

June 2007

The Motor Neurone Disease Research Institute of Australia Inc was established in 1986 to promote medical and scientific research into motor neurone disease. Researchers around Australia are encouraged to apply for funding available through the Institute. All applications are reviewed by our national medical/scientific committee, and grants are awarded to the most worthy applicants.

This year, the closing date for grant applications is Friday 24 August. Please go to www.mndresearch.asn.au for details of available grants. MNDRIA is pleased to announce that the Bill Gole Postdoctoral MND Fellowship will be offered for a period of up to three years, commencing January 2008. Applications are also invited for two PhD Scholarships (one in conjunction with NHMRC).

Reports of research funded by MNDRIA in 2006 are published in this newsletter, together with a report on international research presented at the American Academy of Neurology meeting in May. For more detailed reports, visit our website.

Report from the American Academy of Neurology in Boston 29 April - 5 May 2007

Motor neurone disease and other disorders of the anterior horn cell were a major focus of the AAN meeting in Boston this year. **Dr Bob Brown** from Massachusetts General Hospital delivered the 2007 Robert Wartenberg Lecture entitled **ALS from mutations to mechanisms and medicines**. In Dr Brown's typical fashion, he gave a superb lecture on MND. One of the major focuses of his lecture was whether familial models of MND, such as mutations in SOD1 were valid models for sporadic MND in patients with no family history. He emphasised that there may be common pathways in both familial and sporadic MND such that methods of down-regulating SOD1 might be useful in both familial MND, due to mutations in SOD1, and in sporadic disease. He also discussed mutations in other genes that may lead to familial MND such as VAPB, dynactin and senataxin. He discussed predisposing genes such as VEGF, angiogenin and SMN, and how these genes might lead to protective therapies. Other genes that might give clues to the triggers of MND, such as paraoxonase, were also discussed. On the disappointing side, Dr Brown has been involved in a very large gene 'chip' study looking at 600,000,000 different possible genes in MND, with very little in the way of promising results.

The role of inflammation in the mechanisms that cause MND was another major topic. Somewhat controversially, Dr Brown came down heavily on the side of microglia as being protective in MND, although the role of astrocytes is still unclear. What does seem to be evident is that there is some form of modification of SOD1 that may render it toxic and propagate the disease. Dr Brown sees this as a major target for therapeutics in slowing the progression of MND for both familial and sporadic MND. He brushed on the possibility from large epidemiological studies that aspirin and non-steroidal anti-inflammatory drugs might not be helpful in MND. However, it should be emphasised that his observation does

not come from properly conducted prospective trials, but from retrospective observations.

The problems with drug trials were emphasised, with a current trial of ceftriaxone underway. There are no clear data regarding the effectiveness of ceftriaxone in MND yet. Dr Brown did show some very encouraging animal data showing the ability to shut off SOD1 production to extend the life of experimental animals. Perhaps this therapy will translate into humans in the near future. He only briefly touched on stem cell therapy, which continues to be the 'emperor's new clothes' of MND research. Intriguingly he touched on vaccination therapy in MND, again to inactivate putative toxic proteins. While the studies are yet to be replicated, the prospect for new therapeutics is certainly exciting.

Anterior Horn Cell Platform Sessions

There were many interesting platform sessions on MND, some of which are summarised in this report. There was an important observation from the **wALS group** regarding extrapolating from mouse studies to human therapies in MND. Several years ago there was a vogue for treatment with minocycline in MND on the basis of observations in G93A SOD1 mouse model that this widely used antibiotic improved survival in the treated mice. In a large study that treated over 200 patients in both the placebo and minocycline arms to a maximum dose of 400 mg a day, the patients treated with minocycline deteriorated 23% quicker in measurements of ALSFRS-R, respiratory tests and manual muscle testing than the patients treated with placebo. Interestingly there was no difference in survival between the groups, and no difference in quality of life measures. The major side effect was nausea and diarrhoea. There is no evidence for the use of minocycline in MND.

(Continued on page 4)

Reports on MND research funded by the MNDRIA in 2006

Grants in aid

Dr Robert Henderson, Royal Brisbane Hospital, QLD
Assessing disease progression in motor neurone disease

MND is highly variable in its presentation and progression and there is a need for a marker of the disease that can be used in treatment trials. This project aims to find an objective marker of the disease using new methods of standard neurology tests, and applies a new statistical approach to determine the number of motor nerves supplying muscles. To the end of the year 2006, 49 subjects with MND and 3 subjects with lower motor neurone (LMN) weakness, and 8 normal subjects were studied. Of those subjects, 17 MND subjects and 3 LMN subjects had serial studies performed on them over an average of 189 days. For serial studies, patients come into the Royal Brisbane & Women's Hospital Neurology Department usually every 4-8 weeks for a study to be performed. The nerves studied are the ulnar, median (both in the hand) and peroneal (foot). The number of motor units (the motor nerve and the muscle it supplies) of both the MND and LMN subjects declined over time. This project will provide a new method for assessment of disease progression by determining the number of motor nerves and will allow our involvement in clinical trials to find effective treatments for MND.

Dr Mark Bellingham

School of Biomedical Sciences, University of QLD
Glutamate receptors and ion currents controlling excitability of motor neurons susceptible to death in ALS

Previous work on this grant has shown that riluzole, the only approved treatment for motor neuron disease, significantly reduces the excitability of normal motor neurons. This decrease in excitability is due to a selective suppression of sodium channel proteins which cause persistent electrical activity. This evokes the question of whether motor neuron excitability may be abnormally increased in motor neuron disease. In collaboration with Drs. Martha Constantine-Paton at MIT and Dr. Bob Brown, a leading MND researcher at Massachusetts General Hospital, I have recorded the electrical activity of motor neurons in brain tissue taken from a mouse model of MND shortly after birth of these animals and long before their motor neurons begin to die. These recordings show that motor neuron excitability is significantly increased compared to normal animals of the same age. This hyper-excitability is closely correlated with an increase in the riluzole-sensitive persistent sodium channel activity. In addition, neural responses of these animals are consistent with an accelerated development of motor neurons, suggesting that the eventual death of motor neurons in MND may be the result of premature aging processes.

Dr Wayne Murrell, School of Biomolecular & Biomedical Science, Griffith University, QLD, *Adult stem cells from patients with ALS: Culture of motor neurons*

Six cell lines from ALS patients have been provided by Dr Carolyn Sue's lab, propagated and frozen stocks have been put into storage. The aim of this project was to define

conditions that induce olfactory neural stem cells to become motor neurons. Various combinations of growth factors were tested based on published methods used on other types of stem cells. So far these resulted in differentiation of cholinergic neurons but not bona fide motor neurons. To assay capability to respond to the necessary signals that occur *in vivo* in development of the spinal cord neurons, molecular expression of the various messenger RNAs coding for the correct receptors was undertaken. The results indicate that these cells do have that capability. As well to confirm this, olfactory neurospheres were placed between chick notochord and neural tube floor plate in a co-culture induction experiment. This experiment mimics the situation in embryonic development when motor neurons are specified. This has resulted in very long neurites emerging from the neurospheres. These cells were not shown to express motor neuron markers or those that did express them could not be unequivocally identified as target cells.

Assoc Prof Roger Pamphlett, Depts of Pathology and Molecular & Clinical Genetics, The University of Sydney
Somatic Mutations in Motor Neuron Disease: An analysis of SOD1 exons and introns in MND brain tissue

The great majority of cases of MND do not run in families, and the cause of this "sporadic" form of the disease remains unknown. A possible cause for sporadic MND is a gene mutation that occurs in the central nervous system only. Because the mutation is not in the germ cells (sperm or ova) it is not passed on to the next generation. This mutation could be the same as is found in all cells of the body in familial MND. We are therefore looking at the commonest gene mutated in familial MND, SOD1, to see if mutations can be found in the brains of people who have died of sporadic MND. We are looking for mutations not only in the parts of the gene that make the protein (the exons) but also the large regions between the exons (the introns) as well as lengths of DNA upstream and downstream of the gene. These parts of the gene could be important in regulating the way the gene makes the protein. Studies so far have found differences in introns in two SMND patients, and one of these is now being checked in a larger sample to see if it is a disease-causing mutation or a normal variation. In addition, a new method to detect mutations in very small proportions of brain tissue has been undertaken, and will be used to look at larger numbers of samples.

Jennica Winhammar, David Joffe, Matthew Kiernan and Dominic B. Rowe, Royal North Shore Hospital & Prince of Wales Medical Research Institute, NSW
Diffusion tensor imaging in motor neuron disease

We are using diffusion tensor imaging (DTI) and transcranial magnetic stimulation (TMS) to determine if there is upper motor neurone (UMN) damage in MND. A complete set of data on all measures was obtained from 10 MND patients and 10 healthy controls. The use of DTI has enabled successful fiber tracking to locate the corticospinal tract. Regions of interest were placed in four locations along the corticospinal tract including the cerebral peduncle and the internal capsule.

These values were collected in both hemispheres and then compared to each other, within the patient group and then to the control group. DTI indirectly investigates corticospinal tract problems in MND by looking at the diffusivity of water within the axons of nerves. The idea behind this is that if there is more diffusion of water there may be a break down of the integrity of the nerves. Preliminary fiber tracking results suggest that the MND patient group have more diffusion of water within the nerve suggesting that there may be nerve damage (increased 'leakiness' of the nerve membrane). Other measures suggest that there is less 'organisation' within the nerve of the MND group. We hope that this can be a tool used not only to understand the disease better (which in time will help us find a cure), but also to help with diagnosis and monitoring disease progression.

Post Doctoral Fellowships

Dr Ian Blair, Bill Gole MND Research Fellow
ANZAC Research Institute, NSW. *Identification of novel genes involved in motor neuron degeneration*

The Bill Gole MND Research Fellowship supported my recruitment in 2006 from the Garvan Institute/ University of NSW to the ANZAC Research Institute to establish and lead a new MND research group. Since commencement, I have sought grant funding and recruited staff and students. Our group now comprises five dedicated researchers, all investigating the molecular basis of MND. We have made substantial progress in 2006. We are well poised for 2007.

The motor neuron diseases are a group of related neurodegenerative diseases that cause the selective progressive death of motor neurons. These diseases range from slowly progressive forms to the rapidly progressive disorder amyotrophic lateral sclerosis (ALS). The only proven causes of MND are mutations in genes (including the SOD1 gene) that lead to death of motor neurons. However, the known MND genes only account for about 20% of familial cases (about 2% of all MND cases). The aim of this project is to gain an understanding of the biological basis of ALS through identification of genes that cause the disease among the remaining 80% of familial cases. We have recruited over 100 MND families in which the responsible gene is unknown. We have commenced screening these families using high-throughput genetic techniques to identify shared chromosomal regions that harbour previously unknown MND genes. Several chromosome regions have been identified that potentially contain new MND genes. We have confirmed that one of these genes, which causes a slowly progressive form of MND, is located in a region of chromosome 7 that contains around 70 genes. Work is underway to search these genes to identify the causative mutation. We have also identified chromosome regions that appear to carry mutations that cause rapidly progressive forms of MND (i.e. ALS). Other research groups have also recently reported other new MND genes, and we have tested these within our cohort of MND families. We have identified several new mutations in these genes. Our research is now focusing on how these mutations act to cause disease.

Identification of the genes and mutations that cause MND will lead to a greater understanding of the biology of motor neurons and the basis of familial and sporadic motor neuron

degeneration. This understanding is a prerequisite to effective diagnosis, treatment and prevention of the disease.

Valerie Hansen, Bill Gole MND Research Fellow
Depts of Pathology and Molecular & Clinical Genetics, The University of Sydney. *Susceptibility to enteroviral infection: a cause of motor neuron disease?*

We have used gene chips to look at 500,000 genetic differences in DNA across the chromosomes of people with MND and compared these to people without the disease. We looked at DNA both in the blood and brains of people with MND, to see if the gene differences only occurred in the brain. Two types of change were looked for: (1) changes within individual genes, and (2) differences in the number of whole genes (i.e., gains or losses of "copy number"). We detected a number of copy number differences in MND brains compared to controls. To be considered valid, however, these differences now have to be confirmed using a separate technique. If validated, they would represent a novel mechanism underlying sporadic MND.

I thank the MND Research Institute for granting me the Bill Gole Fellowship that has allowed me to undertake this work.

Clinical Scholarship

Dr Steve Vucic, MND NSW Clinical Scholar
Prince of Wales Medical Research Institute, NSW
Site of origin & patterns of neuronal degeneration in MND

Although MND was first described in the 19th century, we still don't know what causes motor neuron loss or where MND begins. It has been proposed that MND begins in the brain, with the nerves connecting the brain to motor neurons in the spinal cord, called corticomotoneurons, mediating motor neuron degeneration by excessive excitation, the "dying forward" hypothesis. In order to investigate this potential mechanism of neurodegeneration, over the past two-years I have been conducting research on MND patients using a novel technique for measuring cortical excitability, called *threshold tracking transcranial magnetic stimulation (TMS)* which was developed by our group at the Prince of Wales Medical Research Institute in Randwick. By applying threshold tracking TMS, we established that cortical hyperexcitability is an early feature in MND patients in whom there is no family history (sporadic MND). Longitudinal studies over a three year period in familial MND subjects with mutations in the SOD1 gene has established that cortical hyperexcitability precedes the development of clinical features of MND, thereby suggesting that cortical hyperexcitability drives the ensuing motor neuron degeneration. In order to assess whether cortical hyperexcitability represented a compensatory upregulation of the corticomotoneurons attempting to overcome motor neuron dysfunction, patients with Kennedy's disease were studied. The patients with Kennedy's disease exhibited normal cortical excitability, suggesting that cortical hyperexcitability underlies motor neurodegeneration in familial ALS. Further, my research suggests that the threshold tracking TMS technique could be an important diagnostic test for MND, especially in diagnosing MND at an early stage.

I would like to thank MNDRIA for providing the grant support that has enabled this research to proceed.

(Report from Boston continued from page 1)

The group from Strasbourg and Paris presented further data on NogoA, a marker that has increased expression in muscle in patients with MND. In a study of NogoA expression in muscle biopsies of patients with post mortem confirmation of the diagnosis, NogoA expression in muscle has a sensitivity of 89%, specificity of 87% and a positive predictive value of 96%. It is highly likely that NogoA is an antemortem biomarker of MND. The role of NogoA in the pathway of MND is still not known. Whether it is an epiphenomenon, or involved in disease production is yet to be determined. In a related study, Pierre-Francois Pradat from Paris looked at whether NogoA expression at the onset of disease was able to predict whether the patient would develop full blown ALS from a limited lower motor neurone syndrome. In a blinded study of 33 patients, 17 were NogoA positive in a deltoid muscle biopsy, and 16 were NogoA negative. In the NogoA +ve patients, 15 of 17 (88%) went on to develop full blown ALS over the next 2-3 years. The two remaining NogoA + patients had the flail arm variant of MND. Of the NogoA -ve patients, only one went on to develop MND (6%). Therefore NogoA expression in the muscle had a positive predictive value of 88% and a negative predictive value of 94%. NogoA is an aid to help discriminate which patients with LMN syndromes will go on to develop MND, and should aid in the introduction of earlier therapies when they are available.

Tim Miller from UCSD presented data on anti-sense oligonucleotide therapy in a G93A rat model of MND. This therapy delivered to the intraventricular space by pumps, did not influence the age at onset, but slowed the progression of disease such that rats treated with the oligos survived 30% longer than rats treated with placebo. Before anyone gets too excited, this amounted to an increased mean survival of 10 days. The Sheffield group showed data looking at microglial activation from transgenic animals, extending the observations of previous workers who have demonstrated microglial activation in animal models of MND. Whether the microglial activation is protective or toxic is still unclear.

Lastly **Chris Henderson**, from the UK, Marseilles and now in Columbia, New York was awarded the Sheila Essey Award in MND for his original work in MND showing that alternative methods of cell death are present in the pathway to cell death in MND. He demonstrated that expression of mutant SOD1 makes motor neurones more susceptible to non cell autonomous triggers of degeneration. In other words, there is a motor neurone specific pathway to cell death in animal models, and this pathway may well be present in human familial MND as well as sporadic MND.

Poster Sessions

There were many very interesting posters. If you want to look at the abstract titles, the website is <http://am.aan.com/scientific/>. There is a huge research effort into the cause(s) of MND with translation into therapies to slow the disease. It will be interesting to see the new information at the International ALS/MND Symposium in Toronto later this year.

Dominic B Rowe

Office Bearers & Members of MNDRIA

EXECUTIVE COMMITTEE

Chairman: Dr Dominic Rowe, NSW
Honorary Treasurer: David Lamperd, VIC
Honorary Secretary: Paula Trigg, NSW
Public Officer: Professor John Pollard, NSW
Dr Susan Mathers, VIC Peter Whitehouse, SA

MEDICAL/SCIENTIFIC COMMITTEE

Dr Dominic Rowe, NSW Prof Perry Bartlett, QLD
Ass Prof Matthew Kiernan, NSW
Prof Frank Mastaglia, WA Dr Susan Mathers, VIC
Dr Pamela McCombe, QLD
Prof John Pollard, NSW Prof Robert Rush, SA
Prof James Vickers, TAS

AUDIT AND FINANCE COMMITTEE

Dr Dominic Rowe, NSW Bob Howe, NSW
David Lamperd, VIC Paula Trigg, NSW

MND ASSOCIATION DELEGATES

MND Australia: Bob Howe, Helen Sjardin
ACT: Helen Christiansen **NSW:** Paula Trigg
QLD: Lesley Taylor, John Wearne
SA: Peter Whitehouse
TAS: Tim Hynes, Lynette Willis
VIC: Rod Harris, David Lamperd
WA: Marie Macdonald, Emanuel Manolios

MEMBERS:

Graham Lang Kevin Langdon Ian Rodwell

AUDITOR: C M Pitt & Co

EXECUTIVE OFFICER: Janet Nash

MNDRIA extends thanks to Mrs Mavis Gallienne who has resigned her membership of the Institute after ten years of untiring support. Mavis has made a distinguished contribution to MND, serving not only as Chair of the International Alliance of ALS/MND Associations, but also as a member of the Board of MND Victoria. Mavis continues in her role with MND Victoria.

Mr Ian Rodwell is welcomed as a new member of the Institute. Ian Rodwell has a background in advertising (founder of the Adcorp Group) and now has many active interests including being a Director of Diabetes Australia Research Trust. We look forward to the help he will provide as an ambassador for MND research in Australia.

Donations

Research funded by the MND Research Institute of Australia is dependent on donations. If you would like 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). All donations 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 on 02 8877 0990 or email info@mndresearch.asn.au.