND Australia

MND Australia works to improve the lives of all Australians impacted by motor neurone disease by influencing policy, providing trusted information and promoting and funding the best research through our research arm, the MND Research Institute of Australia (MNDRIA).

While there is still much to learn about MND, understanding of this complex condition globally has transformed over the last decade. The Motor Neurone Disease Research Institute of Australia (MNDRIA) has played an integral role in this transformation. Almost $30 million has been invested in Australian health and medical research over 31 years. The community has donated every dollar, including more than $7 million via the State MND Associations. This incredible effort is a testament to the community’s commitment to changing the future of MND. Every dollar of each donation goes to supporting research excellence identified through a rigorous process. MNDRIA funds only the best research with the greatest chance of developing effective treatments and improving the lives of people with MND.

About the MND Australia Research Conference

The MND Australia Research Conference has been held annually since 2005 and has been integral to the development and funding of MND research in Australia. The conference has been held in Sydney, Melbourne and Brisbane to facilitate participation for researchers in different states. With more projects funded each year, the research conference has grown to become an important event in the MND calendar with attendees from all over Australia.

The objectives of the MND Australia Research Conference are:

- To promote sharing of expertise amongst MND researchers in Australia
- To enable interaction of researchers to foster the development of research collaborations
- To provide feedback to a wide audience about the latest developments in MND research
- To demonstrate the value of the funded research to donors to encourage their continuing support.
Acknowledgements:

MND Australia would like to thank the many people and organisations who have contributed to the 14th MND Australia Research Conference:

- Professor Steven Petrou, Associate Professor Bradley Turner and the Florey Institute of Neuroscience and Mental Health
- Session chairs, keynote speakers and presenters
- MNDRIA Research Committee
- MNDRIA Poster Committee
- The many generous donors who provide all the funds that make this research possible
- Biogen providing a grant to support the conference
## PROGRAM

### ORAL PRESENTATIONS

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<td>9.00 am</td>
<td>Welcome on behalf of MND Australia</td>
<td>David Ali (President, MND Australia)</td>
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<td>Welcome on behalf of the Florey Institute of Neuroscience &amp; Mental Health</td>
<td>Professor Steven Petrou (Director)</td>
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<td><strong>Awards presentation</strong></td>
<td>Professor Matthew Kiernan (1)</td>
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<td>Community awards (4)</td>
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<td>9.40 am – 10.30 am</td>
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<td></td>
<td>Dr Catherine Blizzard (Betty Laidlaw Prize 2017)</td>
<td>Investing in innovation: supporting researchers at all stages of their careers</td>
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<td></td>
<td>Professor Naomi Wray (MND Australia Ice Bucket Challenge Grant 2015-2018, NHMRC Partnership Grant 2018 - 2022)</td>
<td>Partnering for progress</td>
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<td>10.30 am</td>
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<td>11.00 am – 12.45 pm</td>
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<td><strong>Chair: Professor Tracey Dickson</strong></td>
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<td></td>
<td>A/Prof Justin Rubio (Jenny Simko MND Research Grant)</td>
<td>A precision genomics approach to dissect the pathogenesis of ALS/MND</td>
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<td>Dr Kelly Williams (Jenny Barr Smith MND Research Grant)</td>
<td>Gene expression profiling in Australian sporadic ALS identifies retroviral activation in patients</td>
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<td>Dr Lezanne Ooi (Benalla Act to d’Feet MND Research Grant)</td>
<td>Targeting the mechanisms governing alterations in motor neuron excitability in MND</td>
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<td>A/ Prof Justin Yerbury (Betty Laidlaw Prize 2018)</td>
<td>The importance of proteostasis at the synapse in ALS</td>
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<td>A/ Prof Peter Noakes (MND and Me Foundation Research Grant)</td>
<td>The neuromuscular junction - the hidden player in MND: studies from MND model mice and MND patients</td>
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<td>Dr Edwin Lim (Lady (Mary) Fairfax MND Research Grant)</td>
<td>Investigating cyanobacteria toxin as a causative environmental factor in pathogenesis of ALS</td>
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<td>12.45 pm – 1.45 pm</td>
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### SESSION 3 – Fostering treatment development and clinical trials

**Chair: Professor Dominic Rowe**

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<td>Professor Roger Chung</td>
<td><em>SuperBall X MND Research Grant</em> New insight into the role of protein degradation pathways in the pathogenesis of familial and sporadic ALS</td>
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<td>Dr Angela Laird</td>
<td><em>MNDRIA Grant-in-aid</em> Testing drugs to induce activity of the autophagy quality control pathway to treat motor neuron disease</td>
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<td>A/Prof Trent Woodruff</td>
<td><em>Charles and Shirley Graham MND Research Grant</em> Multiple protective and pathogenic roles for the innate immune complement system in MND</td>
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<td>Dr Mary-Louise Rogers</td>
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<td>A/Prof Peter Crouch</td>
<td><em>Betty Laidlaw Grant 2016-2018</em> Disrupted copper availability in sporadic motor neurone disease promotes ferroptotic stress and toxic glial activation</td>
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<td>Professor Julian Gold</td>
<td><em>Cure for MND Collaboration Grant 2016</em> The Lighthouse Project: Triumeq</td>
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### SESSION 4 – Enhancing clinical research and improving quality of life

**Chair: Professor David Berlowitz**

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<td><em>Jenny Simko MND Research Grant</em> The elemental signature of motor neurone disease</td>
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<td>Dr Frederik Steyn</td>
<td><em>Marie McGrath MND Research Grant</em> Impact of loss of appetite on weight management and disease progression in MND</td>
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<td>Professor Vicki Flood</td>
<td><em>Jenny Simko MND Research Grant</em> A pilot study to trial the feasibility and effect of swallowing exercises and diet among people with MND</td>
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<td>Nicole Sheers</td>
<td><em>NHMRC/MNDRIA co-funded PhD scholarship</em> Lung Volume Recruitment in Neuromuscular Disease: Can ‘breath-stacking’ improve lung function, respiratory symptoms and quality of life for people with neuromuscular disease?</td>
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### Closing remarks and invitation to poster session

A/ Prof Bradley Turner  
The Florey Institute

### POSTER SESSION AND DRINKS RECEPTION
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<td>Alison Hogan</td>
<td>Macquarie University</td>
<td>Use of in vitro CRISPR-Cas9 genome editing for the identification of novel MND-linked gene mutations</td>
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<td>2.</td>
<td>Marcus Dyer</td>
<td>University of Tasmania</td>
<td>Does mislocalised TDP-43 in the motor cortex drive ALS?</td>
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<td>3.</td>
<td>Emily Don</td>
<td>Macquarie University</td>
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<td>4.</td>
<td>Natalie Grima</td>
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<td>5.</td>
<td>Samantha Barton</td>
<td>Florey Institute of Neuroscience and Mental Health</td>
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<td>6.</td>
<td>Rachel Atkinson</td>
<td>University of Tasmania</td>
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<td>7.</td>
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<td>Metabolic disturbances in glucose metabolism contributing to increased oxidative stress and treatment thereof</td>
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<td>12.</td>
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<td>Mouna Haidar</td>
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<td>Samiha Khan</td>
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<td>17.</td>
<td>Jean Giacomotto</td>
<td>University of Queensland</td>
<td>Versatile genetic systems in zebrafish for unveiling MND genes pathogenicity and generate degenerative models</td>
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<td>Adam Svahn</td>
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<td>Taide Wang</td>
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<td>Therapeutic inhibition of the necroptosis cell death signalling pathway in ALS</td>
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<td>Sharlynn Wu</td>
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<td>4</td>
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<td>University of Queensland</td>
<td>Decreased signalling of EphA4 improves functional performance and motor neuron survival in the SOD1G93A ALS mouse model</td>
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Investing in innovation: supporting researchers at all stages of their careers
Dr Catherine Blizzard, The University of Tasmania

There is increasing evidence for a central role for changes in neuronal excitability in the pathogenesis of amyotrophic lateral sclerosis (ALS). Dr Catherine Blizzard was awarded the Bill Gole Post Doctoral Fellowship (2011-2014) to investigate how site-specific changes in excitability may be contributing it ALS. Over excitation of AMPA receptors in the spinal cord had the potential to cause motor neuron degeneration and the neuromuscular junction die back that characterise ALS. This work indicates that the post synaptic site - where the AMPA receptors reside - may be a primary site for pathology in ALS.

Through grant-in-aid support, culminating in the Betty Laidlaw Prize (2017) Catherine established a program of research investigating how changes at the post synapse may be driving ALS. Her studies have found that TDP-43, the protein most frequently found in cytoplasmic aggregates in ALS, is involved in maintaining neuronal synapses - regulating the number and maturation of dendritic spines. Spine changes occur before symptom onset in the motor cortex, but not the somatosensory cortex, indicating that this is one of the earliest pathological changes associated with TDP-43. The action of misprocessed TDP-43 at the spine may be due to its role in activity-dependent RNA translation. Mutant TDP-43 drives increased expression of TDP-43 in the cytoplasm and causes alterations in the composition and localisation of AMPA receptor proteins. Collectively Catherine’s research has identified that the post synapse may play an important role in ALS and represents a targetable site for therapeutic intervention to tackle this destructive disease.

Partnering for progress
Professor Naomi Wray, The University of Queensland

To draw strong conclusions in research requires well-powered studies and replication. Since ALS is a late onset, fast progressing disease the limited. This means that we have to think beyond individual clinics to achieve the necessary sample sizes to address our in a reasonable time period. The Australian ALS clinical community has a long history of collaborative research and this year has been awarded a 5-year NHMRC Partnership Grant. I will discuss the aims of this grant which builds upon the unified clinical data collection infrastructure and biobanking protocols implemented through the SALSA-SGC (Sporadic ALS Australia-Systems Genomics Consortium) funded by the MNDRIA IceBucket Challenge grant. These partnerships position us well for future national and international collaborations. Working collaboratively maximises research progress and best honours the gifts made by the individuals who consent to participate in research.
SESSION 2 – ADVANCING MND RESEARCH TO UNDERSTAND CAUSES

Associate Professor Justin Rubio, The University of Melbourne

A precision genomics approach to dissect the pathogenesis of ALS/MND

Background: Genetic studies conducted in families and large population-based cohorts of sporadic cases and controls have contributed significantly to our understanding of ALS pathogenesis, but there is still much we don’t know. Advances in single cell genomics and high-throughput next generation sequencing (NGS) are providing opportunities to understand the complex biology underpinning human brain disease. Of particular relevance to ALS, recent studies in healthy brains have shown that somatic variation in the genomes of single neurons is reflective of their developmental and transcriptional history, and also processes of ageing and neurodegeneration.

Hypothesis: We propose that the somatic genome of surviving neurons in the ALS brain and spinal cord will reveal fundamental insight into the life history of these cells, from conception through to pre-clinical and clinical disease, and this will address some of the gaps in our knowledge in relation to disease pathogenesis and progression.

Objectives: To leverage somatic variation in the genomes of single neurons isolated from ALS patients post-mortem to understand molecular and cellular mechanisms of disease pathogenesis.

Results: We have conducted whole genome sequencing of multiple single ALS neurons. Data quality for these single neurons is of a good standard, benchmarking against published data and providing the basis for somatic mutation analysis. Here, we will present preliminary data arising from these initial experiments.

Summary: This study provides evidence of feasibility for a new frontier of genomic discovery in ALS research, which has the potential to transform our understanding of disease pathogenesis and progression.

Dr Kelly Williams, Macquarie University

Gene expression profiling in Australian sporadic ALS identifies retroviral activation in patients

Each year in Australia, over 700 individuals are newly diagnosed with ALS, and of these, 650 are sporadic cases. The only proven causes of ALS are gene mutations that lead to motor neuron death, yet only 5-10% of sporadic ALS patients have known causal gene mutations. For the remaining cases, little is known of the underlying cause of disease. The age of disease onset, site of symptom onset and duration of disease are highly variable among ALS patients. This variable phenotypic presentation of sporadic ALS indicates the presence of disease modifiers that control disease manifestation which may be reflected in aberrant gene expression.

We have applied gene expression profiling to a blood-derived RNA-Seq dataset from Australian sporadic ALS patients (n=95) and matched controls (n=69). Targeted analysis of
18 known ALS genes indicated significant gene expression changes were present in sporadic ALS. Unbiased transcriptome-wide analysis using EdgeR and DESeq2 identified 1151 differentially expressed genes (DEGs) in sporadic ALS compared to controls. Gene Enrichment analysis of DEGs implicated pathway dysfunction in sporadic ALS that may arise from retroviral activation and enrichment of protein clearance. Since genes rarely work in isolation, we evaluated dysregulated pathways based on co-expression of genes. Weighted Gene Correlation Network Analysis identified association of a co-expression gene module with clinical phenotypes (early age of onset and rapid disease progression). Protein interactions within this gene module implicate an enrichment of antiviral response, which supports our DEG data and implicates retroviral activity in ALS.

Dr Lezanne Ooi, The University of Wollongong
Targeting the mechanisms governing alterations in motor neuron excitability in MND

In patients in the clinic and in animal and cell models of MND /ALS it has been observed that there is a change in the properties of motor neurons, termed neuronal hyperexcitability, which is an increase or exaggerated response of the neurons to a stimulus. The factors that instigate these changes in neurons over the course of disease onset and progression are not well understood.

Aims: To compare the excitability of motor neurons in the motor cortex and spinal cord in SOD1G93A mice and their littermate controls and induced pluripotent stem cell-derived motor neurons from ALS patients and controls. Specifically: (1) To measure the changes in excitability before and after symptom onset; (2) to identify the ionic mechanisms that govern excitability in motor neurons and their relevance to disease.

Results: Overall we identified that motor neurons from symptomatic mice were more excitable than those from presymptomatic mice; we found alterations in (i) input resistance, (ii) rheobase, (iii) adaptation of firing (maximal suprathreshold frequency). Finally we identified the ionic mechanisms that regulate these changes in cellular excitability and firing properties.

Conclusions: Our findings indicate that motor neurons from symptomatic mice are significantly more excitable, capable to fire in higher frequencies and at higher oscillation frequencies. We have uncovered ionic mechanisms underlying motor neuron excitability in MND / ALS and our data provides an explanation for motor neuron susceptibility with aging.

Associate Professor Justin Yerbury, The University of Wollongong
Translating research into practice: how do we make research useful to people living with MND?

Mounting evidence suggests that one of the earliest presymptomatic functional and pathological changes associated with ALS occur distally in motor neurons axons and at the synaptic terminals. While it is clear that these physiological changes are intimately linked with ALS pathology, the underlying molecular alterations that result in such physiological outcomes remains largely unknown. Our previous results indicate that protein homeostasis in motor neurons is dysfunctional in ALS.
Recently, it has been estimated that there are over 1000 different proteins required for a functional synaptic bouton, and data suggests that there are on average over 1 million individual protein molecules at the synaptic terminal. Precisely how motor neurons maintain the organelles and proteome of the presynaptic terminal is not fully understood. The challenges in the maintenance of the distal axons and pre-synaptic terminals may make motor neurons particularly vulnerable to cell dysfunction and death. Our analysis suggests that the sub-proteome of the presynaptic terminal (PST) is significantly more supersaturated compared to the entire motor neuron proteome and is thus particularly vulnerable to protein homeostasis dysfunction. Moreover, the sub-proteome of the presynaptic terminal is significantly enriched with some of the most supersaturated proteins in the entire proteome. This data suggests that maintaining proteome homeostasis at the presynaptic terminal is important in the context of ALS.

**Associate Professor Peter Noakes, The University of Queensland**

*The neuromuscular junction - the hidden player in MND: studies from MND model mice and MND patients*

A central event in motor neuron disease (MND) is the early withdrawal of motor nerve terminals from muscle cells, resulting in muscle weakness. In our studies of MND model mice, we have observed declines in synaptic laminins, which are adhesion molecules located between motor neurons and muscle. We have also observed reduced expression in the muscle tyrosine kinase receptor (MuSK), which is needed to stabilize postsynaptic specialisations at NMJs, in response to the motor neuron factor agrin. Importantly, these changes coincide with altered synaptic transmission and disassembly of the neuromuscular junction (NMJ), all of which occur before the loss of upper and lower motor neurons. Our human studies have also revealed a similar loss of synaptic laminins and MuSK from NMJs of muscles from early-diagnosed MND patients. The down regulation of synaptic laminins could explain the changes at MND-NMJs that we have observed including: misalignment of active zones, encroachment of Schwann cells into the synaptic cleft, and motor terminal withdrawal from muscle. The down regulation of MuSK expression could contribute to the dispersal of postsynaptic acetylcholine receptor clusters (AChRs) from NMJs in the muscle of MND patients. The down regulation of MuSK at the NMJs from MND patients also supports our recent studies, which so far have shown that muscle from MND patients appear not to respond to agrin, suggesting a fault in the agrin-MuSK signalling pathway. Collectively, these data add support the idea that alterations of NMJ adhesion and NMJ signalling are early peripheral contributions to MND.

**Dr Edward Lim, Macquarie University**

*Neurotoxin BMAA is a contributor to axonal damage, and its transcellular transmission*

**Background**: The cyanobacterial neurotoxin, β-N-methylamino-L-alanine (BMAA) causes neurotoxicity in whole cells, however, specific effects of BMAA on subcellular compartments such as axons, are unknown. Exposure to BMAA may contribute to increased
susceptibility in MND, but it is unclear how BMAA enters the central nervous system (CNS).

**Objectives:** Determine (1) subcellular neurotoxicity of BMAA on primary murine and human neurons, and (2) if BMAA can be transmitted transcellularly retrograde and anterograde in neuronal cells.

**Results:** Low levels (50µM) of somatic BMAA exposure causes axonal fragmentation, but limited somatic death only at 500µM (p<0.0001). BMAA can be transported anterograde to neurons (62%, p<0.0014) and astrocytes (23%, p<0.05).

A new, improved design of microfluidic chip has been synthesized to investigate retrograde transcellular transmission of BMAA, and is being tested for retrograde transmission.

**Discussion:** These results indicate BMAA can contribute to neuronal network collapse, and BMAA can spread through neuronal pathways. Importantly, low levels of BMAA can cause significant axonal damage indicating that it can contribute to Wallerian-like degeneration, leading to neuronal network collapse and loss of function, even though the cell soma remains intact.

Transport of BMAA through neuronal cells may mimic the focal neuroanatomical spread of neurodegenerative pathologies in MND. This is also a feasible method by which peripheral exposure to BMAA is the entry point for the neurotoxin into the CNS.

From a clinical perspective, this presents an opportunity to rescue damaged phenotypes by encouraging axonal repair or regeneration to restore function; or to halt or delay the spread of BMAA.

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**SESSION 3 – FOSTERING TREATMENT DEVELOPMENT AND CLINICAL TRIALS**

**Professor Roger Chung,** Macquarie University

*New insight into the role of protein degradation pathways in the pathogenesis of familial and sporadic ALS*

The CCNF gene encodes for Cyclin F, which is a component of a complex system that is responsible for ‘labelling’ proteins with a molecule called ubiquitin, marking these ubiquitylated proteins for degradation as part of a cell’s ‘housekeeping’ processes. We have recently discovered mutations in the CCNF gene as the cause of ALS in both familial and sporadic ALS (Williams et al, Nat Comms, 2016).

Over the past 3 years through the support of the MNDRIA, we have discovered that Cyclin F directly regulates the ‘labelling’ of key proteins implicated in ALS pathology in patient motor neurons, targeting them for degradation. Accordingly, overexpression of Cyclin F leads to a reduction in the intracellular levels of these proteins in cultured cells. Conversely, overexpression of ALS-variant Cyclin F results in hyper-ubiquitylation and accumulation of these proteins. Thus, we have identified an endogenous clearance mechanism that removes pathological proteins from cells, which we propose as a tractable therapeutic target (patents filed). We are currently evaluating a variety of strategies to therapeutically regulate Cyclin F levels in motor neurons, and determine whether this can clear pathological protein aggregates and prevent neurodegeneration in appropriate animal models of ALS.
Pathological studies on the brains and spinal cords of motor neuron disease (MND) patients with either sporadic or familial MND frequently reveal apoptotic neurons that contain protein inclusions and aggregated proteins. There autophagy pathway is a protein quality control pathway that acts within cells to aid removal of misfolded and aggregated proteins to prevent formation of these protein inclusions. In many forms of MND the ability of the autophagy pathway to remove toxic proteins is impaired, resulting in accumulation of aggregated proteins within neurons leading to cell death. Drugs that enhance removal of toxic proteins, such as inducers of the autophagy pathway, have potential to be useful for clearing this misfolded and aggregated protein, particularly to counter an impaired level of autophagy activity in MND conditions.

In our studies we have identified four different drugs that can increase activity of the autophagy pathway and improve movement of our zebrafish model of another neurodegenerative disease. Here we have tested the effect of one of these drugs on cultured motor neuron-like NSC-34 cells expressing MND-associated mutant proteins, including FUSP525L, examining the effect of the drug treatments on protein mislocalisation, aggregate formation and cell toxicity. We have also tested the effect of this drug on our zebrafish models of motor neuron disease and monitored the effect on the development of impaired movement and levels of the disease-causing protein. Together the findings of our study provide insight into the potential of this drug for the treatment of motor neuron disease.

Over the last decade, multiple studies have demonstrated that the complement system is upregulated in the blood, CSF and brain of MND patients, suggesting widespread complement activation is at play during MND. There are many functional consequences of complement activation, including opsonisation (driven through C1q and C3b), anaphylatoxin generation and resulting inflammation (driven through C3a and C5a), and direct cell killing (driven through the membrane attack complex), all of which could feasibly be active in MND to propagate neuronal death. Initial studies using SOD1G93A mice deficient in upstream complement factors C1q, and C3 indicated these factors do not contribute to MND pathogenesis. Results from our laboratory however, demonstrate that the anaphylatoxins C3a and C5a play key roles in MND progression by modulating neuroinflammation. SOD1G93A mice deficient in C3a receptors (C3aR) have worsened MND progression, whereas mice deficient in C5a receptors (C5aR1 or C5aR2) have slower disease progression. Intriguingly, both C3a and C5a
are generated following complement activation, suggesting that a homeostatic balance between these two factors controls MND progression. This balance could therefore be tipped towards neuroprotection with pharmacological targeting, a strategy successfully applied with the selective C5aR1 antagonist PMX205, now under clinical development. Downstream terminal inhibition of complement to block membrane attack complex formation is underexplored, but represents another strategy to ameliorate disease currently under investigation in our laboratory. Our results indicate a complex role for complement in MND, which with further study, could lead to multiple drug leads for therapeutic intervention to slow neuronal death in MND.

Dr Mary-Louise Rogers, Flinders University

Pre-clinical validation of growth factors delivered to motor neurons by non-viral gene therapy as a treatment for MND

**Background:**
Our laboratory has developed a non-viral gene delivery method called ‘immunogenes’ comprising a modified antibody that can target and deliver therapeutic genes to motor neurons (MNs) in neonatal mice from the circulation using p75 antibody MLR21. Immunogenes are now needed that can deliver therapy such as neurotrophic genes to adult mice, using an alternate targeting antibody called 2B7. Our current MNDRIA funded project focuses on the development and testing of 2B7 immunogenes in comparison to MLR2-based immunogenes.

**Methods, Results and Future Directions:**
We aimed to demonstrate efficient delivery and expression of immunogenes encoding human IGF-I (hIGF-1) and human GDNF (hGDNF) in MNs in mice and show expression in adult MNs in pre-symptomatic SOD1G93A mice. Construction of immunogenes was as described1. SOD1G93A or neonatal mice (C57Bl/6), n=3-4 per group were injected with either MLR2-PEI-PEG12 or 2B7-PEI-PEG12, carrying pVIVO2-GFP, or pVIVO2-GFP-IGF-I, or pVIVO2-GFP-GDNF via IP injections. Following intraperitoneal injections of MLR2 immunogenes carrying hIGF-I or hGDNF plasmids, RT-PCR analysis of (n=3-4 spinal cords) indicates expression of these genes in the spinal cord. However, in adult SOD1G93A mice, MLR2 immunogenes do not transfect MNs in pre-symptomatic mice, due to low p75 expression. Further studies describing how long transgene expression persists in mice is ongoing in addition to describing 2B7 immunogene transfection in SOD1G93A mice is in progress
Copper and iron are essential for life due to their requirement in fundamental biochemical processes. But if allowed to accumulate to excess, both can be highly toxic and treading the fine line between deficiency and excess is therefore critical. We have demonstrated that the copper drug CuII(atsm) is protective in familial MND mice and that perturbations to copper and cuproenzymes are evident in human, sporadic MND-affected tissue. Here we show that diminished copper availability is associated with iron accumulation. Iron accumulation in MND is supported by MRI scanning of patients in the clinic and is confirmed by our direct assessment of post-mortem tissue.

Moreover, our assessment of copper-dependent ferroxidases indicates that diminished activity of ceruloplasmin may be the basis for iron accumulation in MND. Because ceruloplasmin is a glial protein, we specifically examined the potentially toxic role of glial iron accumulation. We show that mixed glial cultures are sensitive to the iron-dependent form of cell stress known as ferroptosis, and that within these cultures it is the microglia that are exquisitely sensitive to ferroptotic stimuli. Most significantly, we show that mild ferroptotic stress triggers a phenotypic switch in the microglia which causes toxic activation of astrocytes. We provide evidences for these phenomena occurring within CNS tissue obtained from people who had sporadic MND, and for their mitigation in familial MND mice treated with CuII(atsm). We hypothesise that disrupted copper availability leading to ferroptotic stress and toxic glia activation is a significant pathway that leads to neuronal demise in MND.

Professor Julian Gold, The Albion Centre, Prince of Wales Hospital, NSW Health
The Lighthouse Project: investigating the effectiveness of the antiretroviral medication, Triumeq, in people with ALS
Professor Dominic Rowe, Macquarie University

*Inflammation in Amyotrophic Lateral Sclerosis*

There is considerable evidence for inflammation in patients with ALS. This talk will outline the evidence for both peripheral and central inflammation in ALS. Recent data from ongoing human studies at Macquarie University will be presented. Therapeutics focussed on attenuating the inflammatory response will be discussed as they may well represent an opportunity to slow the progression of ALS.

Associate Professor Dominic Hare, The University of Melbourne

*The elemental signature of motor neurone disease*

At a base level, all diseases are characterised by subtle changes in the fundamental chemical makeup of affected tissue. Long before the onset of clinical symptoms or motor neurone death in MND, pathological chemical reactions begin a cascade of cellular events that lead to neurodegeneration. Identifying these chemical changes is a challenging task; the subtlest of shifts in the chemical environment of at-risk neurones requires extremely sensitive analytical equipment that can detect changes at the fundamental level of all matter – the chemical elements. Both healthy and diseased cells have characteristic ‘elemental signatures’ that directly reflect the biochemical processes driving MND pathology. Supported by the Simko Family MND research grant, we have commenced an ambitious project to identify the elemental signature of MND, using a combination of imaging technology, element-specific mass spectrometry and advanced machine learning data analysis methods to build a comprehensive database of how the basic building blocks of life are altered in this disease, with the aim of identifying the precise moment when typical neurochemistry becomes pathological. With this information at hand, we can identify new targets for novel therapies, assess efficacy of emerging treatments that target early disease processes, and potentially develop new early diagnostic tests that give such therapies the best chance at modifying disease outcomes.

Dr Frederik Steyn, The University of Queensland

*Impact of loss of appetite on weight management and disease progression in MND*

**Background:** In MND, malnutrition and weight loss are associated with faster disease progression and shorter survival. Here we use the Council on Nutrition Appetite Questionnaire (CNAQ) to determine the
prevalence and impact of loss of appetite in MND. METHODS: We assessed 62 patients with clinically definite or probable MND and 40 controls. Patients with PEG as sole source of nutrition were excluded. Retrospective data for body weight, anthropomorphic measures and clinical outcomes were sourced from ongoing metabolic studies at the RBWH/UQCCR. Anthropomorphic measures were determined by whole body air displacement plethysmography (BodPod, Cosmed).

Results: Loss of appetite (indicated by a CNAQ score of ≥28pts) was more prevalent in MND patients than controls [38.7% vs. 12.5%, odds ratio=4.5 (1.6-13.2); p<0.005] and was associated with greater weight loss (p<0.001), reduction in BMI (p<0.001), and loss of fat free mass (p<0.001). Loss of appetite was associated with decline in fat mass (p<0.001), whereas intact appetite was associated with increased fat mass (p=0.002). Loss of appetite correlated with a faster functional decline (p<0.001; change in ALSFRS-R). There was no association between CNAQ scores and bulbar dysfunction. Conclusion: We confirm that loss of appetite is prevalent in MND and is associated with greater weight loss, loss of fat free mass, and faster functional decline. Loss of appetite is not associated with bulbar dysfunction, suggesting that dysphagia is not a sole contributor. Identification of factors that contribute to loss of appetite in MND could inform nutritional management and improved care.

Professor Vicki Flood, The University of Sydney

A pilot study to trial the feasibility and effect of swallowing exercises and diet among people with Motor Neurone Disease

Background: Swallowing problems and poor nutrition intake compromise quality of life among people with MND, and contribute to poor muscle function and fatigue. The aim of this study was to investigate the feasibility and impact of a moderate intensity swallowing exercise program and diet regime of 20% energy from extra virgin olive oil (EVOO).

Methods: Participants with MND were randomised into three groups: 1. exercise only (lingual and laryngeal exercises) (Ex); 2. EVOO only (Diet); and 3. exercise combined with EVOO (Ex+Diet). The intervention occurred over four weeks and included: 2x/week exercises with a speech pathologist, and used surface electromyography (sEMG) biofeedback; and weekly support from a dietitian and included guidelines to consume EVOO. Baseline and follow-up assessments of swallow function, dietary intake and weight were investigated.

Results: Preliminary data from the pilot study (n=12, mean age 66 years, 75% male, mean baseline ALSFRS-r 35.4) indicated good compliance and tolerability of diet; participants consumed the prescribed EVOO orally or via tube feeding, and had a non-significant mean weight gain of 1.0kg (Diet and Ex+Diet) (n=8), compared to 0.1kg in the Ex group (n=4). Participants randomised to the exercise groups (n=8), were observed to have variable fatigue and ability to complete the exercises, with an overall reduction in pharyngeal residue.

Discussion: This pilot study suggests that diet intervention of 20% energy from EVOO is feasible and well tolerated, and may support weight maintenance. The swallowing exercises were feasible among most included people with MND. Additional assessment is required to confirm the findings.
Nicole Sheers, The University of Melbourne

**Lung Volume Recruitment in Neuromuscular Disease**

Weak respiratory muscles, decline in breathing capacity and poor cough are problems commonly experienced by people with MND and other neuromuscular disorders. Respiratory complications are associated with substantial morbidity and ultimately it is respiratory failure that will be the cause of death for the majority of people with MND. Non-invasive ventilation helps breathing overnight and increases survival in people who can tolerate it, but it is not designed to help with deep breathing and coughing.

Respiratory therapies that aim to improve cough ability and lung volume are recommended in clinical guidelines, yet evidence for their efficacy is limited. Regular deep breathing exercises, specifically lung volume recruitment, a method of assisting lung inflation, may prevent the respiratory system from getting stiff, slow the decline in lung function, improve cough effectiveness and help prevent chest infections.

This randomised controlled trial is the first of its kind to evaluate the clinical, physiological and quality of life effects of daily lung volume recruitment in this population. This project is in the final stages of participant recruitment and data collection. However, preliminary analysis of baseline respiratory function in the first 60 participants suggests this cohort of people with MND have better preserved lung capacity, are less stiff and experience lower rates of respiratory infection compared to participants with slowly progressive neuromuscular diseases. Although these differences support the project hypothesis, results from the clinical trial will provide much needed data about the benefits versus burden of regular respiratory therapy in the management of people with MND.

Dr Sam John (for Dr Thomas Oxley), The University of Melbourne

**Minimally invasive brain-controlled communication**

Brain Machine Interfaces (BMI) are artificial communication channel between the brain and external devices. BMI’s have the potential to return independence to people with paralysis through direct brain-control of assistive technologies. Despite several advances a fully portable BMI that people can take home has not been accomplished due to the invasive method of implanting the device into the brain. Unlike existing, highly invasive technologies, we developed a minimally invasive interface (the Stentrode™) that avoids the need for open-brain surgery by recording brain signals from within a blood vessel. This minimally invasive method significantly increases the safety and efficacy of the neural interface allowing for a take home Brain Machine Interface. In previous work we showed that the Stentrode can record high-fidelity brain signals over a chronic duration, supporting the potential of direct brain-control of external devices while mitigating risks associated with open brain surgery. We aim to implant the Stentrode in a world-first human trial in 2019, translating this technology for in-home use by people with motor neuron disease (MND). Here we describe our progress toward a first in human trial of a minimally invasive brain machine interface that will enable people with Motor Neuron Disease to control a computer to do everyday tasks such as typing.
19. Azin Amin, The University of Melbourne

*Development of autophagy-inducing peptides as a potential therapy for MND*

The pathological hallmark of a range of neurodegenerative diseases including Motor Neurone Disease (MND) is the presence of protein inclusions and aggregates in affected neurons. Aggregates or their precursors, misfolded proteins, progress continually from the site of onset to disturb cellular processes, ultimately resulting in neuron degeneration. The only intracellular degradative pathway that can purge the cells of these misfolded proteins, aggregates, and dysfunctional organelles is autophagy. Since impaired/reduced autophagy may contribute to MND pathogenesis; upregulating autophagy offers a promising potential therapeutic option. This study aimed to develop and synthesize novel pharmacological agents that have non-toxic autophagy-inducing properties in motor neuronal NSC-34 cells. In this study, we synthesized different analogues of the Beclin1 peptide, the master regulator of autophagy. In order to enhance the cellular uptake and increase the cytosolic bioavailability, the Beclin peptides were synthesized with a cell-penetrating peptide domain (a fragment of apolipoprotein E (ApoE)), and an endosomal escape domain, hemagglutinin-2 protein (HA2). These Beclin peptides increased autophagy levels by increasing the conversion rate of cytosolic-associated LC3-I to autophagosome-associated LC3-II, and increasing the degradation rate of the autophagy receptor and substrate, p62. However, Beclin peptides did not alter VDAC1 degradation indicative of mitophagy, a selective form of autophagy responsible for mitochondrial clearance. Additionally, the most potent autophagy-inducing peptides, HA2-ApoE-Beclin1 and HA2-ApoE-Beclin3 significantly reduced the most severe and aggregate-prone form of mutant SOD1 (A4V) soluble monomeric and oligomeric protein levels in vitro. This study provides evidence for a novel autophagy-inducing approach that could have a potential therapeutic benefit in MND and other proteinopathies.

7. Rachel Atkinson, The University of Tasmania

*Does TDP-43 pathology cause axon traffic problems?*

The aim of our work is to determine the effect of pathological TDP-43 mislocalisation on the axon structure and function. To do this we have developed a novel model using the visual system, which allows investigation of the ultrastructural alterations to optic nerve axons following genetic manipulation of the retinal ganglion cells (RGCs), as well as downstream effects on the synapse. AAV2 virus was used to introduce fluorescently tagged human WT-TDP-43 and TDP-43 with a mutation in the nuclear localisation signal (-NLS) into RGCs and the effect on neurons examined histologically after 3 months. AAV2 transduced over 60% of RGCs, with WT-TDP-43 expression confined to the nucleus and NLS-TDP-43 expressed throughout the cytoplasm. WT-TDP-43 expression resulted in subtle changes to RGCs including a significant (p<0.05) increase in the numbers of synapses in the inner plexiform layer of the retina, but did not significantly affect the axons. NLS-TDP-43 expression resulted in axonal pathology including a significant (p<0.05) increase in degenerative profiles in optic nerves. Electron microscopic analysis revealed that these degenerative
profiles resulted from accumulation of autophagic vesicles. Autophagic vesicles are formed in the axon terminal and retrogradely transported, thus our data suggests that cytoplasmic TDP-43 “trapping” in the cytoplasm results in disruption to retrograde transport, which is linked to a dying back pathology. Our future studies will investigate specific transport cargos affected by ALS pathology as well as whether NLS TDP-43 induced axon pathology is linked to known axon degeneration mechanisms such as Wallerian degeneration and developmental axon pruning.

5. Samantha Barton, Florey Institute of Neuroscience and Mental Health

_The characterisation of oligodendrocytes derived from induced pluripotent stem cells from ALS patients harbouring point mutations in the TDP43 gene._

**Introduction:** TDP-43 pathology is common to >95% of ALS patients and has been identified in glia, including oligodendrocytes. Oligodendrocytes have two main roles in the brain, with both being critical for maintaining neuronal health and function, which are myelination and metabolic support. The effect of TDP-43 pathology on oligodendrocyte function remains unknown. **Methods:** We derived oligodendrocytes from induced pluripotent stem cells (iPSC) from patients harbouring separate point mutations in the TDP43 gene, namely G298S and M337V. We investigated the effect of these mutations on oligodendrocyte TDP-43 subcellular localization, cell morphology, and function in vitro and in vivo. Using advanced CRISPR-Cas9 technology we generated an isogenic control line for comparison and also generated oligodendrocytes from an unrelated control. Specifically, we aimed to ascertain the effect of these TDP-43 mutations on the myelinating and metabolic capacity of oligodendrocytes compared to controls using a ‘disease in a dish’ approach. **Results and Conclusions:** For the first time, we demonstrated pathogenic TDP-43 protein mislocalization in iPSC-derived oligodendrocytes that was not present in the isogenic control or unrelated control. Despite this TDP-43 pathology, the oligodendrocytes did not have a developmental or morphological deficit and there was no effect of the TDP43 mutations on the oligodendrocytes’ ability to myelinate or on their metabolic capacity.

11. Britt Berning, The University of Queensland

_The Golgi apparatus of neurons is an early feature of disease in TDP-43 ALS mice._

The Golgi apparatus is a dynamic organelle critical for protein post-translational modification and intracellular transport. Amyotrophic lateral sclerosis (ALS) is a motor neuron disease characterised by rapidly progressive neurodegeneration causing paralysis and death. Golgi apparatus fragmentation is found in the spinal cord motor neurons with the TDP-43 pathology that is characteristic of ALS, in patient autopsy samples. However, it is unknown how early Golgi fragmentation occurs and how it contributes to neurodegeneration. We therefore studied the transgenic rNLS TDP-43 mouse model of ALS, which express human TDP-43 (hTDP-43) with a defective nuclear localisation sequence (NLS) in neurons, prior to symptoms and throughout disease. Golgi morphology was examined by transmission electron microscopy and confocal microscopy. Also, we used quantitative proteomics to identify proteins of altered abundance in rNLS mouse cortex and
spinal cord, and candidate proteins of interest were validated by immunoblotting and immunohistochemistry. Even prior to disease, rNLS mice exhibited dramatic fragmentation of the Golgi apparatus into discrete stacks that were dispersed throughout the cytoplasm of neurons. This was particularly evident in motor neurons, and persisted throughout the disease course. Using proteomics, immunoblotting, and immunofluorescence, we detected increased levels of several Golgi apparatus-related proteins in the rNLS mice compared to controls, even prior to neurodegeneration. Golgi apparatus changes therefore represent an early mechanism in disease that may reflect a compensatory response to cytoplasmic TDP-43 accumulation. These studies suggest that Golgi apparatus fragmentation and trafficking defects may contribute to development of ALS.

35. Karin Borges, The University of Queensland

**Metabolic disturbances in glucose metabolism contributing to increased oxidative stress and treatment thereof**

Energy metabolism impairments in MND have long been known. To investigate specific alterations in glucose metabolism in glycolytic, pentose phosphate and TCA cycle pathways, we injected labelled 13C-glucose to wild type and hSOD1G93A mice at symptom onset (80 days). Using liquid chromatography-tandem mass spectrometry, levels of metabolites were determined in extracts of cortex and spinal cord. In addition, the activities of several enzymes involved in glucose metabolism were quantified. In the spinal cord, the levels of pentose phosphate pathway (PPP) intermediate ribose 5-phosphate (p=0.039) were reduced by 37% in hSOD1G93A mice, while the % 13C enrichment in glucose 6-phosphate were increased three-fold. The activities of the glucose 6-phosphate dehydrogenase were reduced by 24% in the spinal cord (p=0.005), pointing to abnormalities in the PPP. This is expected to result in increased oxidative stress due to a lowered availability of NADPH and glutathione. The total amounts of the glycolytic intermediates pyruvate and lactate were significantly reduced by 20 % in hSOD1G93A mice (p=0.037). Also, the activity of the glycolytic enzyme, pyruvate kinase was reduced in cortex by 31% (p=0.002), indicating alterations in pyruvate handling. In CNS mitochondria OGDH activity was decreased by 13-22%, an indicator for oxidative stress. Overall, this study revealed decreased activity of the PPP in spinal cord and confirms earlier findings of alteration in glycolysis in hSOD1G93A mouse CNS at early symptomatic stages. Triheptanoin, an alternative fuel with anti-oxidant properties, can improve some of these problems in MND and is being tested in a clinical trial.

9. Rosemary Clark, The University of Tasmania

**Investigating the Neuropeptide Y system as a neuroprotective strategy for ALS**

**Introduction:** Neuropeptide Y (NPY) is widely expressed in the central and peripheral nervous system and plays important roles in a variety of physiological processes, including inhibiting excessive glutamate release, stimulating autophagy, neurogenesis and regulation of neuronal excitability. Several lines of evidence indicate that NPY may have a neuroprotective role in chronic neurodegenerative disease. We recently provided evidence
that brain NPY levels are altered in human and rodent models of amyotrophic lateral sclerosis (ALS). In this study we focus on NPY as a neuroprotective agent that can be harnessed to ameliorate pathogenic excitability and excitotoxicity in ALS. **Methods:** Here, we investigated the NPY receptor expression in cortical cultures derived from G93A SOD1 E15.5 embryos. Primary cortical cultures were treated with the excitotoxin kainate for 24 hours at 14 days in vitro and assessed using cell viability assays, western blot and immunohistochemistry. **Results:** In naïve conditions there was two-fold increase in NPY-Y1 receptor levels in G93A SOD1 cortical cultures compared to WT cultures (p<0.01). Exposure to kainate caused a similar increase in NPY-Y1 receptor levels in WT cultures (p<0.01), a treatment effect that was not observed in G93A SOD1 cultures. Assessment of NPY-Y2 receptors in naïve conditions found no significant difference (p>0.05), however, kainate caused a four-fold increase in both G93A SOD1 and WT cultures (p<0.01). **Conclusion:** These results suggest NPY-R1 has a role in the pathogenicity of the ALS-causing G93A SOD1 mutation. Ongoing works assess the potential of targeting the NPY system to confer neuroprotection against pathogenic excitability associated with ALS.

22. Alana De Luca, Macquarie University

**Proteomics reveals pathogenic ALS/FTD mutations in CCNF triggers apoptosis via the Bad/Bax signalling pathway**

Mutations in the CCNF gene have been identified as a novel cause of ALS/FTD1. CCNF encodes cyclin F, an E3 ubiquitin ligase that forms part of a Skp-Cul-F-Box (SCF) complex that ubiquitylates proteins for degradation by the ubiquitin-proteasome system. We have shown that expression of the cyclin FS621G mutation leads to defective protein degradation, motor axonopathy, and signature features of ALS pathogenesis in vitro and in vivo2-4. In this study, we investigated the effect of other mutations (K97R, S195R, S509P, and R574Q) in cyclin F on their E3 ligase Lys48-specific ubiquitylation activity, and how this may contribute to dysfunctional proteostasis. We used an unbiased label-free quantitative proteomics strategy to determine the differential expression of proteins in transfected cells to uncover changes to biological processes and cellular pathways. Proteomic analysis of cells expressing cyclin F wild-type, K97R, S195R, S509P, R574Q, and S621G identified >5000 quantifiable proteins. Principal component analysis revealed proteomic similarities between cells expressing cyclin F wild-type and R574Q, while the proteomes of cells expressing K97R, S195R, S509P and S621G mutations clustered closely together. Interestingly, these four mutations have been identified to be ALS/FTD causative mutations, while the R574Q mutation is a database SNP. Ingenuity Pathway Analysis predicted variations to multiple canonical pathways and potential mechanism(s) responsible for motor neuron death, one of which was the activation of the Bad/Bax apoptosis signalling pathway in all four mutations. These findings demonstrate that gene discoveries are valuable to generate an “ALS/FTD proteomic profile” that will advance our knowledge of ALS/FTD disease mechanisms.
38. Emma Devenney, The University of Sydney

**C9ORF72 Kindreds and major psychiatric disorders: A study of 1,414 family members**

**Background:** Psychotic symptoms are common in carriers of the C9orf72 expansion. It has been suggested that the C9orf72 phenotype might extend beyond motor neurone disease (MND) and frontotemporal dementia (FTD) to include psychiatric disorders. 

**Objective:** This study aimed to systematically explore this potential association by i) determining if psychiatric disorders occur more frequently in C9orf72 positive than C9orf72 negative kindreds, ii) determining if psychiatric disorders aggregate within these kindreds, and iii) considering whether the inclusion of psychiatric diagnosis is warranted when considering familial ALS and FTD.

**Method:** The family history of 1,414 first and second-degree relatives of FTD and ALS patients was obtained by means of a validated semi-structured interview. In addition to routine assessments, patients also underwent a clinical interview to determine if psychotic symptoms were present, and were tested for the C9orf72 expansion. Statistical analyses employed both the hazard ratio (HR) and relative risk ratio (RR) to determine the risk profiles within families.

**Results:** The study found that relatives of C9orf72 carriers were more likely to be diagnosed with Autism Spectrum Disorder (ASD) than non-carrier family members (HR=2.7, 95% CI: 1.1 - 6.9, p=0.03). In addition, relatives of C9orf72 carriers were more likely to develop schizophrenia compared to non-carriers (HR=4.9, 95% CI: 1.9 - 13.9, p=0.003), and were also more likely to experience an episode of late-onset psychosis, unrelated to schizophrenia, in comparison to non-carriers (HR=17.9, 95% CI: 2.2, 143.2, p=0.007). The probability of suicide was also significantly higher for family members of C9orf72 carriers (HR=2.7, 95% CI 1.2,6.2, p=0.02). A positive association was identified between psychosis in probands and mental health disorders in family members (p=0.04).

17. Emily Don, Macquarie University

**A Zebrafish Model of FUS Histopathology for the Study of Autophagy Inducers in MND**

Our aim is to understand the cellular pathology observed in Motor Neuron Disease (MND) caused by mutations in Fused-in-Sarcoma (FUS) with the long-term goal of therapeutic testing. We have utilised transgenic zebrafish to create models of MND. We have generated stable transgenic zebrafish lines expressing either wild-type or mutant (R521C) human FUS. The zebrafish expressing mutant human FUSR521C recapitulated distinctive histopathological features of MND pathology, including significant mislocalisation of FUS to the cytoplasm and the formation of protein aggregates. We are now investigating autophagy inducers to determine if increased levels of autophagy lead to reduced proteinopathies in this model. We believe that the human mutant FUS expressing transgenic zebrafish will be a valuable tool for studying the effects of FUS proteinopathies and testing therapeutics targeted at correcting these.
6. Marcus Dyer, The University of Tasmania

**Does mislocalised TDP-43 in the motor cortex drive ALS?**

The accumulation of TAR DNA binding protein 43 (TDP-43) in the cytoplasm of neurons is a characteristic hallmark of amyotrophic lateral sclerosis (ALS). We have generated a mouse model of doxycycline (dox) suppressible TDP-43 with a deleted nuclear localisation signal (TDP-ΔNLS) under control of the CAMK2a promoter to target expression of TDP-43 to the cytoplasm of forebrain neurons, where the motor cortex resides. Immunolabelling in the cortex of TDP-ΔNLS mice show mislocalised TDP-43 in a subset of neurons throughout cortical layers 1-5 in the forebrain. Double labelling with the excitatory neuronal marker NeuN demonstrated that the cells expressing TDP-ΔNLS were predominately excitatory neurons. Behavioural tests (rotarod for motor function and Y-maze for cognitive function) were performed and we report significant (p<0.01) deficiencies in motor function observed on the rotarod in TDP-ΔNLS mice in comparison to wildtype control TDP-43 mice. Alterations in the overall activity (p<0.05), time spent in maze centre (p<0.001) and no direct revisits into the same arm of the Y-maze were also observed in TDP-ΔNLS mice, which is indicative of a cognitive phenotype in this model. Understanding the effect of cytoplasmically targeted TDP-43 in upper motor neurons will enable us to investigate the role of the motor cortex in ALS and determine how pathogenic changes in the motor cortex may drive disease.

18. Jean Giacomotto, The University of Queensland

**Versatile genetic systems in zebrafish for unveiling MND genes pathogenicity and generate degenerative models**

We have recently established a platform aiming at evaluating the pathogenicity of MND-risk genes and generate stable degenerative models for drug screening. Although we are now incorporating gain-of-function (GOF) approaches in our pipeline, we have initially taken advantage of a recent RNAi technology developed for the zebrafish allowing to study the partial loss-of-function (LOF) of our risk-genes. We hypothesized that pLOF (as opposed to total absence of one gene function - knockout), coupled or not to GOF, is key to understand the pathogenic contribution of MND risk-genes. Confirming the relevance of our approach, our preliminary data showed that total absence or strong reduction of the gene tbk1 is embryonic lethal while its partial-LOF triggers early motor defects and premature death in zebrafish. These results are promising and suggests that our approach will help to better understand the role of tbk1 in MND, and generate a useful model for the search of therapeutics. That being said, our tbk1-pLOF animals die before sexual maturity, thereby hampering the generation of stable lines required for further investigation and screening. To fix this issue, we are working at establishing an innovative inducible system allowing the generation of healthy animals that can be activated on demand. This system will present the advantage to be compatible with both pLOF and GOF (as well as polygenic approaches), thereby of considerable potential for MND research. In short term, this will allow us to fully investigate the pathogenic role of tbk1 and generate versatile models for drug discovery.
21. Natalie Grima, Macquarie University

*Genetic and immunopathological analysis of CHCHD10 in Australian amyotrophic lateral sclerosis and frontotemporal dementia*

Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are believed to belong to a common disease spectrum. Approximately 15% of ALS patients exhibit co-morbid FTD, and up to 50% of patients will develop some degree of cognitive impairment. To date, the only proven causes of ALS and FTD are gene mutations. Recently, variants in CHCHD10 have been identified as a cause of, or associated with, pure ALS, FTD and ALS-FTD in families and sporadic patients of European ancestry. We sought to uncover the prevalence of CHCHD10 mutations among 81 Australian familial ALS (FALS) patients negative for known ALS gene mutations, 628 sporadic ALS (SALS) and 108 FTD patients. No pathogenic or disease-associated variants were identified among FALS or FTD patients. Two known CHCHD10 variants (p.P34S and p.P80L) were identified in six and two SALS patients respectively. In addition, CHCHD10 immunopathological analysis was performed on ALS, FTD and ALS-FTD central nervous system tissue from patients with known or unknown genetic causes. Immunopathological analysis in ALS patient spinal cord tissue identified a significant decrease in CHCHD10 levels compared to controls and an absence of CHCHD10 in TDP43-positive inclusions in all but 2 SALS cases. CHCHD10 expression levels were variable in ALS motor cortex and ALS/ALS-FTD frontal cortex tissues however expression was restricted to the cytoplasm of neurons. Our preliminary findings suggest that CHCHD10 mutations are not a common cause of ALS or FTD in Australian patients of predominately European ancestry. However, pathological changes suggest a role for CHCHD10 in these neurodegenerative diseases.

27. Mouna Haidar, The Florey Institute of Neuroscience and Mental Health

*Validation of a combinatorial viral approach to target UMNs and LMNs for selective neuronal activation in vivo*

Hyperexcitability of brain motor neurons (upper motor neurons, UMNs) and spinal cord motor neurons (lower motor neurons, LMNs) is linked to excitotoxicity and cell death in MND. UMN hyperexcitability is present in sporadic MND, while occurring prior to symptom onset in familial MND. Likewise, LMN hyperexcitability is a consistent feature of MND. However, the anatomical site at which neuronal dysfunction initiates remains contentious. The aim of this project is to determine the neuroanatomical origin of progressive neurodegeneration in MND. Specifically, we are examining whether chronic neuronal activation of UMNs (Aim 1) and/or LMNs (Aim 2) results in UMN/LMN loss, muscle weakness and subsequent motor deficits.

In preliminary studies to validate this approach, a retrograde tracer expressing Cre-recombinase (Canine Adenovirus 2 Cre, CAV2-Cre) was injected into the spinal cord lumbar region via a laminectomy followed by bilateral stereotaxic injection of a Cre-dependent excitatory DREADD (AAV2-hSyn-DIO-hM3Dq-mcherry) into the motor cortex of adult mice. To examine LMN targeting specificity, AAV2-hSyn-DIO-hM3Dq-mcherry was injected into the ventral horn of adult ChAT-Cre mice. Following 4-weeks recovery, viral expressing mice were treated with clozapine-N-oxide (CNO, 3 mg/kg twice daily) or saline (n = 3 p/group). Using immunohistochemistry, we observed effective and specific transduction of UMNs and
LMNs, and CNO administration resulted in activation of hM3Dq expressing motor neurons, relative to saline treated mice. This experimental model provides a novel tool to model neuronal hyperexcitability occurring in MND placing this project in a unique position to definitively address the contribution of UMNs and LMNs to pathophysiology of MND.

10. Emily Handley, The University of Tasmania

The motor cortex is vulnerable to TDP-43-mediated alterations to dendritic spine dynamics

Dendritic spine turnover is central to maintaining neuroplastic cortical networks, and disruptions linked to synaptic dysfunction and the progression of neurodegenerative disorders. We have found that TDP-43- the protein most frequently found in cytoplasmic aggregates in ALS- is involved in maintaining neuronal synapses in mouse models of ALS, regulating the number and maturation of dendritic spines. Spine changes occur well before symptom onset in the motor cortex, but not the somatosensory cortex, indicating that this is one of the earliest pathological changes associated with TDP-43. We have advanced these finding through the application of 2-photon live imaging in the motor cortex in real time. Cranial window were surgically implanted over the motor cortices (MC) of male Thy1-YFP::TDP-43A315T mice and Thy1-YFP controls, and 2-photon live imaging used to capture the gain, loss and morphology of dendritic spines on Layer V pyramidal neurons. We report an important role for TDP-43 in the development, maturation and turnover of dendritic spines at a pre-symptomatic time point in the TDP-43A315T mouse model. The male TDP-43A315T MC displayed a significant reduction in overall turnover of spines compared to controls, specifically in mature morphologies. These changes occur presymptomatically in a cortical region most susceptible to disease insults, and highlights the vulnerability of the motor cortex to TDP-43-mediated alterations at the dendritic spine. This research provides insights into early disease events that can be utilised to understand activity-dependent disease pathology for future research and therapeutics aimed at mitigating this devastating disease.

29. James Hilton, The University of Melbourne

Perturbed copper and cuproenzyme function are significant features of sporadic motor neurone disease (MND)

Improving bioavailability of copper using CuIl(atsm) has been proposed as treatment strategy for MND and phase 1/2 testing is currently underway. Mouse models of familial MND show that endogenous copper availability fails to satiate changed central nervous system (CNS) requirements for copper as disease develops, and improving CNS copper availability via CuIl(atsm) is effective at ameliorating disease progression. To better understand the relevance of therapeutically modulating copper availability to sporadic cases of MND we used laser ablation-inductively coupled plasma-mass spectrometry to quantitatively and anatomically map copper distribution in sporadic MND-affected CNS tissues relative to controls. We also assessed abundance and activity of various cuproenzymes. Endogenous copper distribution and activity of multiple cuproenzymes are
extensively disrupted in the sporadic MND CNS. Anatomically, a decrease in total copper levels in the ventral horn grey matter of the spinal cord was observed. Biochemically, whilst functionality of some cuproenzymes was impaired (ceruloplasmin and dopamine-β-hydroxylase), others were unchanged (SOD1) or even increased (lysyl oxidase). Herein we present evidence for copper and multiple cuproenzymes being disrupted in sporadic MND. Rather than supporting a simplistic scenario of copper-deficiency, our results indicate that when requirements for copper in MND-affected tissues are altered, a copper redistribution occurs that favours certain cuproenzymes over others and is limited by the naturally slow turnover for CNS copper. We propose that the natural availability of copper in the CNS becomes perturbed when requirements are changed in MND, and improving copper bioavailability may be an important mechanism for CuII(atsm) treatment of sporadic MND.

1. Alison Hogan, Macquarie University

*Use of in vitro CRISPR-Cas9 genome editing for the identification of novel MND-linked gene mutations*

Gene mutations remain the only proven cause of motor neuron disease (MND) and appear to contribute to both familial and sporadic forms of the disease. Identification of genetic risk factors has provided great insight into the biology of MND. However, MND-linked variants are yet to be identified in 40% of familial and 90% of sporadic patients. Many MND-affected families display reduced disease penetrance i.e. not all family members who carry a mutation develop MND. Genetically, these families appear to lie between classic familial and sporadic MND. This presents an opportunity to identify novel genetic risk factors that underlie both forms of the disease. However, gene discovery in these families is challenging as multiple variants that segregate with disease are commonly present, any one of which may have pathogenic effects. Our laboratory aims to establish a functional pipeline that will differentiate pathogenic from benign variants. This project will establish one component of the pipeline by utilising CRISPR-Cas9 genome editing tools to perform targeted modification of patient fibroblasts. Potential MND-linked variants identified in that patient will be “corrected’ to the wildtype allele and the effect of this “correction” on disease relevant characteristics will be determined. This will provide a strong indication of variant pathogenicity in a genetically relevant model and consequently contribute to the identification of novel MND-linked genes. Additionally, the unedited and genome edited fibroblasts will provide models with which to investigate the mechanisms of variant pathogenicity and provide tools for the preliminary testing of potential therapeutics.

12. Cortina Chen Hsiao-Jou, The University of Queensland

*Poly(GA) dipeptide repeat proteins generate IL-1β in primary microglia through the NLRP3 inflammasome*

An intronic (G4C2)n hexanucleotide repeat expansion in C9orf72 is the most common genetic cause of motor neuron disease (MND). Co-aggregating dipeptide repeat (DPR) proteins synthesised from these expanded repeats, including poly(GA) from the sense transcript, display a propensity to aggregate and exhibit high neuronal toxicity. However the microglial response to these DPR proteins is not clear. Our prior studies have
demonstrated a link between sensing of SOD1 and TDP43 protein aggregates by microglia and activation of the NLRP3 inflammasome complex, leading to the generation of IL-1β. In the present study, we aimed to determine if the NLRP3 inflammasome is similarly activated in response to DPR poly(GA) proteins. We synthesised 1-10 repeats of the DPR, poly(GA), using a solid-phase peptide synthesis strategy and confirmed the formation of protein aggregates by poly(GA)10 using the thioflavin assay. The inflammatory response elicited by poly(GA)1-10 was further tested in primary mouse microglia. Following incubation with poly(GA)10, there was substantially increased IL-1β production from LPS-primed microglia which was both dose- and time-dependent. Smaller repeat sizes were unable to aggregate and did not generate IL-1β from microglia. The involvement of NLRP3 in poly(GA)10-mediated IL-1β production was confirmed using a specific NLRP3 inhibitor, MCC950. Furthermore, the IL-1β response was not observed following poly(GA)10 incubation in microglia isolated from NLRP3-/- mice. Together, these results demonstrate the importance of NLRP3 inflammasome in regulating the inflammatory response following aggregated DPR proteins which could be a potential therapeutic target to slow propagative neuroinflammatory cell death in C9orf72 cases of MND.

36. Chi Hsuan Hu, Calvary Health Care Bethlehem

*Do patients with Motor Neurone Disease experience disorders of olfaction?*

**Background:** It has been established that people with Motor Neurone Disease (MND) can suffer from non-motor problems. Olfactory deficits have been observed through small sample studies. However, evidence is conflicting and there’s a paucity of studies assessing correlations with clinical sub-types and the diagnostic utility of smell impairment.

**Objectives:** Examine the prevalence of smell abnormalities in MND and its relationship with various clinical parameters and evaluate the clinical usefulness of a smell test. **Method:** Our cross-sectional study has (to date) assessed 60 MND patients and 20 matched controls with the Brief Smell Identification Test (B-SIT) and a cognitive test. Patient data was collected on clinical phenotype, behavioural changes, disease duration, disease severity and respiratory function. We performed non-parametric and receiver operating characteristic (ROC) curve analyses. **Preliminary results:** 31.67% of MND participants displayed smell abnormalities. B-SIT scores were significantly lower than controls (MND=9.03/12 vs. Controls=10.25/12, p<0.05). Scores didn’t differ between bulbar and limb-onset groups. Analysis of current data shows no correlation between olfactory performance, and frontotemporal changes, disease duration, severity nor respiratory function. ROC analysis showed poor diagnostic accuracy of the B-SIT (area under curve=0.58) in identifying MND participants. **Conclusion:** Olfactory impairment is significantly more common in people with MND, however our results do not support smell testing as a useful biomarker. Analysis so far does not show correlation between B-SIT scores and the other clinical variables. We aim to study more patients and controls to validate these findings. Pathological studies would benefit our understanding of the involvement of olfactory structures.
32. Kai Kysenius, The University of Melbourne
Capturing the elemental signature of motor neurone disease

The elemental composition of biological material reflects organic diversity, giving each tissue a unique “elemental signature”. Measuring perturbations in the natural elemental signature of a given tissue can provide valuable insight to the cause of disease and potential opportunity for therapeutic intervention. Broader understanding of the elemental signature of motor neurone disease (MND), across multiple regions of the CNS, is likely to expedite targeted therapy development, as evidenced by the successful translation of copper-containing CuII(atsm) into clinical trials. We used laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) to assess anatomically defined changes to magnesium, manganese, iron, copper, zinc, calcium, sulphur and rubidium in multiple CNS regions from MND cases and controls. This technique involves constructing 2D elemental maps of the sections by vaporising material with a laser, which is then analysed by ICP-MS. Our analysis of human MND CNS tissue revealed significant anatomical elemental changes primarily in the spinal cord and motor cortex, two primary sites of MND pathology. In the spinal cord, the ventral horns presented with decreased copper levels and increased iron levels compared to controls. Additionally, zinc levels were increased throughout the spinal cord. In the motor cortex, both iron and zinc were increased in the grey matter, whereas only iron was increased in the white matter. We hypothesise that these localised changes to copper, zinc and iron distribution reflect biochemical alterations of the metalloproteins and their function, suggesting novel targets for therapeutic intervention.

16. Isabella Lambert-Smith, Macquarie University
Evaluation of autophagy induction to ameliorate mutant FUS disease phenotypes in cell culture MND models

A universal pathological feature of brain and spinal cord motor neurons (MNs) of familial and sporadic MND patients is the presence of ubiquitylated protein inclusions. A correlation between toxic gain-of-function mechanisms of MND-associated mutant proteins, accumulation of misfolded proteins in MNs and cellular toxicity has been demonstrated in several studies. These findings suggest that cellular mechanisms involved in protein quality control and degradation are impaired in MND. Furthermore, the autophagy pathway has been shown to be impaired in MND caused by mutations in SOD1, FUS and C9ORF72. This raises the idea that induction of autophagy may have the potential to clear mutant proteins and ameliorate MND phenotypes. Here we present findings from our investigation into the effects of pharmacological induction of autophagy in cell culture models of mutant SOD1, FUS, CCNF and TDP-43-linked MND. MN-like NSC-34 cells expressing MND-associated mutant FUSP525L, SOD1A4V, CCNFS621G or TDP-43M337V exhibit distinct disease phenotypes of cellular toxicity, protein mislocalisation and inclusion formation. Sytox Blue cell staining coupled with flow cytometry enables evaluation of cellular toxicity, while protein mislocalisation and inclusion formation are examined using confocal microscopy. We have examined selected FDA approved drugs and small compounds for their ability to improve these phenotypes, decrease the levels of the mutant proteins and augment autophagy activity. These studies provide insight into autophagy induction as a potential therapeutic strategy for MND.
13. John Lee, The University of Queensland

**Investigating the role of complement C5a receptor, C5aR1 in TDP43 based models of Motor Neuron Disease**

The complement system is upregulated in MND, with recent studies indicating that the activation product C5a may accelerate disease progression via its receptor, C5aR1 in SOD1G93A mice. However, a comprehensive examination of C5a-C5aR1 signalling in other transgenic MND models has not been performed. This study therefore aimed to determine the expression of C5aR1 in two novel MND mouse models with the familial MND-linked TDP43Q331K mutation as TDP43 pathology is found in the majority of MND patients. TDP43Q331K and TDP43Q331KxWT transgenic mice were examined at different ages of disease progression. Expression of C5a and C5aR1 were examined using real-time quantitative PCR and enzyme-linked immunosorbent assay. Localisation of C5aR1 within the lumbar spinal cord was also investigated using immunohistochemistry. C5a and C5aR1 increased in both TDP43Q331K and TDP43Q331KxWT mice during disease progression, suggesting overall increased complement activation with immunolocalisation demonstrating expression on motor neurons and microglia surrounding the regions of motor neuron death. These results indicate that similar to SOD1 transgenic animals, local complement activation via increased C5a-C5aR1 signalling may contribute to motor neuron death in the new TDP43 based mouse MND models. This further validates C5a-C5aR1 signalling as a potential therapeutic target to slow disease progression in MND. Current studies are now investigating the impact of the therapeutic blockade of C5aR1 on disease outcomes in these models.

8. Katherine Lewis, The University of Tasmania

**TDP-43 at the synapse: Investigating the role of TDP-43 in synapse formation at the dendritic spine using in vitro cortical neurons**

Altered cortical excitability is an early event in the brain of people with amyotrophic lateral sclerosis (ALS), and synapse dysfunction occurs prior to symptom onset in ALS mouse models. An important synaptic role for TAR DNA binding protein 43 (TDP-43), a protein whose mislocalisation and aggregation are key pathological features of ALS, has been proposed. However, the relationship between ALS-linked TDP-43 mutations, excitability, and synaptic function is not fully understood. We investigated the role of ALS-linked mutant TDP-43 in synapse formation in mouse primary cortical neurons expressing human TDP-43A315T. Compared to control neurons, we found reduced dendritic spine density, reduced action potential generation, and alterations in the subunit composition of AMPA glutamate receptors at the dendritic spine. We hypothesise that these changes are mediated by the cytoplasmic mislocalisation of hTDP-43A315T. To further investigate changes instigated by cytoplasmic TDP-43, we are currently performing primary neuron cultures from the hTDP-43TET®NLS mouse model, in which hTDP-43 lacks its normal nuclear localization sequence and is mislocalised to the cytoplasm. Expression of hTDP-43TET®NLS in these cultures is limited to excitatory projection neurons under the Camk2a promoter, and is inducible using tetracycline antibiotics. We are performing immunocytochemical analysis and molecular analyses such as western blot, RT-qPCR and isolation of cell-surface proteins, to examine the expression of AMPA receptor subunits in these hTDP-43TET®NLS cortical neurons.
Further studies into the mechanisms underlying AMPA receptor-mediated excitability changes within ALS cortical circuitry, particularly in response to the cytoplasmic mislocalisation of TDP-43, may yield novel therapeutic targets for treatment of ALS.

28. Jeffrey Liddell, The University of Melbourne

**Protective action of Cull(atsm) in models of MND is mediated through copper-dependent upregulation of astrocytic Nrf2**

The copper complex, Cull(atsm) is currently in early stage clinical trials, initiated on the basis of outstanding preclinical results: Cull(atsm) delays symptom onset and extends lifespan in multiple transgenic mouse models of motor neuron disease (MND) and is the first therapy ever to be independently verified by the ALS Therapy Development Institute. In spite of this, the molecular targets of Cull(atsm) remain unclear. The predominantly glial transcription factor Nuclear factor erythroid 2-related factor (Nrf2) is a master regulator of antioxidant genes. We demonstrate that human sporadic MND spinal cord exhibits mild activation of Nrf2 signaling. Similarly, SOD1-G37R mice also exhibit mild Nrf2 activation, which is stimulated by therapeutic treatment with Cull(atsm). In vitro, Cull(atsm) activates Nrf2 in cultured astrocytes but not neurons or Nrf2-/- astrocytes, and in human astrocytes derived from MND patient iPSCs more strongly than in healthy controls. Astrocytes treated with Cull(atsm) promote neuron survival and neurite extension in an Nrf2-dependent manner. The mechanism of Nrf2 activation by Cull(atsm) involves inhibition of Nrf2 nuclear export by GSK3 via copper-dependent serine/threonine phosphatase inhibition.

In conclusion, we show that endogenous activation of Nrf2 in human MND spinal cord is clearly inadequate to prevent motor neuron failure, and MND-model mice recapitulate this phenotype. Cull(atsm) activates Nrf2 in MND-model mice and cultured human and mouse astrocytes, and promotes neuron survival in an Nrf2-dependent manner. This is the first report of copper-associated Nrf2-mediated neuroprotection and has important considerations for the therapeutic treatment of MND.

30. Tanya McDonald, The University of Queensland

**Bioenergetic changes in the skeletal muscle of the SOD1G93A mouse model of MND**

Increasing evidence indicates that deficiencies in energy metabolism play a crucial role in the development and progression of MND. In particular, skeletal muscle oxidative stress, mitochondrial dysfunction and other bioenergetic disturbances have all been identified as hallmarks of MND pathology. However, the exact biochemical changes that lead to these energetic disturbances in skeletal muscle have not been determined. Using the SOD1G93A mouse model of MND we profiled energy metabolism in the fast twitch tibialis anterior (TA) and extensor digitorum longus (EDL), the slow twitch soleus and the mixed gastrocnemius muscles at pre-defined onset and mid stage of disease. The activities of regulatory enzymes involved in several pathways of both glucose and fatty acid metabolism were assessed in the different muscle types, with each muscle displaying a unique metabolic profile at the mid stage of disease. In brief, the SOD1G93A mice display a loss of glycolytic activity in the mixed and fast twitch muscles, whereas, mitochondrial metabolism was
impaired in the mixed and slow twitch muscles. These changes in energy metabolism will be correlated with established MND pathologies such as the loss of neuromuscular junction innervation, change in muscle fiber type distribution and the infiltration of macrofages along with the distribution of the glucose transporter, GLUT4, on the plasma membrane. Overall, this study will determine the correlation between metabolic changes in the SOD1G93A muscle tissues, and previously established muscle pathologies for this model.

37. Camille Paynter, The University of Melbourne

*Communication impairment and healthcare decision-making in MND: A narrative review*

**Rationale:** For any condition, person-centred healthcare relies on sharing of information and a mutual understanding of the person’s needs and preferences. Decision-making in MND becomes more complex as there is no curative treatment and a high probability of co-morbid cognitive or communication difficulties. **Objective:** To identify the reported impact of cognitive or communication impairment on patient and carer involvement in healthcare decision-making in MND. **Methods:** Databases were searched (January 1998 to July 2018) using terms: MND; motor neuron* disease; ALS; amyotrophic lateral sclerosis; decision making; advance care plan*; advance directive; communication; communication impairment. **Results:** Forty articles were screened. Five articles were eligible for review. Data synthesis used the validated Framework Method. Data from the results sections related to communication, cognitive impairment or communication impairment in the context of decision-making was extracted. An iterative analysis process grouped data according to common ideas and allowed construction of overarching themes. Four major and 11 subthemes were identified. Major themes are: enablers of patient autonomy; barriers to patient autonomy; the undertaking of decisions; patient values and preferences. **Conclusion:** Our review highlights a paucity of research addressing the impact of cognitive or communication deficits on decision-making in MND. Involving people with MND who present with cognitive or communication impairment, and their carers, in healthcare decisions across the continuum of their disease is vital to providing person-centred care and supportive care for carers. Yet, how to best do this is currently unknown. A longitudinal study is being undertaken to identify how communication impairment impacts healthcare decisions.

20. Nirma Perera, Florey Institute of Neuroscience and Mental Health

*Monitoring the dynamics of autophagic flux in vivo in a mouse model of motor neuron disease*

Autophagy is the main intracellular catabolic pathway that clears misfolded proteins, aggregates and damaged organelles associated with ageing and neurodegeneration. Autophagy is impacted in motor neuron disease (MND). However experimental manipulation of autophagy in mouse models of MND has yielded often contradictory outcomes due to an inability to accurately measure basal neuronal autophagic flux in vivo and following experimental drug treatments. We therefore sought to comprehensively monitor autophagic flux in mutant SOD1G93A mice in vivo using a newly generated autophagy reporter mouse.
SOD1G93A mice were crossbred with RFP-EGFP-LC3 mice to generate double transgenic SOD1G93A;RFP-EGFP-LC3 mice and control RFP-EGFP-LC3 mice. Brains, spinal cords, peripheral nerves and hindlimb muscles were collected from mice at presymptomatic, disease onset and symptomatic ages. Tissues were analysed for autophagic flux using confocal microscopy to quantify abundance of autophagosomes and autolysosomes in motor neurons. In addition, separate RFP-EGFP-LC3 mice were treated with autophagy enhancers; rapamycin (2 mg/kg) and rilmenidine (10 mg/kg) for 2-weeks to promote autophagic flux.

In adult RFP-EGFP-LC3 mice, we observed basal autophagy in spinal motor neurons at ~10% autophagosomes and ~40% autolysosomes. Autophagy enhancer treatment significantly augmented the autophagic flux in spinal motor neurons. We demonstrate the power of RFP-EGFP-LC3 reporter mice to accurately measure autophagic flux in spinal motor neurons in vivo and predict drugs that effectively promote autophagic flux in target neurons, allowing us to clearly distinguish autophagy activation and inhibition, and reconcile effects of experimental manipulation of the autophagy pathway in MND.

24. Emma Perri, Macquarie University

*The Endoplasmic Reticulum (ER) chaperone, Protein Disulphide Isomerase (PDI), is protective against novel ALS mutant protein, Cyclin F*

A major hallmark observed in Motor Neuron Disease (MND) is the accumulation of misfolded proteins, which form aggregates in the cytoplasm of degenerating motor neurons. Nonetheless, the pathogenic mechanisms of disease in MND remain poorly understood. Recent evidence suggests that dysfunction to the Endoplasmic Reticulum (ER), resulting in ER stress, is increasingly implicated in MND pathogenesis. Protein Disulphide Isomerase (PDI) is an ER chaperone upregulated during bouts of ER stress, which also functions as an oxidoreductase, utilising its disulphide interchange activity to oxidise, reduce and isomerase disulphide bonds, thus restoring proteostasis. Our laboratory has previously demonstrated that PDI overexpression is protective against mutant forms of MND proteins, SOD1, TDP-43 and FUS, in cell culture. Therefore, it was examined here whether PDI overexpression is also protective against mechanisms of pathogenesis triggered by mutant forms of MND protein, Cyclin F.

Results demonstrated that the overexpression of PDI was protective against mutant Cyclin F and decreased mechanisms of MND pathogenesis in cell culture, such as Cyclin F’s redistribution from its normal localisation in the nucleus to the cytoplasm, ER stress and dysfunction of the Ubiquitin-Proteasome System (UPS). Furthermore, PDI overexpression also decreased the proportion of cells expressing mutant Cyclin F undergoing apoptosis. These results suggest that PDI is protective against a novel protein recently implicated in MND, and that therapeutics based on the protective activity of PDI may be beneficial in the advancement of an effective treatment for MND.
Amyotrophic lateral sclerosis (ALS) is a motor neuron disease (MND) that has no effective therapeutic options to date. The lack of effective treatment is largely driven by an inability to model sporadic forms of the disease, which represents majority of cases. Since the discovery of induced pluripotent stem cells (iPSC), patient cells can be reprogrammed into iPSCs retaining the patient’s genetic information. These iPSCs can be differentiated into affected cell types for various applications. This approach provides a powerful tool capable of modelling the disease process in sporadic patient motor neurons, promising great development and testing of candidate therapeutics in order to treat MND. Additionally, allowing the implementation of a large scale, high throughput drug screening program aimed at accelerating the identification and validation of potential treatments for Motor Neuron Disease using patient derived iPSCs.

100 sporadic, 20 familial ALS patients across a range of clinical phenotypes and healthy family members were recruited for the generation of iPSC lines from skin fibroblasts. These iPSC lines were subsequently differentiated into spinal cord motor neurons using established differentiation protocols, and their cellular phenotype characterised to identify a subset of representative lines for drug screening. Drug screening will be conducted using an automated liquid handling robot to identify candidate drugs capable of increasing motor neuron survival.

This program signifies an important advancement in the search for disease modifying therapeutics for the treatment of MND. Furthermore, iPSC lines generated will provide a valuable resource for future research into MND in Australia.

Mutations in intron 1 of C9ORF72 is a major cause of familial Motor neuron disease (MND). We have focused our efforts on one aspect of pathogenesis, namely, repeat-associated non-AUG (RAN) translation of the sense and antisense transcripts of the repeats which generate five poly-dipeptide repeat sequences. Previous studies have revealed that poly-proline-arginine (poly-PR) peptides are particularly toxic to cells and that this is thought to arise by disrupting ribosome biogenesis (REF). We examined the transient expression of 10x and 100x poly-PR repeat lengths which confirmed they were highly toxic and formed puncta in the nucleus of neuroblastoma cell culture models of disease. Quantitative proteomic analysis of the interactome of GFP-(PR)100 identified ribosomal proteins, translation initiation factors and translation elongation factors. Of note was ribosome splitting factor ABCE1, which suggested that translation of the polyPR dipeptide induces ribosome stalling, rather than impairing ribosome biogenesis which was previously suggested from experiments that involved the addition of exogenous peptides to cells rather than expressing them directly. To test this hypothesis, we employed an assay of ribosome stalling, which involves testing the expression of two fluorescent proteins from a single mRNA sequence separated by the polyPR sequence. poly(PR)100 led to a robust...
stalling whereas another RAN product, poly(AP)100, which is not toxic did not. We propose that the positive charges of the poly(PR)100 electrostatically interact with the negatively charged residues of the ribosomal exit tunnel and hence that the toxicity arises from a broad failure of ribosome activity.

15. Rebecca San Gil, The University of Queensland

Investigating chaperones to inhibit the aggregation of cytoplasmic mutants of TDP-43

In all sporadic and most familial cases of MND, TAR DNA-binding protein-43 (TDP-43) is the primary aggregated protein that forms large inclusions in motor neurons and has been correlated with the degeneration of these neurons. One possible therapeutic strategy for people living with MND is to prevent the aggregation of TDP-43 and subsequent neurotoxicity. Recent, unpublished findings from our laboratory have demonstrated by quantitative proteomics that there are significant changes in protein levels of key molecular chaperones, called heat shock proteins (Hsps), at early stages of disease in the hTDP-43ΔNLS mouse. Therefore, the aim of this study was to establish the mechanisms of action of several different Hsps on the aggregation of a mutant isoform of TDP-43 in cell-based assays. We expressed cytoplasmic aggregation-prone TDP-43 mutants fused with EGFP in cell-lines and primary mouse motor neurons. We observed a significant decrease in the proportion of cells with cytoplasmic TDP-43 inclusions in the presence of Hsps compared to cells co-expressing EGFP invisible (negative control) by immunocytochemistry and flow cytometry. Likewise, there was a significant decrease in insoluble mutant TDP-43 in cells co-expressing Hsps compared to cells expressing EGFP invisible by immunoblot analysis. Taken together, these findings suggest that a subset of Hsps can act as inhibitors of cytoplasmic TDP-43 aggregation. Future studies will establish the mechanisms of action in neurons and investigate the effects of Hsps on neuron viability and MND-related pathologies. These findings suggest that Hsps are promising targets to take forward into preclinical testing in the hTDP-43ΔNLS mouse model of MND.

31. Darren Saunders, The University of Wollongong

Enhancing proteostasis via the ubiquitin-proteasome system as a potential therapeutic strategy in motor neurone disease

Mounting genetic and functional evidence points to a central role for dysfunctional proteome homeostasis (proteostasis) in the pathology of both familial and sporadic forms of ALS. These include the growing list of genes controlling protein degradation pathways with ALS-associated mutations, and the widespread presence of ubiquitin (Ub) positive inclusions in affected motor neurones. Ubiquitination is one of the most abundant protein modifications in cellular signaling, controlling numerous cellular pathways. The Ub system is vital for normal motor neuron function, and restoring capacity of the system through overexpression of key component genes is protective in mouse and zebrafish models of SMA. We hypothesise that disrupted cellular Ub homeostasis is an underlying cause of ALS. Our genetic screens and ubiquitin proteomics identified a number of genes in the ubiquitin system as modulators of ALS-associated cellular toxicity. Further, exogenous expression of key genes in the ubiquitin pathway (but not catalytically inactive mutants) is protective in
mammalian cell culture models of ALS. Hence, augmenting components of the Ub system can restore cellular viability and function. These data provide novel mechanistic insight into fundamental molecular mechanisms in ALS, and predict that increasing the capacity of the ubiquitin system in motor neurons will be protective regardless of the underlying genetic basis for disease. Hence, a number of Ub system components are promising therapeutic targets for disease intervention and we are now moving to develop gene therapies targeting augmentation of the Ub system, along with pre-clinical validation of this approach in animal models and patient-derived cell lines.

26. Ronald Sluyter, The University of Wollongong

*A central nervous system-penetrant P2X7 antagonist does not alter disease progression in SOD1G93A mice*

The ATP-gated P2X7 ion channel has emerging roles in ALS. Pharmacological blockade of P2X7 with Brilliant Blue G (BBG) can ameliorate disease in SOD1G93A mice. Recent data, however, reveals that this P2X7 antagonist displays poor penetration of the central nervous system (CNS). Therefore, the current study aimed to determine whether the CNS-penetrant P2X7 antagonist, JNJ-47965567, could ameliorate ALS progression in SOD1G93A mice. Female and male SOD1G93A mice were injected i.p. with JNJ-47965567 (30 mg/kg) or vehicle (control) three times a week from disease onset until end-point. In contrast to BBG, JNJ-47965567 did not impact weight loss, clinical score, motor (rotarod) coordination or survival compared to control mice. NanoString analysis of spinal cord RNA revealed that JNJ-47965567 reduced expression of NQO1 (P = 0.0897) and GGA1 (P = 0.0967) but not P2RX7 (P = 0.353). Flow cytometric analysis revealed no differences in the frequencies of regulatory T cells or activated dendritic cells in the lymph nodes of JNJ-47965567 and control mice. LEGENDplex analysis also revealed no differences in serum cytokine concentrations between these mice. Serum cytokines displayed a rank order of IL-10 >IL-27 >IFNβ >IL-1β, IL-6, IL-17A, GM-CSF >IL-23, CCL2 >TNFα, IFNγ >IL1α >IL-12p70 in both groups. Combined, the BBG and JNJ-47965567 studies suggest that systemic P2X7 blockade in the absence of CNS P2X7 blockade ameliorates ALS progression, but this effect is lost if CNS P2X7 is also inhibited. This data provides a potential paradigm shift concerning the role of P2X7 in ALS. Funding: 2017 MNDRIA Stanford Family MND Research Grant.

34. Frederik Steyn, The University of Queensland

*Extended phenotyping of a patient with MND with a Novel TBK1 mutation*

**Background.** Motor Neurone disease (MND) is a progressive neurodegenerative disease that is variable in terms of age and site of symptom onset, and rate and pattern of disease progression. Genetic differences could account for some of the heterogeneity of disease. TBK1 is a gene that has recently been associated with MND. Objective: Exploring factors that could contribute to disease variability, we conducted a detailed characterisation of a patient with familial MND with a novel TBK1 mutation. **Methods.** A 62-year-old male patient presented with a 3-month history of gait unsteadiness, weakness of the right arm and emotional lability. He was subsequently diagnosed with MND and received routine clinical care. He participated in genetic, metabolic, neuropsychological and brain imaging research. **Results.** The patient had a novel pVal700fs TBK1 mutation. Compared to a
heterogeneous population of MND patients, he had severe disease characterised by rapid weight loss, fast functional decline and earlier death. Hypermetabolism and loss of appetite occurred alongside hypothalamic atrophy. Widespread brain atrophy was matched by a cognitive and behavioural profile reflecting a degree of primarily frontal lobe dysfunction, indicative of MND with behaviour changes. **Discussion.** This study demonstrates that an extended phenotype can be described using brain imaging, and neuropsychological and metabolic testing to quantify the clinical features of MND. The concurrent presentation of hypermetabolism and reduced appetite occurring alongside hypothalamic atrophy lend support to the emerging hypothesis that defects in neural processes that maintain energy homeostasis might contribute to altered disease outcome.

14. Adam Svahn, Macquarie University

*Inhibition of microglia reveals the process of TDP-43 redistribution in injured motor neurons and TDP-43 transfer to the extracellular environment in vivo*

The clinical progression of motor neuron disease (MND) suggests a spreading process of neurodegeneration within the nervous system. In vitro evidence has emerged for the spreading of aggregates between cells [1], similar to other misfolded proteins (e.g. β-amyloid). Fluorescently labelled TDP-43 was tracked in motor neurons of the zebrafish spinal cord [2]. In uninjured motor neurons tagged TDP-43 was localised predominantly to the nucleus in a diffuse form or in highly motile, droplet-like structures. We used high-speed confocal imaging to track and quantify these structures. To characterise neurodegeneration and the fate of TDP-43 in vivo, we applied selective UV laser injury of single motor neurons [3]. In the absence of microglia following antisense PU.1 (spi1b) knockdown, injured motor neurons underwent neurodegeneration that followed a stereotyped time-course. TDP-43 redistribution was observed from the nucleus and into the extracellular space. The axons of injured neurons underwent progressive anterograde degeneration and we observed redistribution of TDP-43 into the axon during this phase of degeneration. This represents the first in vivo experimental evidence for the theory of MND spreading via axonal tracts.

3. Taide Wang, Florey Institute of Neuroscience and Mental Health

*Therapeutic inhibition of the necroptosis cell death signalling pathway in ALS*

Amyotrophic lateral sclerosis (ALS) is characterised by severe motor neuron loss in the brain and spinal cord. Recently, it has been demonstrated these motor neurons may undergo a form of cell death known as necroptosis, a form of programmed necrosis. Necroptosis activation is triggered by the phosphorylation of receptor interacting kinase (RIPK) 1 and 3, and is ultimately executed by the pseudokinase mixed lineage kinase domain-like (MLKL). To examine to role of necroptosis in ALS, transgenic SOD1G93A mice were inter-crossed with MLKL knockout (KO) mice on identical C57BL/6 backgrounds to generate 3 genotypes for analysis: SOD1G93A;MLKL KO, SOD1G93A, MLKL KO. In addition, WT mice were also used for comparison. Mice were weighed and tested weekly for loco-motor function from 60 days of age. Interestingly, female SOD1G93A;MLKL KO mice are trending to be consistently underweight compared to female SOD1G93A mice. However, disease onset,
which is determined by the age of peak body weight was not statistically different between female SOD1G93A;MLKL KO (123±3.3) and SOD1G93A mice (122±4.5). Genetic ablation of MLKL did not affect the life span, Rotarod nor Grip-Strength test performance for both male and female SOD1G93A mice. Future experiments will include biochemical analysis of the brains and spinal cords collected from the cohort to investigate necroptotic regulator levels and activation with Western blotting. These factors include MLKL, RIPK1, and RIPK3. In addition, localisation of necroptotic regulators and motor neuron survival will be determined by immunohistochemistry.

23. Sharlynn Wu, Macquarie University

*Elucidating the potential pathogenicity of novel MND candidate variants in vitro*

Despite the recent discovery of novel gene mutations responsible for causing motor neuron disease (MND), 39% of Australian MND families remain unsolved. To uncover the genetic causes of MND in these remaining families, we developed a strategy that comprises of whole exome sequence data and several custom filtering pipelines. This approach provided us with a set of high priority candidate variants from each family. To further distinguish the causative gene mutation from benign candidates, unbiased in silico and cell biology-based in vitro pipelines were designed and applied. For one of these families—the MQ1 family—two candidates were independently prioritised by both pipelines. Thus, this study aims to identify biochemical changes in cells expressing these candidates. Our solubility fractionation experiments show that the candidate variants are more insoluble than their wild-type counterparts. This suggests that proteostasis was compromised in these cells and that the variants might be misfolded. Our time-course experiment shows that the expression of our top candidate—variant A—was lower than wild-type and decreased over time. Proteomics data further suggests that essential ubiquitin-proteasome system components, were upregulated in the insoluble fractions. Taken together, these results suggest that candidate variant A may form aggresomes that continuously recruit soluble proteins, which may eventually interrupt proteostasis. Next, candidate variant B will be assessed in a similar manner. Interestingly, the proximity of both genes suggests that they are likely to be inherited together. Hence, we will investigate whether the biochemical profile of cells over-expressing these variants together is exacerbated.

2. Fatemeh Zanganeh, The Florey Institute of Neuroscience and Mental Health

*Gene expression profiling of spinal motor neurons during early developmental stages of the SOD1G93A mouse model of ALS*

ALS is an adult-onset neurodegenerative. However there has been increasing evidence for a protracted preclinical period of MN damage before diagnosis of ALS. This preclinical period in ALS may span years or decades before symptom onset. The goal of this study is transcriptomic analysis of spinal MNs isolated at very early stages of development in the SOD1G93A mouse model of ALS to identify primary mechanisms that drive MN vulnerability.
We have implemented a fluorescent reporter mouse to unambiguously identify and isolate spinal MNs from SOD1G93A or wild-type (WT) mice using FACS at embryonic day (E)12, E17, postnatal day (P)3 and P8 (n = 6 per genotype, per age) which overlap with critical stages of MN development and maturation. The RNA was isolated and differentially expressed genes were determined using RNA sequencing.

We have successfully isolated spinal MN population from SOD1G93A and WT mice and differentially expressed genes were identified. Pathway analysis uncovered several remarkably dysregulated pathways in the disease context. Most significant dysregulated genes candidates from the network will be used for further validation and intervention studies in SOD1G93A mice.

Data obtained from this transcriptomic study identified lower MN gene candidates and dysregulated pathways at very early stages of pathogenesis in SOD1G93A mice. These pathways may potentially provide new insights into early mechanisms of ALS pathogenesis and prove to be effective targets for future therapeutics.

4. Jing Zhao, The University of Queensland

*Decreased signalling of EphA4 improves functional performance and motor neuron survival in the SOD1G93A ALS mouse model*

Amyotrophic lateral sclerosis (ALS) is an untreatable, progressive, neurodegenerative disease specifically affecting motor neurons. Recently, the tyrosine kinase receptor EphA4 was directly implicated in ALS disease progression. We report that a long-lived mutated form of the EphA4 antagonist EphA4-Fc (mutEphA4-Fc), which blocks EphA4 binding to its ligands and inhibits its function, significantly improved functional performance in SOD1G93A ALS model mice, as assessed by rotarod and hind-limb grip strength tests. Further, heterozygous motor neuron-specific EphA4 gene deletion in SOD1G93A mice promoted significant improvement in functional performance during the disease course and a delay in disease onset relative to control mice. Importantly, mice in the heterozygous deletion group showed significantly improved survival of motor neurons and architecture of endplates of neuromuscular junctions compared with control and homozygous EphA4-deletion groups. Our novel results show that EphA4 signalling directly regulates motor neuron survival and that mutEphA4-Fc is a promising therapeutic candidate to slow disease progression in ALS.

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