mnd Research Institute of Australia

Advance

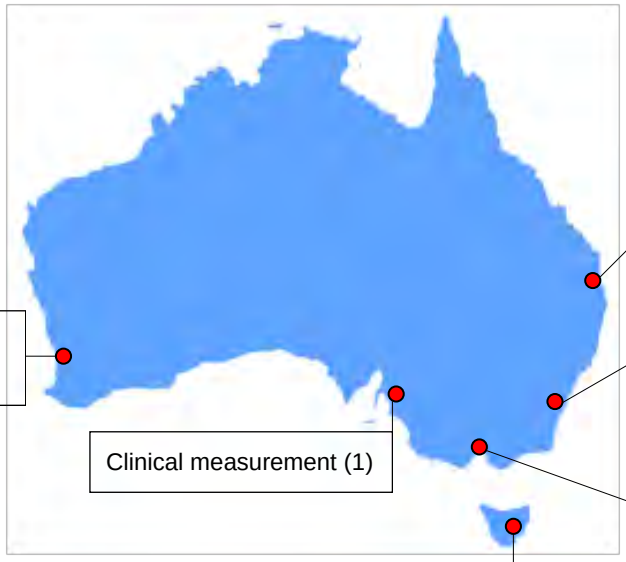
NEWSLETTER OF THE MND RESEARCH INSTITUTE OF AUSTRALIA

June 2014

The Motor Neurone Disease Research Institute of Australia (MNDRIA), the research arm of MND Australia, is committed to supporting a broad spectrum of research projects nationally.

MNDRIA aims is to ensure that that every dollar received is invested in research that has the best chance of understanding the causes, developing effective treatments, establishing evidenced-based care management and finding a cure for MND.

Spectrum of projects supported by MNDRIA across Australia in 2013 and 2014



The starting point of many projects is involved with understanding the cause of MND. This can cover many categories of research including genetics, proteomics, cell biology, metabolism, immunology, environment/ epidemiology and more. Discoveries about causes of MND form the basis for translation to effective treatments and measurement of disease progression so the efficacy of potential treatments can be assessed.

While we cannot change the future for MND without basic research, it is essential that the best possible care and management of symptoms is available to all people living with MND now. Health care research seeks to provide evidence for the best available treatments and the delivery of the right care in the right place at the right time. Health care research also seeks to find ways to relieve carer burden and ensure provision of accurate information.

MNDRIA encourages grant applications from researchers working in all these fields on projects that are relevant to MND. Some research centres comprise collaborative groups working across different fields, while others concentrate on one specialised area.

The map above details the breadth of research projects receiving grants from MNDRIA in 2013 and 2014. Only the best research that has the greatest chance of providing benefit for people with MND is selected to receive funding, but this diversity of fields covered spreads the chance of 'backing a winner'.

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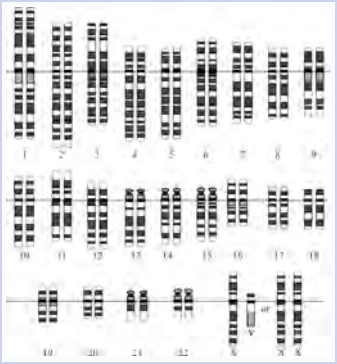
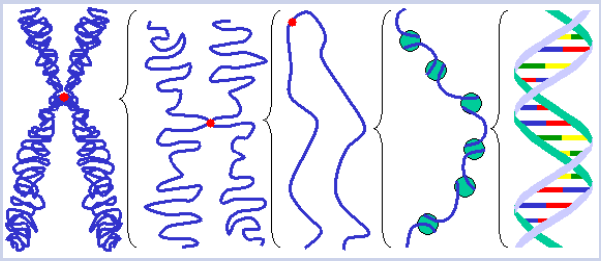
GENETICS / GENOMICS

Advances in technology have lead to rapid discovery of mutations in genes involved in motor neurone disease in recent years. Australian researchers are at the forefront of these discoveries with 70 percent of genes that cause or confer risk for familial MND now known. Gene defects have also been found in some sporadic cases of MND. Discovery of these genes contributes to understanding of the cause of MND and allows the development of animal models with the faulty gene. These animal models (mice, rats, fish, worms, flies ...) provide a way to study disease progression at a cellular level and to test the efficacy of drugs for potential treatment.





Development of next generation sequencing (whole genome or whole exome sequencing), at a fraction of the cost of genome sequencing ten years ago, has paved the way ahead for discovery of all the gene mutations that cause MND or predispose to MND.

MND Australia Leadership Grant 2013 to 2016

Associate Professor Ian Blair

MND Research Centre, Macquarie University, NSW

Investigating the pathogenic basis of familial ALS

There is a pressing need to develop more effective diagnostic tools and treatments for MND. To date, the only proven causes of MND are gene mutations that lead to motor neuron death. Despite recent gene discoveries, current insights have been insufficient to develop effective treatments. As part of collaborative studies, Associate Professor Blair's laboratory previously made breakthroughs in MND through identification of defective genes that cause inherited forms of MND. These discoveries have opened new chapters in MND research. Despite this, the genes are yet to be identified for around 40% of Australian familial MND cases.

Recently, Associate Professor Blair's group, together with international collaborators, identified new defective genes that cause familial MND. These include ERBB4 and SS18L1, with mutations identified in both inherited and sporadic forms of MND. Another gene discovery is responsible for disease in a subset of MND families from Australia, Canada, Spain, Italy, the UK and USA.

The ongoing aims are to better understand how these newly discovered MND genes cause motor neuron degeneration. In addition to better understanding the causes of MND, these studies should lead to development of new diagnostic tests for familial MND and, in the long-term, provide tools for investigating proposed new treatments.

Grants-in-aid

MNDRIA Grant-in-aid

Professor Grant Morahan

University of Western Australia

Discovery of novel genes protecting against MND

The cause of 20% of cases of inherited MND is a mutation in the *SOD1* gene. Some people with mutations in *SOD1* do not develop the disease or develop it much later in life. We propose that there are genes which can prevent MND caused by *SOD1* mutations. We have developed a powerful new system to find these genes in mutant *SOD1* mice. The research project is still underway. We have detected one strain of mice that appears to be protected when crossed to the mutant *SOD1* mice. We will continue mating further Gene Mine strains with the goal of identifying more protective strains. When completed successfully, this project will have two very significant outcomes: (i) It will identify gene(s) and molecular mechanisms that can protect against MND symptoms in otherwise genetically susceptible individuals with SOD1 mutations.
(ii) Gene identification could enable development of drugs that can mimic the actions of the protective genes to develop treatments for MND.

Peter Stearne Grant for Familial MND Research

Professor Garth Nicholson

ANZAC Research Institute, University of Sydney

Finding new MND gene variants

This project aims to find new gene variants that might contribute to the development of MND. We are examining variants in and around genes causing familial MND to see if these variants are more common

in some families with MND. New state of the art computer programs have been developed to search the DNA data files obtained by sequencing all genes in MND families. We aim to locate particular DNA variants in common in some families with MND. This may assist us to find new DNA variants and genes that increase the risk of developing MND.

MNDRIA Grant-in-aid

Dr Tony Roscioli

Sydney Children's Hospital

Genetic diagnosis and gene discovery in motor neurone diseases

This project has brought together people with motor neurone diseases, their families, clinicians and genetic scientists to understand disease mechanisms and to identify genetic causes of motor neurone diseases. We use exome sequencing to analyse all 20,000 genes at one time to look for disease-causing mutations. The families in this project mainly have childhood onset motor neurone and related diseases.

Results have confirmed the genetic cause for disease in 3 of 11 families, including confirmation of the *FBX038* gene's involvement in adult Spinal Muscular Atrophy in one family. Testing in a fourth family has led to the potential discovery of a new gene, which is not currently known to cause disease in humans.

For the families where a cause has been identified, this has helped them in genetic counseling and with the availability of IVF/pre-implantation genetic diagnosis and prenatal testing.

The cost of this new Next Generation Sequencing is being compared to traditional ways of testing genes one at a time. We have shown, after detailed analyses in two families, that genomic testing has saved $4,000 - $6,000 for each family compared to traditional non-genomic testing. Cost analysis of other families will lead to evidence that Next Generation Sequencing will be cost-effective for a wide range of genetic conditions.

POSTDOCTORAL FELLOWSHIPS

Bill Gole Postdoctoral Fellowship 2010 – 2012

Dr Shu Yang

MND Research Centre, Macquarie University, NSW

Investigating the role of recently identified mutant genes in MND pathogenesis

One of the milestones in MND research was the discovery of gene mutations in the superoxide dismutase 1 (*SOD1*) gene. More than 100 different mutations have been found in *SOD1* which cause 10-20% of familial MND cases. More than one decade later, two more disease causing genes encoding the TAR DNA binding protein 43 (TDP-43) and the fused in sarcoma (FUS) protein were identified. Our laboratory played key roles in the identification of these two genes and we continue to search for other unknown MND genes. My research aims to identify the mechanisms through which defects in TDP-43, FUS and other new MND genes cause the disease. This will give insights that are relevant to other familial and sporadic MND cases. With support from a Bill Gole fellowship, I have established experimental strategies using patient skin and blood cells, mouse motor neuron cells and transgenic mice. These strategies will serve as reliable platforms to study MND mechanisms and to test potential treatments. We reproduced MND-like cellular features in the laboratory. One of the hallmarks of MND is the presence of protein aggregates, or inclusions, in the brain and spinal cord. We treated cells with different stresses, some of which induced inclusions with similar composition to those seen in MND patients. We can also examine pathology associated with any known or new MND protein using patient post-mortem tissues. We are currently optimising our transgenic mouse studies and are using different stresses to evaluate whether they will be useful in future studies of MND biology.

As part of our established DNA sequencing program, we have identified mutations in a new MND gene within a subset of patients. This candidate gene plays a role in coordinating essential cell cycle events by regulating degradation of abnormal proteins. We found that the mutant protein synthesised from this gene slows down the protein degradation system. As a result, abnormal proteins failed to be broken down and accumulate in the cell. This accumulation causes internal stresses to the cells that may lead to cell death. We also found that the mutant protein physically interacts with other known MND proteins, indicating that they may be functionally related. Our results suggest that although MND can be caused by defects in multiple genes, there are common biological pathways. Identification of these mechanisms will ultimately assist the development of MND therapeutics.

Bill Gole Postdoctoral Fellowship 2011 – 2013

Dr Rachel Duff

Western Australian Institute for Medical Research

The application of new generation genetic techniques to MND

Although many genes responsible for MND have been discovered, there are still many people with MND who do not have a mutation in a known gene. This project uses a technology that allows every gene in a person's DNA to be searched for a mutation (whole exome sequencing).

Further investigation of several candidate disease variants is currently underway as a result of these large scale sequencing projects. Our laboratory has developed and implemented a new method for rapidly and accurately diagnosing the genetic basis of hundreds of known neuromuscular diseases, including MND. This screening test has been performed on nine of the samples included in this project and resulted in the genetic diagnosis of three samples.

Another aim of this project is to determine why some

GENETICS / GENOMICS

people with a mutation in the *SOD1* gene do not develop MND. In collaboration the MND Research Group at Macquarie University, we have identified dozens of samples that will be included in this study.

Bill Gole Postdoctoral Fellowship 2013 – 2015

Dr Kelly Williams

MND Research Centre, Macquarie University, NSW

Investigating the molecular basis of ALS

With the support of a Bill Gole Fellowship, I have recently identified a new defective gene that appears to cause both familial and sporadic MND in MND populations from diverse geographical locations. The discovery of new gene defects will add to existing genetic diagnostic testing in MND families and provide an opportunity to investigate the causes of motor neuron degeneration in both familial and sporadic MND.

In the proportion of Australian MND patients with known causative gene defects, the course of disease varies among patients, including variable age of disease onset, clinical progression and duration of disease, even among those with identical gene mutations. This suggests the presence of disease modifiers. We will use our extensive cohort of Australian MND patients that are discordant for features of the disease (e.g. patients with early or late-onset; fast or slow progression) to search for these disease modifiers. Preliminary data indicates dysregulation of specific pathways that have previously been implicated in neurodegenerative disease. The identification of crucial genetic modifiers will enhance our understanding of the biology of MND, and potentially lead to therapeutic discovery.

PhD Top-Up Grant

MNDRIA PhD top-up Grant 2013-2015

Jennifer Fifita (PhD candidate) and Associate Professor Ian Blair (Supervisor)

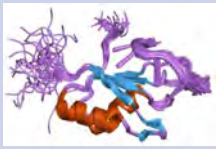
MND Research Centre, Macquarie University, NSW

Examining the role of novel molecules causing MND.

Genetic mutations are the only known cause of MND. Known mutations account for about 60% of familial MND cases, and less than 5% of sporadic cases in Australia. The aim of my PhD project is to discover and examine novel genes that cause familial MND, and determine the role of their encoded proteins in causing MND. In the first year of my PhD, I have successfully sequenced the entire exome (all the genes) of five Australian MND families and produced a list of possible MND-causing gene mutations for each family. Work is underway to filter out the true, disease causing mutation. I will use a zebrafish model of MND to determine the effect of each candidate mutation on zebrafish motor neurons in the laboratory, to identify novel genes causing MND. I have successfully used this zebrafish model to determine the effect of eight mutations in a novel MND gene discovered by our group. The identification of novel genetic mutations can significantly affect families living with MND. The identification of the causative mutation in a family can allow for diagnostic testing of individual family members and pre-implantation genetic diagnosis. On a research basis, the discovery of novel familial MND proteins can provide insight into sporadic MND cases as well as insight into disease mechanisms.

PROTEOMICS

***PROTEOMICS** is the large scale study of the protein complement of a living organism.*
*The term proteome comes from **prote**in and gen**ome** to make an analogy with genomics, the study of the genome.*
Proteomic studies investigate the proteins produced by the faulty genes associated with MND.

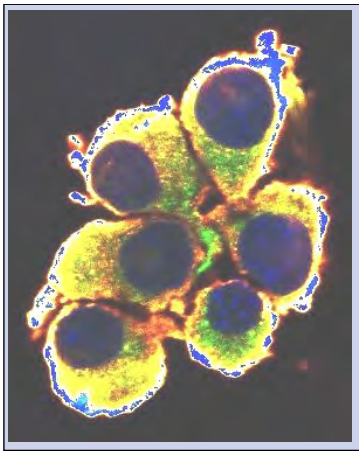


Mick Rodger Benalla MND Research Grant

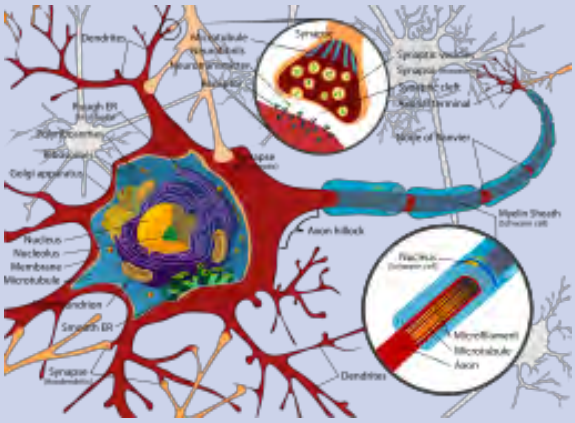
Dr Rachel Tan

Neuroscience Research Australia, NSW. *Are polyglutamine repeats the mystery proteins in the novel p62 lesions in MND*

The recent identification of novel p62 inclusions in the brains of patients with MND and the new C9ORF72 gene abnormality suggests that there are additional proteins involved in the degeneration occurring in MND, and that these proteins have a much broader affect on the nervous system. The p62 protein labels other proteins for degradation in cells. It will be important to identify the labelled protein to determine what cell pathways are involved. It was recently identified that variability in another gene, the ataxin-2 gene, can produce slightly longer ataxin-2 proteins due to increased polyglutamine repeats, and that people with this variability are much more likely to get MND. In other research it has been shown that p62 binds ataxins and contributes to assembling them for degradation. People with very long ataxin proteins (some with MND) get degeneration in the cerebellum and spinal cord. Based on the distribution of the novel lesions in the cerebellum of MND patients, and knowledge that p62 binds ataxins, we will investigate if the mystery protein in the novel p62 inclusions is ataxin-2 with increased polyglutamine repeats. Identifying the mystery protein involved in these novel lesions in MND will identify the cellular pathway that is abnormal, which can then be targeted for therapeutic treatment.



CELL BIOLOGY *research studies the components of nerve cells, interactions with their environment, mechanisms that cause cell death and the search for interventions that will potentially stop cell death. This is done both at a microscopic and a molecular level.*



GRANTS-IN-AID

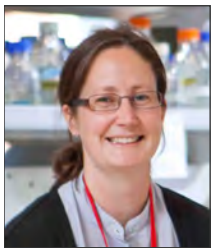
Rosalind Nicholson MND Research Grant

Dr Julie Atkin La Trobe University, Victoria

The role of extracellular misfolded proteins in the pathogenesis of ALS

This proposal investigated how motor neuron death is targeted in MND. The disease is usually initiated in a characteristic manner, beginning in one localised site, and subsequently spreading to surrounding areas within the nervous system. However the basic cellular mechanisms that spread pathology in MND remain undefined. In previous studies we have found that the death of motor neurons occurs by the same cellular mechanism in the many diverse forms of disease, involving specific compartments of the cell, termed the 'ER-Golgi'. In this study we found that misfolded proteins SOD1 and FUS are released by motor neuron cells in culture, where they are subsequently taken up into surrounding cells. These extracellular misfolded proteins trigger ER-Golgi pathology typical of MND after they are taken up into cells, resulting in death of the motor neurons. We have also demonstrated that other misfolded proteins linked to MND, C9ORF72 and optineurin, are present extracellularly where, subsequently, they can be taken up into motor neurons. Hence, this mechanism leads to the spreading of disease amongst motor neurons in cell culture, suggesting this is how it may be propagated throughout the nervous system in human MND. Our work also shows that each protein is taken up by a distinct mechanism and we are currently investigating these mechanisms in more detail. Understanding these processes will enable the development of more effective therapies in the future.

Zo-ée MND Research Grant



Associate Professor Tracey Dickson
Menzies Research Institute, University of Tasmania
Interneuron dysfunction in amyotrophic lateral sclerosis: A new target for potential therapeutics?

Our research group has new evidence that suggests that ALS may be caused by the dysfunction of a particular type of nerve cell called an interneuron which has an important job in neuronal communication in the brain and spinal cord.
Using a range of experimental approaches that we have developed in our lab, we have been investigating the pathological changes in these interneurons in human brain and spinal cord tissue donated through the Victorian Brain Bank Network.
We have also been investigating pathological changes in two widely used mouse models of ALS. We have found that in the SOD1 transgenic model there is evidence of a particular population of interneurons being lost. We have also found that early in the course of disease in this mouse model there is evidence of interneuron specific changes in morphology that are likely to affect neuronal circuit function and which may account for some of the symptoms of ALS. In a second model of ALS, the TDP43 mouse model, we have found evidence of dysfunction in the specific connections between neurons that are responsible for balancing the function of the neuronal circuit – these are called inhibitory synapses.
Collectively this work increases our understanding of the cause of ALS and reveals important new targets for developing a treatment or cure. Ongoing investigations will further explore the mechanisms of interneuron dysfunction and will use a new in vitro experiment to start trialing potential interventions.

Mick Rodger MND Research Grant

Dr Anna King

Wicking Dementia and Education Centre, University of Tasmania
Axonal protection in ALS

In ALS nerve fibres or axons degenerate resulting in a loss of connection between the nervous system and the muscles. The result is a loss of movement. Many of the therapeutic agents that have been trialed in ALS protect the nerve cells from dying but do not protect the axons. In our research we are interested in finding the mechanisms by which axons degenerate in order to find therapeutic agents that protect the axons. In our cellular investigations we have discovered that components of axons, called microtubules,

become unstable in conditions of disease. We have also shown that this instability occurs early on and therefore may be a good therapeutic target to prevent axons from degenerating. We have found that drugs that stabilise microtubules protect axons from degenerating in our cell models and we are now testing these drugs in mouse models of disease-induced axon degeneration. Importantly, as part of this project we firstly established that these drugs can be tolerated by healthy mice and do not have any effect on the nervous system at both a cellular (tissue pathology analysis) and whole organism level (behavioural analysis). Information from these necessary pilot investigations has been used to guide design of the full trial. We hope that therapeutic agents that protect nerve fibres will aid in the treatment of ALS and other neurodegenerative disorders, either alone or in combination therapies.

MNDRIA Grant-in-aid

Dr Lachlan Thompson

Florey Neuroscience Institute, VIC

Generation of spinal motor neurons from stem cells and transplantation in an animal model of MND

We need to improve our basic understanding of how stem cells behave in models of disease if we are to develop safe and effective stem cell based therapies.

We have found that human stem cells can be grown in the laboratory and used to produce 'cortical' neurons. When transplanted into the brains of a rat 'model' of MND these cells delay the development of the MND symptoms. Unfortunately, this therapeutic benefit is only transient and the grafted cells do not survive for long enough to provide a more lasting benefit. The purpose of this research was to ask 'why'? We speculated that either the cells were being rejected by the host immune system OR that the MND disease-like environment was causing the cells to die.

The main finding from the research project was that the host-immune system, rather than the MND-like environment, is having the major impact on graft rejection. This is in itself a positive and important result because it suggests there is scope for further improving the efficacy of stem cells grafts by promoting long-term survival through suppression of the host immune system.

A second important and unexpected finding was that suppression of certain aspects of the host immune-system (in order to allow for better survival of the grafted cells) actually made the disease progression worse. This has lead our beginning to develop a new and potentially very important research avenue to explore how manipulation of the immune system affects MND disease progression.

In summary, these results show that while there is good support for the general principal that stem cells can have therapeutic benefit for MND, there are important but surmountable practical hurdles related to the host immune -system that must be addressed if we are to continue basic research that can lead to development of effective stem cell therapies with long-term benefits to the patient.

Susie Harris Memorial MND Research Grant

Dr Bradley Turner

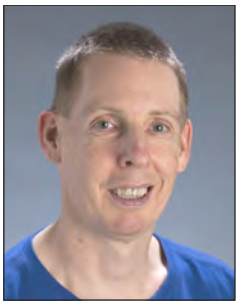
Florey Neuroscience Institute, Victoria. *Autophagy dysregulation in MND*

Accumulation of damaged proteins inside nerve cells is a signature pathology of MND. Strategies that reduce the burden of damaged proteins in MND may offer a promising potential therapeutic approach for MND. This project examined whether autophagy, a protective process which breaks down damaged proteins inside cells, is activated in MND. We show that autophagy is highly active in motor neuron cultures and spinal cords of mice with MND. Autophagy occurs at symptom onset in MND mice, suggesting it may be an important player in disease progression. We also show that stimulating autophagy is beneficial and clears damaged proteins in MND models. Importantly, we have discovered that a certain type of autophagy called "CMA" is involved in MND, which opens up a new potential drug target.

Our future studies will focus on testing approaches to enhance autophagy and clearance of damaged proteins in MND models.

Cliff Smith MND Research Grant

Associate Professor Anthony White



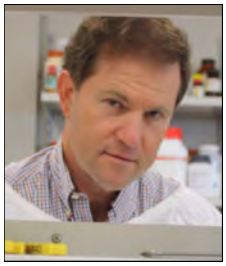
University of Melbourne

The role of RNA-binding protein hnRNP K in motor neuron degeneration

Proteins known as hnRNPs have essential roles in maintaining health of neurons by controlling gene expression and new protein production in each cell. Recent studies have shown that in MND, there are changes to hnRNPs including a shift in their normal cell localisation and sometimes accumulation of the proteins. It is believed that these changes are important for the subsequent death of motor neurons but the reasons are not known. We have found that a mutation in the protein TDP-43 in some cases of MND leads to dramatic changes in another hnRNP known as hnRNP K. We have found that when cells experience oxidative stress in MND, the mutant TDP-43 causes accumulation of hnRNP K in some regions of the cell and is lost from others. We are investigating how and why this occurs and our data suggests that this is due at least partly to the way mutant TDP-43 affects the ability of the cell to signal to hnRNP K regarding where it should be localised. We are also investigating the consequences to the cell. hnRNP K binds RNA, controlling gene expression. This normal function is disrupted when mutant TDP-43 affects hnRNP K localization and accumulation. The change in gene expression can then affect cell function and survival. We are investigating which gene targets change in the presence of mutant TDP-43. This research may lead to development of new treatment approaches targeting hnRNPs.

MNDRIA Grant-in-aid

Professor Mark Wilson



University of Wollongong, NSW

Protein aggregation and chaperones: key players in MND

1. We succeeded in developing techniques to recover protein inclusions from inside cells in MND patient spinal cord tissues. However, even using the most sensitive approaches currently possible, the amounts of protein contained in the inclusions isolated were too small to identify the specific proteins they contain.
2. In response to 1. (above), we established a genetically engineered cell model to form inclusions and used this to generate and isolate larger quantities of inclusions. These inclusions have been analysed by gel electrophoresis and multiple bands detected. We are continuing to optimize the best technique to make the proteins in the inclusions soluble, which will aid in their specific identification.
3. As previously reported, even using a wide range of approaches, it is very difficult to express soluble TDP-43. We have now, however, succeeded in expressing (small amounts of) soluble TDP-43, and used this to show that the chaperone clusterin can prevent the aggregation of TDP-43 *in vitro.*
4. We developed a novel technique to measure, in individual cells, changes in the distribution of TDP-43 between its normal location in the nucleus and the cytoplasm, and will in the future use this to probe what "goes wrong" to cause proteins to accumulate and aggregate inside MND patient nerve cells.

Extensions of this work should allow us to (i) identify major proteins making up the inclusions found inside MND patient nerve cells, (ii) determine how chaperones affect the aggregation of (full-length) TDP-43, and (iii) identify process(es) important in triggering the accumulation of TDP-43 in the cytoplasm which is implicated in MND pathology. Collectively, these outputs will help identify key proteins and processes in the onset and development of MND which could serve as targets for the development of new therapies.

POSTDOCTORAL FELLOWSHIP

Bill Gole Postdoctoral Fellowship 2011 – 2013

Dr Catherine Blizzard

Menzies Research Institute
University of Tasmania.

Investigating the cause of site-specific excitotoxicity in ALS

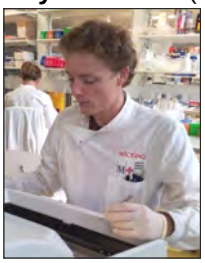
MND is caused by a loss of function of the nerve cells controlling the muscles. This loss of function of the nerve cells may be due to over excitation of nerve cells, either at the muscle or at the site of the nerve cell bodies, the spinal cord. These two possibilities of toxic site leading to nerve cell degeneration was explored by establishing a model of over excitation in either the spinal cord or hind limb of a mouse. Over excitation at the spinal cord has a very different effect to over excitation at the muscle. Spinal cord over excitation leads to motor neuron degeneration and death, which may be similar to the motor neuron death that occurs in MND.

The neuromuscular junction degeneration that occurred prior to motor neuron death was then investigated in 'real time' using state of the art multiphoton microscopy. These studies revealed an unknown potential for neuromuscular junction remodeling prior to degeneration. Understanding this remodeling and degeneration better may hold the key is finding new therapeutics to target this devastating disease.

PHD TOP-UP GRANTS

MNDRIA PhD top-up Grant 2013-2015

Jayden Clark (PhD candidate)



and Associate Professor Tracey Dickson (Supervisor)
Menzies Research Institute, University of Tasmania

Axonal protection in ALS

The focus of my PhD is to identify the therapeutic potential of protecting the motor nerve cell's axon from degenerating. In ALS the axon has been found to degenerate, thus it is a therapeutic target. The drug that I am trialling, Epothilone D, targets a structure in the axon that is involved in the transport of virtually every cellular protein from the cell body down the axon. It also makes up a part of the cytoskeleton. In ALS this structure, called the Microtubule, breaks down and fragments. Epothilone D prevents this from occurring. Epothilone D is already available for the treatment of some types of aggressive cancer and, at very high doses, stops cancer cells from dividing. At low doses it has been shown to have beneficial effects in animal models of other neurological diseases, such as Parkinson's disease. However, when used at the wrong dose, this drug can have detrimental effects. I have established that at the dose chosen for the treatment of our ALS mouse model, there are no off-target effects due to the administration of Epothilone D. Due to this positive result I have begun a full drug trial on our ALS mouse model, investigating changes in pathology, motor function and longevity between treated and untreated mice.

This study will be comprehensive, identifying three elements that are important in therapeutic trials of ALS (pathology, motor function and longevity). This means that I can establish if there are improvements in quality of life, changes that may shed light on disease mechanisms and whether this drug can extend life. Following this initial drug trial, any results will be validated using cell culture methods that will recreate the ALS environment 'in a dish'. All of these methods, from using animal models to cell culture in a therapeutic trial, are necessary to gain a fundamental understanding of the drug's interactions with individual cells, and the organism as a whole. This will allow us to gauge if it is a worthy candidate for a human trial.

CELL BIOLOGY

MNDRIA PhD top-up Grant 2013-2015

Rosemary Clark (PhD candidate)

and Associate Professor Tracey Dickson (Supervisor)
Menzies Research Institute, University of Tasmania
Interneuron dysfunction in ALS: A new target for potential therapeutics?

Interneurons are a key regulatory population in the central nervous system where they exert an inhibitory effect to regulate the excitability of local network circuitry, particularly the excitatory pyramidal neurons. In MND there is substantial evidence to suggest a disturbance in the balance of excitation and inhibition is an early feature of disease. This is evidenced by increased excitability of circuitry at a stage in disease preceding motor neuron losses.

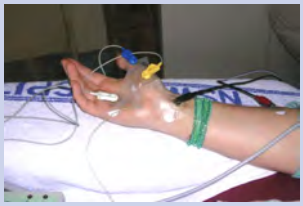
We are investigating if decreased inhibition, or interneuron dysfunction, is central to observed changes in excitability. We have found evidence to suggest that specific interneuron populations are altered in a regional- and lamina-specific manner in response to a key ALS-linked gene mutation, hSOD1, in cortical circuitry. This suggests a differential ability of interneurons to respond to disease, both at end-stage and pre-symptomatic time points. The unique involvement of different interneuron populations highlights the importance of understanding the environment in which motor neurons are lost, as this may hold clues to understanding their unique vulnerability in this disease.

We hope to further elucidate the significance of these findings by completing an extensive time course of investigations to determine a) when these cells become altered in disease, and b) if responses observed are static or change throughout the disease progression.

We hope that this work will eventually translate into a new candidate for the targeting of therapeutics in MND that may restore a functional balance to MND cell circuitry, at an early stage before motor function is lost.

CLINICAL MEASUREMENT / HEALTH CARE

CLINICAL MEASUREMENT / HEALTH CARE RESEARCH



The exact location of onset and subsequent spread of disease in MND is still not completely understood. Imaging, electrical testing and biomarkers are essential tools to facilitate improvements in the way MND is diagnosed, to follow progression of MND and to provide a way to measure efficacy of treatments.

Health care research will help to identify and develop strategies and guidelines to improve the diagnostic experience, planning of services, carer support, professional training and end of life care. Health care research is vital for the development of evidence-based interventions to improve quality and length of life. Health, disability and aged care professionals will benefit from having evidence-based support techniques and interventions available and will be better equipped with skills to support the person with MND, their carer and families.

GRANTS-IN-AID

MND Victoria Research Grant

Dr James Burrell

Neuroscience Research Australia, NSW
The impact of language dysfunction on patient quality of life and carer burden in MND

Motor neuron disease (MND) and frontotemporal dementia (FTD) overlap significantly. Language disturbance is a feature of FTD, and patients with MND may have similar language problems, over and above problems with speech clarity. In this study, the investigators have successfully adapted and applied FTD language assessment tools to investigate higher language disturbance in MND.

So far, more than 30 patients with MND and FTD-MND have undergone detailed language assessments and MRI scans, and the project remains on track to meet our recruitment goals. This data has been combined with pilot data from 2012 to enable initial analyses,. These were presented in poster format at the ALS/MND Symposium in Milan in 2013, and in an oral platform at the annual scientific meeting of the Australian and New Zealand Association of Neurologists in May 2014.

Two manuscripts are currently being prepared for publication. The first study has demonstrated that deficits in word and object knowledge (i.e. semantic deficits) are common in MND and are related to anterior temporal pathology. The second study has demonstrated that errors in the understanding of grammar (i.e. syntactic deficits) also occur in MND and FTD-MND.

Once the language deficits encountered in MND have been fully elucidated, practical therapeutic options will be developed. This important work would have been impossible without the generous support of MND Victoria, for which the investigators are extremely grateful.

Charles and Shirley Graham Grant

Dr Robert Henderson

University of Queensland Centre for Clinical Research

Use of biomarkers to understand ALS

Assessment of disease progression and testing of new therapies is difficult in MND. With the support of the large RBWH multidisciplinary MND clinic, we have explored biomarkers using blood samples, a new type of MRI and expanded the electrical test that is used to diagnose MND. With the funding from the MNDRIA, we have employed a research assistant who has been dedicated to MND research. The person has established a large database for all samples. They have processed all of the blood samples which were serially collected by our research nurse and stored and analysed them. The serial samples have allowed the exploration of a novel blood biomarker pnf-H. This is a marker of the death of motor nerves. The results of our research have shown that it is elevated early in the disease and correlates with survival. We will continue to explore this marker and our imaging and electrophysiology marker and see if it is reliable for use clinically and potentially in treatment trials. The database that the research assistant has created has also been invaluable for a small project by a research fellow which explores the effect of limb dominance on how MND occurs and spreads.

MNDRIA Grant-in-aid

Dr Mary-Louise Rogers & Professor Robert Rush

Flinders University, SA

Biomarker for determining outcomes of motor neurone disease treatments in animal trials

We have identified a candidate marker in the urine of a mice model of MND and in humans living with MND. The project has shown that a urinary protein is significantly elevated not only in MND mice but also people living with MND and the levels are higher than healthy controls. So far, our studies suggest that the urinary marker is a candidate for evaluation in larger studies. We are now aiming to determine if the urinary marker can help to stage disease progression in the mouse model of MND and in humans. This involves looking at the levels of this marker in urine of people with a genetic predisposition for MND over the course of disease and also in mice treated with riluzole. This work is ongoing.

Our project is of importance for people living with MND as biomarkers are urgently needed to assist assessment of potential new drugs, for earlier diagnosis and also for monitoring disease progression.

PhD Scholarships

MNDRIA/ NHMRC PhD Scholarship 2013 -- 2015

Dr Parvathi Menon

University of Sydney and Westmead Hospital, NSW

Pathophysiology of ALS: Evidence to support the dying forward hypothesis

The aim of my research study has been to better understand the mechanisms underlying the degeneration of motor neurons in ALS, specifically, whether the dysfunction is primarily driven by the 'corticomotorneurons' which arise in the brain. We have developed a simple diagnostic index in ALS called the Split-Hand Index. This is based on a unique pattern of hand muscle involvement with preferential wasting of muscles around the thumb. The index can reliably differentiate ALS from mimic neuromuscular disease and further studies are aimed at assessing its ability to predict disease progression.

My research has involved assessment of central and peripheral connections to different hand muscles to assess differences between the preferentially involved and relatively preserved muscles. Motor neuron function was assessed early in the disease to determine if the dysfunction appears earliest in the 'brain' or 'spinal' motor neurons. The conclusion of the series of studies supports a dominant role for the 'corticomotorneuron' in the progressive motor system dysfunction noted in ALS. We would, therefore, identify the 'corticomotorneuron' as a potential target for future treatments.

Future research will be aimed at better understanding the mechanisms driving motor neuron loss so that treatments can be directed toward altering the process. We also plan to identify disease markers which help with early reliable diagnosis and consequent early effective treatment.

MNDRIA/ NHMRC PhD Scholarship 2012 - 2014

Dr Neil Simon

Neuroscience Research Australia, NSW

The distribution of motor system dysfunction in MND

The precise location of onset and subsequent spread of disease in MND is still not completely understood. This project explores these issues using serially collected clinical information, combined with neurophysiological and imaging testing of the brain, spinal cord and peripheral nerves. Clarifying the mechanisms of disease onset and spread in MND may have significant implications for patients in that it may allow further understanding of the causes of degeneration of motor nerves, as well as guiding exploration for new disease-specific treatment modalities.

Since the last update, the project has evolved to include novel imaging measures of peripheral nerve

degeneration, including diffusion tensor imaging of peripheral nerves and dynamic muscle ultrasound to detect denervation changes and fasciculations. I have obtained additional ethics approval at the University of New South Wales, Prince of Wales Hospital and the University of Sydney to specifically explore these imaging markers of disease progression. We are continuing to collect data, particularly with regards to longitudinal imaging measures. Studying these novel non-invasive imaging modalities has opened up opportunities to more fully evaluate motor system dysfunction in MND patients and improve the diagnostic process for patients with MND.

PhD Top-Up Grant

Top-Up award announced in February 2014

MNDRIA PhD top-up Grant 2014-2016

Dr Rebekah Ahmed (PhD candidate)

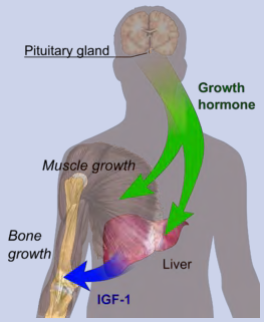
and Professor Matthew Kiernan (Supervisor). Neuroscience Research Australia ,NSW

Eating, autonomic and sexual dysfunction in MND and FTD

This project aims to examine the incidence and underlying causes of eating, autonomic and sexual dysfunction in MND and frontotemporal dementia (FTD). Eating abnormalities have been well documented in FTD, this study aims to extend this work into MND. There is increasing evidence for an overlap between FTD and MND at a clinical and pathological level which has gained impetus with the recent discovery of the C9orf72 gene mutation in cases with familial FTD, FTD-MND and MND.

FTD refers to a group of neurodegenerative disorders characterised by shrinkage of the frontal and anterior temporal lobes of the brain. FTD presents with changes in personality and behaviour and/or progressive language problems. Change in eating patterns including reduced satiety, binge eating, increased carbohydrate consumption and stereotypic eating is a major criterion for the diagnosis of behavioural variant FTD. It is not known whether these same eating abnormalities are present in MND, but given the increasing evidence of an overlap, it is possible that they are. In FTD patients it has been found that those with eating abnormalities, have shrinkage of the posterior hypothalamus. The hypothalamus also controls the autonomic nervous system, which regulates internal bodily functions such as blood pressure control, stomach, bowel and urinary function. The hypothalamus is also involved in controlling sexual function. This project aims to examine the incidence of eating, autonomic and sexual symptoms in MND and FTD and, by imaging methods and endocrine analysis, examine the possible underlying causes and then possible interventions to reduce the impact of these symptoms on the quality of life of patients and carers.

METABOLISM



refers to all the chemical processes that go on inside the body. To carry out these processes, our body converts energy from the food we eat. Metabolism is controlled by hormones and the nervous system.

People with MND show several defects in energy metabolism, including weight loss. Although weight loss can partly be explained by loss of muscle, clinical evidence suggests that defective energy metabolism contributes to the disease process. Understanding the reasons for altered energy metabolism in MND could help with development of interventions to slow the progression of the disease.

Grant-in-aid

MNDRIA Grant-in-aid

Professor Pamela McCombe

University of Queensland Centre for Clinical Research

Investigating the causes and consequences of growth hormone dysfunction in MND

Changes in endocrine factors that control metabolism may contribute to MND. Results from a clinical study suggested that growth hormone (GH) deficiency might play a role in MND. However, the contribution of GH deficiency in MND has not been determined. Because GH is an anabolic hormone that plays an important role in controlling metabolism and body composition, this project aimed to investigate the underlying cause of GH dysfunction in MND, and to investigate the consequences of altered GH secretion on MND pathogenesis.

At disease onset, we observe more GH secreted into circulation in hSOD1G93A mice compared to healthy mice of the same age. This increase in GH occurs at the same time as an increase in the amount of IGF-1 in skeletal muscle. Because GH and IGF-1 promote muscle mass and muscle repair, we believe that changes in GH/IGF-1 during the early stages of disease are a normal response that helps to protect muscle and to maintain muscle function.

Current data suggests that hSOD1G93A mice consume more food than their wild-type controls of the same age, that they have increased metabolic need at rest, and that as soon as they consume a meal, the use of carbohydrates (as energy) from that meal is almost immediate.

We aim to determine whether changes in GH/IGF-1 were matched to oxidative stress in skeletal muscle. We conducted dihydroethidium staining in muscle from hSOD1G93A mice and will examine WT SOD mice to see if changes in oxidative stress are purely due to

overexpression of a SOD gene. We will measure how mitochondria in skeletal muscle are using glucose and fat for energy. This will provide better insight into oxidative stress and mitochondrial function, and will help validate our results.

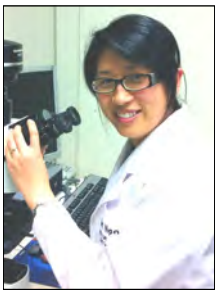
To study the consequences of altered GH secretion in MND, we generated hSOD1G93A animals with partial or complete loss of GH signalling. Our data shows that hSOD1G93A animals with less GH signalling have a similar disease course to hSOD1G93A mice. Interestingly, hSOD1G93A mice with no GH signalling have a very significant delay in the onset of muscle weakness. Despite this delay in the onset of symptoms, hSOD1G93A mice without GH signalling do not appear to have improved survival. This data strongly supports a role for the GH axis in modifying the course of disease.

Our observations provide insight into the role of GH in MND, and suggest that changes in GH secretion occur as a consequence of disease. By further understanding how changes in GH signalling can delay the onset of motor symptoms, but not improve survival in hSOD1G93A mice, we may identify key mechanisms that could be targeted with directed therapies.

POSTDOCTORAL FELLOWSHIP

Bill Gole Postdoctoral Fellowship 2012 – 2014

Dr Shyuan Ngo



University of Queensland

Investigating the mechanisms underlying defective energy metabolism in MND.

While motor neuron loss is the primary pathology in MND, some MND patients have growth hormone deficiency, insulin resistance, glucose intolerance, and hypermetabolism. My research aims to investigate the impact of altered metabolic balance in MND progression.

I have found that changes in growth hormone secretion in the SOD1G93A mouse model of MND are related to the severity of muscle pathology. While these changes may play a vital role in maintaining muscle function during early disease, it appears that changes in growth hormone balance may have negative consequences on disease progression by affecting how the body uses carbohydrates and fat to meet the increased metabolic need that is seen in MND. This may be the underlying cause for a number of the metabolic disturbances that are seen in MND.

My research is now focussed on determining the way in which changes in the regulation of energy flux may contribute to disease. I am monitoring changes in resting metabolism, food intake, activity, and use of carbohydrate or fat in the SOD1G93A mouse relative to healthy mice. Because glucose is a vital source of energy in the body, I am using positron emission tomography (PET) combined with computed tomography (CT) or magnetic resonance imaging (MRI) to track how glucose is taken up by the brain, spinal cord and muscle. I will measure the products of glucose metabolism in order to determine if this glucose is being used effectively. I am coupling this information with observations of how and when fat is used for energy, and whether this contributes to mitochondrial dysfunction in skeletal muscle, and the rapid loss of fat mass that is seen in MND. Current data is promising and suggests that early and persistent defects in glucose metabolism underlie the preferential use of fat as an energy substrate to meet energy demand in MND. This is providing insight into whether early defects in metabolic capacity contribute to the onset and progression of MND.

Initial assessment of metabolic markers in plasma samples obtained from MND patients is indicative of malnutrition. I am obtaining data on glycogen and fat accumulation in skeletal muscle biopsies from MND patients. Current observations suggest that there are defects in glucose metabolism in skeletal muscle. Finally, I have obtained additional skeletal muscle and fat biopsies from MND patients and these will be assessed for glucose and fat metabolism profiles. Understanding the role of altered metabolic balance in MND and identification of avenues for assisting the body to sustain optimal energy needs. This will give greater understanding of MND disease mechanisms with the hope to develop strategies to modify the course of disease and improve prognosis.

PHD TOP-UP GRANT

MNDRIA PhD top-up Grant 2012-2014

Alexandra Mot (PhD candidate)

and Dr Peter Crouch (Supervisor), University of Melbourne, Victoria

Investigating energy metabolism in models of MND to elucidate the mechanism of action of the potential therapeutic Cu^{II}(atsm)

Our group has found that a compound called Cu^{II} (atsm) delays the onset of disease symptoms in mouse models of ALS. Outcomes of this project to date include the development of cell culture models that replicate the conditions of impaired energy metabolism seen in ALS. Such models are essential for the screening and testing of potential therapeutic compounds.

Of significance is the recent signing of a commercial agreement between the University of Melbourne and U.S.-based drug development company Procypra Therapeutics. The nature of this agreement is for Procypra to further refine and generate more effective analogues of Cu^{II}(atsm) as a potential ALS therapeutic. These new analogues of Cu^{II}(atsm) will need to be tested using ALS-like models. To this end, we have recently been focusing our efforts on the development of cell-based models that replicate the ALS-like conditions of impaired energy metabolism. By undertaking this research we expect to expedite the development of therapeutic compounds and to provide new understanding of the fundamental causes of ALS.

MND Research Institute of Australia: Office Bearers and Members 2014

MND Australia is the principal member of the MND Research Institute of Australia.
The operations of both organisations are the responsibility of MND Australia.
The board of the MND Research Institute is the same as the board of MND Australia, consisting of an independent elected President and a nominated representative from each member MND Association board, the chair of the MNDRIA research committee and up to three co-opted special tenure directors.

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Diversity of grant support

MNDRIA PhD scholarships (co-funded with the National Health and Medical Research Council of Australia) and **MNDRIA PhD scholarship top-up grants** are three-year grants which aim to encourage and support our brightest young researchers to develop a specific interest in MND research.

MNDRIA postdoctoral fellowships provide salary support for three years for the very best emerging scientists in their chosen field of MND research.

Grants-in-aid provide an incentive for successful researchers to continue to move forward in their chosen area of research. These grants also help researchers to initiate new projects with the hope they can 'grow' to produce data that can attract more significant funding from government grant schemes.

Project grants are a goal for the future. These grants typically provide funding at a more significant level for a longer period, usually three or four years, allowing the opportunity to follow through on a major project. Researchers generally rely on government for grants of this magnitude, but as government funds become less reliable there is pressure for MNDRIA to fill the gap. If MNDRIA donations continue to increase as they have in recent years, project grant funding will become a reality.

In the meantime MNDRIA will continue to support both early career and established researchers as they strive in their many different fields of research to keep on going until, collectively, they unlock the mysteries of motor neurone disease.

This newsletter not only provides information about the latest results from MND researchers in Australia, but also serves as a source of feedback to the donors who provide the funds that make this research possible. We thank and applaud the many donors who have contributed to the realisation of these outstanding results and continue to provide hope that we will achieve a world free from MND.

Donations

Research funded by the MND Research Institute of Australia is dependent on donations.
To contribute to this vital work, please send your gift to:

MND Research Institute of Australia
PO Box 990, Gladesville NSW 1675

Donations can be made by cheque (payable to MND Research Institute of Australia) or credit card (Visa or MasterCard) or online at www.mndresearch.asn.au.
All donations of $2 and over are tax deductible.

Bequests

Your Will can provide an important way of making a gift that can have lasting influence on MND research and give hope for the future.

If you would like to consider the MND Research Institute of Australia in your Will by providing a Bequest from your Estate, please contact your solicitor.

For more details,
phone Janet Nash, Executive Officer Research on 02 8877 0990 or email research@mndaust.asn.au.

ACKNOWLEDGEMENT: We wish to thank Snap Printing, North Ryde, NSW for their generous support in printing this newsletter.