

Mick Rodger MND Research Grant

Project title: Effect of metallothionein and exercise on progression of motor neurone disease

Principal investigator: A/Prof Meng Inn Chuah

Institution: Menzies Research Institute, University of Tasmania

The International Symposium on ALS/MND brought together biomedical researchers, clinicians and health care professionals to share new developments in the understanding, treatment and management of ALS/MND. I gave a poster presentation under "Theme 1 Therapeutic Strategies" at the ALS/MND Symposium. My poster presented preliminary data that metallothionein-IIA appears to increase survival by delaying late-stage disease progression in G93A SOD1 mice. It also showed that as early as 15 minutes after intramuscular and intraperitoneal injection, MT-IIA can be detected in the vascular system and the parenchyma of the kidney. The poster was well received and I gained valuable feedback. The Symposium also gave me the opportunity to discuss ongoing experiments with other attendees, specifically current experiments investigating possible beneficial effects of combined metallothionein injection and exercise in SOD1 mice.

There were many meeting highlights due largely to the unique nature of participants coming from various specialty backgrounds. The symposium has given me a more balanced view of the topical issues relating to ALS, and enlightened me on the multiple strategies that researchers and professionals are applying to understand and develop better treatment for this disease. One of the major highlights is the opportunity to attend talks by world-renowned researchers such as Prof V Lee, Prof A Al-Chalabi and Prof PN Leigh. I have learnt much from the oral presentation and posters, too many to discuss in detail here but a few bear particular relevance to my work:

- A poster (P8) presented by E Tokuda and S-I Ono which showed that metallothionein-I injected intraperitoneally led to longer survival and better functional outcomes in SOD1 mice. They also showed that the injected metallothionein could be found in cells of the spinal cord, suggesting that the injected metallothionein crossed the blood-brain barrier and acted directly on the motor neurons. My study involves intramuscular injection of metallothionein at a slightly lower dosage but combined with a treadmill exercise regimen. It would be interesting to compare the results by Tokuda and Ono with ours to establish whether the beneficial effects of metallothionein can be enhanced by exercise.
- The use of exercise as an additional therapy for MND patients is being trialed by the Central California Neuroscience Institute. K Volez and J Rosenfeld (CW241) reported that four patients are currently undergoing partial body weight bearing treadmill training. Preliminary results appear to be promising with three of the four patients showing improved timing in performing 25-foot walk and better lower extremity function.
- S Rutkove (C60) introduced electrical impedance myography as an innovative method to quantify motor neurone loss. This measurement which provides a physiological measure in addition to structural atrophy that is commonly observed in ALS, could be applied to my project.

In summary, I have benefitted greatly from attendance at this Symposium; it has given me fresh impetus to continue our work on ALS. I would like to thank MNDRIA and the Mick Rodger MND Research Grant which have provided the financial support to allow me to participate in this event.

Meng Inn Chuah

Mick Rodger Benalla MND Research Grant

Project Title: Investigating the role of biometals in abnormal metabolism of TDP-43

Principle Investigator: Dr Anthony White

Institution: Department of Pathology, The University of Melbourne

Symposium Attendee: PhD candidate Nastasia Lim

The International Symposium provided a good number of speakers from different countries working on similar projects. This gave an excellent overview of current work relating to MND and how our own work could fit into what is currently known or how to further develop our own hypotheses in relation to the rapid advancement of the field. What was also important was the additional focus on the current care of MND patients. There were exhibits from a number of international companies and presenters showing the current therapies available to ease the suffering of MND patients, which provided a good reminder of what we are aiming for with our studies.

Of particular interest at the symposium were presentations relating to animal models, including exciting data on MND models, as our lab is currently doing research using mouse models of MND (e.g. SOD and TDP-43 models). Mice with a TDP-43 G384C mutation showed a decrease in performance in an accelerating rotorod and Barnes maze (Julien). The G384C mice also showed increased TDP-43 aggregates, inflammation, cytokines and smaller axons compared to the control and A315T (another TDP-43 MND model) mice. Another mouse model presented had a SOD1 D38G mutation, associated with familial MND and producing abnormal dismutase activity (Joyce). The D38G mouse showed decreases in weight, rotorod performance, grip strength, startle response and produced tremors, as well as a ~20% loss in motor neurons and increased astrogliosis. These models reflect the MND human pathology and could be useful for our own studies in future, particularly with development of our metallo-complex-based therapeutics and attempts to understand how altered TDP-43 processing induces MND.

Proteins of direct interest to our work were also discussed during the symposium. *Drosophila* overexpressing TDP-43 showed an increase in Notch targeted genes (Tibbetts). UG-rich sequences for TDP-43 binding were further identified as a target (Lagier-Tourenne). Chronic excitotoxicity was reported to increase the phosphorylation of TDP-43 and increase ubiquitin immunoreactivity in spinal cord cultures (Ayala). The phosphorylated TDP-43 was then found aggregated into ubiquitin positive SH-SY5Y cells. ERK inactivation was also found in this study to cause TDP-43 fragmentation, phosphorylation and aggregation, thus ERK inhibition could be protective (Ayala). The HFE H63D protein was shown to be associated to increasing the sensitivity of cells to ER stress (Liu). This is similar to our work showing that JNK controls TDP-43 aggregation during stress. Increased caspase-3 activity is seen in cell cultures with reduced H63D expression and in a HFE mouse model (Liu). Trafficking pathways were also shown to be altered in mutant SOD1 cell models (Atkin). Decreased BDNF secretion blocked ER and Golgi trafficking in mutant SOD1 cells. Dyenin and COPII, both involved with transport, were also shown to interact with mutant SOD1 (Atkin). These proteins could be of interest to our own work and will be further investigated in our studies.

My poster presentation related to work with TDP-43 in cell culture models, trying to understand how TDP-43 accumulates in cytoplasmic stress granules during oxidative stress. Our findings showed that with SY5Y cells treated with paraquat (to induce oxidative stress), TDP-43 accumulation in stress granules is dependent on JNK activation, increased caspase activity and C-terminal cleavage. Low amounts of the cell permeable bis(thiosemicarbazone) copper complex Cull(atsm), which has been shown to be neuroprotective in vivo, inhibited the formation of stress granules and TDP-43 accumulation, thus protecting neurons from paraquat toxicity. Our studies are the first to demonstrate a pathway for TDP-43 accumulation in stress granules and inhibition by a copper complex. It was well received at the symposium during the poster presentations.

Nastasia Lim

Zo-eè MND Research Grant

Project Title: Emotion recognition and social communication in MND: impact on behaviour and carer burden.

Principle Investigator: Dr Fiona Fisher (Clinical Neuropsychologist),

Institution: Calvary Health Care Bethlehem, Victoria

From the grant funding received, two poster presentations were able to be presented at this year's 2010 International Symposium on MND/ALS:

- Fisher, F., Howe, J., Reardon, K. & McPhee M. (2010). *The clinical utility of routine primitive reflex examination in Motor Neurone Disease.*
- Fisher, F., Pavlis, A., Howe, J., Reardon, K. & Mathers, S. (2010). *The challenges of care-giving: contributions of cognitive, behaviour, and social cognition difficulties in Motor Neurone Disease.*

In addition, I attended a range of presentations and shared ideas with a number of researchers during the poster sessions.

Knowledge gained from attending the symposium:

During my attendance at the symposium, I was exposed to international interdisciplinary clinical management and research practices. The exposure provided insights and interesting comparisons into how services around the world provide high quality support for both patients and carers.

Throughout the symposium I attended talks by neurologists and neuropsychologists from around the world. Discussion included the utility of examinations of frontal and temporal dysfunction to assess cognitive and language disruptions, as well as the clinical and pathological implications of assessing cognition. Each year there is increasing recognition of the importance of early detection and recognition of cognitive changes in MND. The importance of examining the impact of cognitive and behavioural changes on both patients and carers was also highlighted by a number of prominent keynote speakers. The international recognition of the importance of continued research and public education in area was definitely the highlight of the symposium.

Discussion with other researchers highlighted our study as being of similar calibre to those conducted abroad, while enabling the fertilization of ideas between like-minded professionals. Potential future collaborations with Kings College in London and Penn state in the USA are now being considered, in order to draw international comparisons of the results we obtained here.

Networking with international researchers enabled the fertilization of ideas between like-minded professionals. Discussions of the challenges of conducting clinical research with other researchers facilitated the sharing of ideas to address common hurdles.

This year's symposium again provided an opportunity for researchers to come together to expand their knowledge and share their experiences and insights. In addition to the invaluable networking opportunity the conference afforded, attendance at the symposium furthered my knowledge into the diverse approaches to assessing cognitive, behaviour and social communication changes in MND. The information obtained from this symposium will most certainly inform our team's ongoing research practices and has even provided a number of ideas for future directions.

Fiona Fisher

MNDRIA Grant-in-aid

Project Title: Investigating the in vivo targets affected by a novel therapeutic agent for motor neuron disease

Principle Investigator: Dr Qiao-Xin Li, Senior Research Fellow

Institution: Department of Pathology, The University of Melbourne

I would like to express my thanks and gratitude in funding this trip to present my research poster at the 21st International Symposium on MND/ALS in Orlando, USA.

This is my first time attending the annual international symposium on MND/ALS, and it has been a very beneficial experience. I had the opportunity to meet with many researchers and clinicians in this field. I am inspired by their enthusiasm and hard work in trying to help people living with MND/ALS. Through the discussion, I had the chance to learn about the studies that they are doing as well as exchange information and ideas. I also received valuable and constructive feedback on my poster presentation which will be helpful in progressing my research. We have learnt so much about the disease but yet we are still struggling to find a cure. I hope it will come in the near future.

This symposium also has many good discussions on the clinical ground that emphasised current drug trials and patient care and support. The conference room for the clinical session was full with attendees. This shows that the communities are trying hard to help those people living with MND/ALS.

In conclusion, this symposium has provided me with fruitful knowledge and thoughts on MND/ALS and is valuable in helping me improve on my research project. Thank you once again for funding my trip to the symposium. It has been a memorable experience.

Qiao-Xin Li

MNDRIA Grant-in-aid

Project Title: Survival motor neuron protein deficits occur in ALS

Principle Investigator: Dr Bradley Turner

Institution: Florey Neuroscience Institutes, Victoria

Last December, the international MND research and care community converged at the theme park capital of the world to share, discuss and debate scientific and clinical strides in the field over the past year. While difficult to resist the temptation of Mickey Mouse and friends, there were plenty of thrills and surprises in the scientific program and daredevil researchers pioneering new technologies to spur development and pursuit of effective treatments for MND.

One of the dominant scientific themes of the conference was RNA biology, lending itself to the increasing belief that RNA defects may partly or even solely cause MND. RNA is the genetic blueprint or message used to build and maintain all cells. Like any message, RNA must be correctly proof-read, handled and posted to often remote locations in cells. Surprisingly, defects in RNA processing steps appear to selectively strike out motor neurones, suggesting that these nerve cells are unusually vulnerable to RNA defects. Here is an account of these sessions and other scientific highlights from the conference.

Shoot the messenger

Day one of the Symposium was largely devoted to TDP-43; the disease signature molecule for MND. TDP-43 forms junk piles in the brain that may kill nerve cells in MND, however the precise action of TDP-43 in disease may be more subtle. Understanding the normal function of TDP-43 may therefore help unravel its rogue function in MND. Clotilde Lagier-Tourenne from UCSD and Janice Robertson from Toronto University shed some light on TDP-43 function in normal brain using a technique to capture its possible partners in crime, namely RNA. Although TDP-43 is highly promiscuous, they showed that it naturally seeks out and binds RNA that promotes motor neuron survival. In contrast, these RNA targets of TDP-43 are abnormal in MND patients, providing the first disease link between TDP-43 and RNA defects in MND. These findings may provide early clues for treatment in MND since RNA defects are likely to be early, if not causative, in the disease process and are highly amenable to gene therapies.

Programmed motor neurones

In an exciting development, Tom Maniatis from Columbia University and iPierian independently reported on preliminary analysis of induced pluripotent stem (IPS) cell-derived motor neurones from MND patients. IPS cell technology allows researchers to isolate skin cells from patients and “reprogram” them into any desired cells which are genetically matched to their donors. This technology was successfully applied to MND in 2008. In this installment, motor neurones grown from MND patients with TDP-43 or FUS gene defects were maintained long-term and subject to next-generation “deep sequencing” which can identify RNA defects. An abnormal RNA profile was indeed demonstrated in these cells, suggesting that RNA defects in motor neurones may be central to MND. More importantly, IPS cell technology offers the research community a robust supply of patient-derived motor neurones that model the gene complexity of MND, particularly sporadic MND, for applications such as drug discovery.

Greasy cells important for MND?

In a session dedicated to SOD1, the most common genetic defect in MND, Don Cleveland from UCSD reminded the audience that MND caused by SOD1 is likely the sum of damage arising in motor neurones and neighbouring cells. Thus, treatments should be targeted at both motor neurones and glial cells. He outlined two such clinical trials: the first involving direct brain infusion of molecules to block SOD1 commenced 2010, and the second involving spinal transplantation of astrocyte precursor cells commencing 2014. Jeff Rothstein from Hopkins continued the theme of glial cells, implicating a potential new player in MND caused by SOD1. He showed compelling data, although very preliminary, that SOD1 damage with oligodendrocytes, the fatty cells that enwrap motor neurones, strongly influences disease initiation in MND mice. If correct, then these glial cells offer an innovative potential treatment target for MND, in addition to affected motor neurones.

News from Oz

In the poster session, I was confronted with the usual dilemma: patrol my poster or indulge in other posters! Finding a compromise, I presented recent data from our lab funded by MND Australia showing that SMN, the determining factor of the childhood disorder SMA, is abnormal in MND patients. These findings may be relevant to the field since SMN closely resembles TDP-43, predicting that RNA defects will also arise from SMN abnormality in MND. I was enthused by the feedback of experts in the field who shared my prediction and offered collaboration. Julie Atkin from La Trobe University continued to wave the Aussie banner in her session presenting fresh evidence that proximal cell transport is disrupted early in MND models. These studies highlight a novel pathway for drug intervention in MND which is currently under intense investigation.

Third culprit in three years

To cap off 2010 on a high note, Bryan Traynor from NIH announced exciting news about a new MND gene called VCP, previously implicated in a hereditary muscle wasting dementia syndrome. Using powerful next-generation “exome sequencing” technology capable of unprecedented rapid screening of every known gene within a person, they identified that gene defects in VCP were linked to forms of MND with family history. VCP is a logical candidate for MND because it was previously established that defective VCP leads to TDP-43 junk piles in nerve cells. Together with TDP-43 and FUS, VCP is the third major MND gene to be reported in the last 3 years, adding to a growing list of culprits that help guide researchers to the relevant pathways and mechanisms in MND. These findings also illustrate application of a new and improved technique for efficient genetic diagnosis of MND.

In summary, this conference provided key updates on current MND research themes, advances, breakthroughs, directions and importantly flagged upcoming clinical trials and publications, particularly in the fast evolving area of RNA biology in MND. The results presented this year affirm my belief that MND is becoming the flagship neurological disease for application of cutting edge technologies including IPC stem cells, deep sequencing and exome sequencing platforms. This information should yield better animal models with improved predictive power for clinical trials. The interaction with national and international basic scientists, clinicians, PALS, CALS and support groups at this venue not possible in everyday science was also invaluable, encouraging international recognition and appraisal of our work and productive collaborations aimed at our goal to change the course of MND.

Bradley Turner