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Research News Special Report

20th International ALS/MND Symposium

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SOCIÉTÉ CANADIENNE DE LA SCLÉROSE LATÉRALE AMYOTROPHIQUE

Introduction



The Brandenburg Gate, Berlin

In 2009, worldwide attention focused on the city of Berlin as it celebrated the 20th anniversary of the fall of the Berlin Wall. Just one month later, a record number of delegates from around the world met in Berlin for the 20th annual International Symposium on ALS/MND from December 8–10. The symposium aimed to break down walls in understanding and managing the disease through co-operative discussions between leading scientists and clinicians.

The meeting opened with a look at patient care from Ilora Finlay, FRCP, FRCGP, from the department of palliative care at Cardiff University, highlighting the need for physical, psychological and emotional support for patients and their families from the moment of diagnosis.

Turning to research efforts, Wim Robberecht, MD, PhD, from the University of Leuven, Belgium, provided an overview of past efforts to find treatments for ALS, which framed a discussion of new and exciting opportunities.

“The future has never been so hopeful,” said Robberecht, urging researchers to explore every lead.

The symposium, organized by The Motor Neurone Disease Association (U.K.) and hosted by The German Association for People with Neuromuscular Diseases, comprised parallel basic science and clinical research meetings. Scientific topics included biomarkers, disease models, genes and proteins, inflammation and axonal transport. Clinical discussions focused on disease detection and progression, clinical trial results and design, cognitive change, exercise and nutrition, and respiratory management.

“There’s no better place for breaking down walls.”

~ Kathy Mitchell, RN, nursing professor at Algonquin College in Ottawa, Ontario, and winner of the International Alliance of ALS/MND Associations’s Humanitarian Award for her work with ALS patients and their families in war-torn countries around the world

Enhancing Speech for BiPap Users

Many patients using bi-level positive airway pressure (BiPap) for respiratory support find it difficult to communicate over the airflow in the mask. A new speech-enhancing system, developed by Samuel Chua, an engineer at the University of British Columbia, can overcome the communication challenges imposed by the BiPap apparatus.

The system features a small microphone suspended near the top of

the mask, which captures the noise from the airflow and subtracts it from the sound of the BiPap user's voice. Feedback circuits continuously improve the sound quality.

Preliminary tests in non-patients resulted in almost complete noise removal, allowing listeners to clearly hear the user's voice.

Chua is now working on making the device smaller and more portable. He is also trying to develop software

that would recognize and enhance dysarthric (inarticulate or slurred) speech, which would allow patients to communicate effectively for longer.

Chua's innovation is the result of a collaboration with Andrew Eisen, MD, Professor Emeritus at the University of British Columbia. Chua plans to start testing the device on a cohort of patient volunteers soon. ●

Respiratory Management

The most life-threatening symptom of ALS is respiratory insufficiency. Patients should be offered ventilatory support at the first signs of respiratory failure, such as shortness of breath, fatigue and headaches. In a session on respiratory management, the following studies assessing the effectiveness of different methods of artificial ventilation and a novel procedure called diaphragm pacing were discussed.

Non-invasive ventilation

Non-invasive ventilation (NIV) has been shown to improve the quality of life and even extend survival in ALS patients—particularly when it's implemented at the onset of respiratory symptoms. Owen Johnson, a respiratory consultant with the Mid-Yorkshire National Health Service Trust in West Yorkshire, England, stressed the importance of recognizing the earliest

signs of respiratory symptoms and that multidisciplinary care teams are key in the detection process. Johnson also reported that initiation and management of NIV in the patient's home is safe and effective and might reduce the need for hospital visits.

Even after NIV is prescribed, monitoring its effectiveness should continue, according to a prospective study presented by Amy Atkeson, MD, from the Columbia University College of Physicians and Surgeons in New York, which examined the benefits and pitfalls of NIV used throughout the night. In the United States, nighttime NIV (nNIV) is prescribed and adjusted non-objectively based on the patient's feedback—a process for which the effectiveness had not been assessed. Atkeson monitored 20 patients who reported “successful” nNIV use. She found that, contrary to patients' beliefs, nNIV use was un-

successful at improving disordered breathing during sleep and sustaining respiratory muscle strength in about half of patients.

Diaphragm pacing

Since the 2005 introduction of diaphragm pacing—a laparoscopically implanted device that stimulates diaphragm contraction and breathing—a large, multicentre trial was conducted to evaluate its impact on respiratory decline and survival in ALS.

Raymond Onders, MD, a surgeon from Case Western Reserve University School of Medicine in Ohio and the principal investigator of the study, provided an overview of the trial results, which suggested that diaphragm pacing can maintain respiratory function, decrease respiratory decline and prolong survival.

Onders and his team observed 145 ALS patients, 106 of whom had a pacing system implanted between 2005 and 2009. He reported that the number of adverse respiratory events decreased by a third from that expected based on published studies. The death rate was cut in half.

In patients who received diaphragm pacing implantation and percutaneous endoscopic gastrostomy (PEG) in the same surgical session (an advantageous strategy in terms of surgery time) one-year survival was 74 per cent—a significant advantage over subjects who received PEG alone (approximately 23 per cent one-year survival).

Onders recommended that all implanted patients also use NIV to rest accessory muscles, noting that additive effects were observed in patients using both (82 per cent of patients in the study). He concluded that diaphragm pacing is safe, tolerated and effective in diminishing respiratory decline in patients declining prior to implantation. He noted, however, that there is no benefit from pacing in patients with involvement of the phrenic nerve—the nerve controlling diaphragm contraction.

Now Onders is waiting for approval from the United States Food and Drug Administration, with hopes that diaphragm pacing will be considered under the humanitarian device program.

Onders' colleague, Jesus Gonzalez-Bermejo, MD, from the Groupe Hospitalier Pitié-Salpêtrière in Paris,

France, presented the results from his related study examining the effects of diaphragm pacing on sleep efficiency in ALS patients. Respiratory insufficiency can cause major disturbances in sleep leading to a decreased quality of life. In a smaller subset from Onders' larger patient pool, Gonzalez-Bermejo reported a significant improvement in sleep efficiency after only four months of diaphragm pacing. Although the nature of this improvement remains unknown, Gonzalez-Bermejo suggested that it might be related to the unloading of inspiratory muscles during sleep.

Tracheostomy

Respiratory failure is the major cause of death in ALS. While many patients use non-invasive respiratory support like bi-level positive airway pressure (BiPap), only a small proportion of patients in North America opt for tracheostomy and permanent mechanical ventilation—a procedure that can prolong life indefinitely.

Amelia Conte, MD, from the department of neurosciences in Rome, Italy, presented the results of her study, which examined the survival of 99 patients who underwent tracheostomy. She reported a median survival of 40 months following the procedure, with younger patients surviving longer. Four patients progressed to a locked-in state—meaning they were unable to communicate. Infections were the cause of death in 80 per cent of patients.

Conte's presentation raised a number of ethical concerns from the audience. In Italy, it is illegal to remove

a patient from a ventilator—even if he or she is “locked in.” Is it ethical to perform a tracheostomy without patients being able to change their minds? Would the cognitive changes known to occur in a subset of patients affect their competence in making such a decision in the first place?

Next, Andrea Calvo, MD, from the University of Torino, Italy, presented her study, which investigated factors influencing the decision to go on a ventilator. She found that male gender, younger age, NIV and PEG use, and access to a multidisciplinary ALS clinic all increased the likelihood of a patient choosing long-term mechanical ventilation. In her study, the mean survival time after tracheostomy was just over eight months. Better survival was associated with younger age, the time of tracheostomy after diagnosis, NIV and PEG use, ALS clinic access, the ability to live at home, and being married.

The exorbitant costs and the need for around-the-clock nursing care make the decision to go on a ventilator impossible for many patients in North America. However, the quality of life for the patient and the burden on the caregiver are also major considerations, raising ethical concerns from the audience about Conte's and Calvo's studies, in which patients could not go off the ventilator after choosing to go on it (because of Italian law). Many meeting attendees acknowledged the need to better educate patients about the long-term outcomes of going on a ventilator and advise them to make end-of-life decisions early on. ●

Clinical Phenotypes and Disease Progression

The speed at which ALS progresses varies dramatically between patients. To offer a more individualized prognosis, researchers are trying to identify surrogate markers that accurately reflect disease processes.

Jan Kassubek, MD, from the department of neurology at the University of Ulm, Germany, has been investigating the use of MRI-based neuroimaging as a “dry” surrogate marker for disease progression, with hopes of ultimately identifying measures that correlate with prognosis. He stressed the need for more longitudinal studies with standardized protocols. Co-operation between scientists and the establishment of large, high-quality, multinational databases is key, Kassubek said.

Currently, the most commonly used measure of progression and predictor of survival is the revised ALS functional rating scale (ALSFRS-R). The ALSFRS-R consists of 12 questions that probe patients’ abilities to perform everyday tasks such as breathing, walking and swallowing. Nazem Atassi, MD, from the department of neurology at Massachusetts General Hospital in Boston, presented data suggesting that while most questions on the ALSFRS-R offer helpful information about disease progression, certain questions are better predictors of survival than others. For example, questions about climbing, writing and salivation were found to be good predictors of survival, while ones about breathing were not. Atassi’s data also

indicated that patients with a slowly progressing disease are more likely to participate in clinical trials than those with a more aggressive disease phenotype. Finally, he found that ALSFRS-R-measured functional decline is more likely to accelerate after percutaneous endoscopic gastrostomy (PEG) or bi-level positive airway pressure (BiPap) use even when questions about swallowing and breathing are removed from the questionnaire. He did not explain whether this was a result of the procedures themselves or a reflection of when in the course of disease progression such procedures are performed.

It has been suggested that in some cases the genotypes of patients with genetic causes of ALS might predict the phenotypes they express. William Camu, MD, from the ALS Centre at the Centre Hospitalier Universitaire in Montpellier, France, studied the disease phenotype of nine patients with mutations in the gene encoding TAR DNA-binding protein (TDP-43). Clinical features in these patients included upper limb onset (in seven of nine) and slower disease progression. Although his sample was small, Camu’s results suggest that TDP-43 mutations might result in a particular disease phenotype. Camu said it was too early to know whether subgroups of patients with specific mutations in the gene express specific phenotypes.

Mutations in superoxide dismutase 1 (SOD1) run in families and can ultimately cause ALS. Mi-

chael Benatar, DPhil, MBChB, from the department of neurology at Emory University in Atlanta, presented the details of his new, systematic study of asymptomatic, SOD1 mutation-positive individuals — one of the only populations known to be at high risk for developing ALS. In studying these individuals prospectively, Benatar hopes to characterize the pre-symptomatic phase of ALS, identify environmental factors that may modify disease onset or progression, develop biomarkers for early diagnosis, and evaluate the use of arimoclomol — a drug that was successful in a mutant SOD1 mouse model of ALS — in SOD1 mutation-positive patients.

Benatar is currently enrolling subjects identified from familial ALS pedigrees in a “Pre-Familial ALS” observational study. Most of the participants have elected to undergo genetic counselling to learn whether or not they carry SOD1 mutations. They will undergo neuropsychological tests and neuroimaging, complete environmental exposure surveys and provide tissue specimens annually. This is the first prospective study of individuals with ALS-causing SOD1 mutations who do not yet have ALS. Benatar has also begun screening newly diagnosed SOD1 mutation-positive patients for a phase II/III randomized control trial of the drug arimoclomol. ●

Cognitive Change

It is now widely accepted that one in two ALS patients exhibits mild cognitive or behavioural changes. Such changes have significant implications for patients and their caregivers, often resulting in poor compliance with treatment programs and reduced survival rates. Recognizing these subtle changes in patients during a routine clinic visit poses several challenges relating to limitations on the neurologist's time and, in some instances, the patient's ability to communicate.

Using the criteria for the diagnosis of frontotemporal syndromes in ALS proposed in 2009 by Michael Strong, MD, University Hospital and the Robarts Research Institute in London, Ontario and his colleagues, Julie Phukan, MD, from the department of neurology at Beaumont Hospital in Dublin, Ireland, was able to categorize patients into subgroups and track the evolution of cognitive and behavioural impairments over time. Phukan presented results at the international symposium which indicated that early deficits in verbal fluency — a measure of cognitive function — predicted cognitive decline that was rare in patients without such deficits.

In the following talk, Cathy Lomen-Hoerth, MD, from the ALS Center

at the University of California in San Francisco, compared old interpretations of FTD-like behaviours, such as depression and denial, with new interpretations based on cognitive and behavioural aspects of frontotemporal dementia, such as apathy and lack of insight. She acknowledged the huge advances in the field that have stemmed from the international workshops on Frontotemporal Dementia in ALS, held in London, ON, in 2005, 2007 and 2009.

Lomen-Hoerth presented results from her study on the use of a 30-minute cognitive screening exam in identifying patients with cognitive and behavioural changes. Out of 14 patients tested for cognitive changes, 12 diagnoses made using the screening exam matched those made using a more comprehensive, time-consuming and costly testing battery. Five out of seven patients tested for behavioural changes had matching diagnoses. Lomen-Hoerth suggested that the screening tool may be effective and efficient in identifying patients with cognitive and behavioural changes but noted that it should not replace the full battery as a diagnostic tool.

With a similar goal of developing a reliable and realistic test for clinic

use, Claire Flaherty-Craig, PhD, from the department of neurology at the Penn State College of Medicine in Hershey, Pennsylvania, described the application of the Penn State Brief Exam of Frontal and Temporal Dysfunction Syndromes. Diagnoses made using the brief exam were validated through a comparison with those made using a more intensive testing battery in 35 patients. Flaherty-Craig stressed that the benefit to this exam, in addition to being short enough to administer during a routine clinic visit, is its ability to identify difficulties in decision making and problem solving — both cognitive functions that have important implications for treatment planning.

These studies reflect the tremendous amount of effort directed at developing reasonable and reliable tools to assess cognitive and behavioural impairments in ALS patients. Although researchers are still exploring ways to better detect these impairments, the consensus recently reached on the criteria for frontotemporal syndromes in ALS was an important first step toward this goal. ●

Electrophysiological Diagnosis and Monitoring of ALS

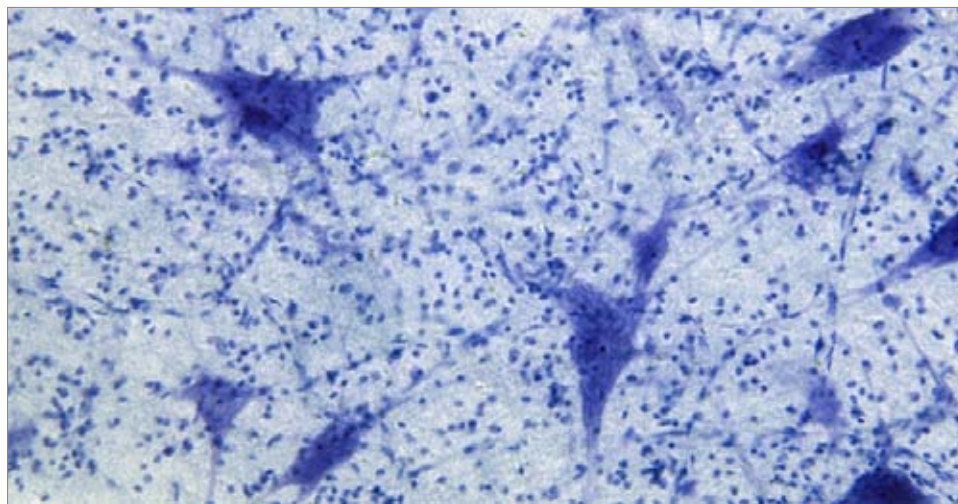
Central to the diagnosis of ALS is the detection of abnormalities in both upper motor neurons running down to the spinal cord from the brain, and lower motor neurons projecting out to the muscles. Clinical signs of these abnormalities, such as spasticity or weakness, are not always easy to detect, but electrophysiological tests can identify abnormalities that might otherwise go unnoticed.

The El Escorial World Federation of Neurology Criteria for the Diagnosis of ALS defines ALS as definite, probable, probable laboratory-supported, possible or suspected based on the extent of upper and lower motor neuron involvement as indicated by clinical, electrophysiological, neuroimaging or neuropathological tests.

Two revisions to the long-standing El Escorial diagnostic criteria were recently proposed, with an aim to improve the sensitivity without diminishing the specificity. The revisions, called “the Awiji modification,” eliminate the diagnostic category “probable laboratory-supported ALS.” Werner Boekestein from Medisch Spectrum Twente in the Netherlands presented the results of a retrospective study

investigating the usefulness of the modification. He concluded that the Awiji modification might substantially improve the sensitivity (reporting a 16 per cent improvement in his study) without losing specificity. This

using modified incremental MUNE to test progression in a standardized way across ALS clinics to pool data for clinical trials. Shefner concluded that modified incremental MUNE was more sensitive in detecting the rate of



Microscopic view of motor neurons

means that patients with early-stage ALS might meet the criteria for a diagnosis sooner.

Motor unit number estimation (MUNE) is a technique used to assess disease progression. Jeremy Shefner, MD, PhD, from SUNY Upstate Medical University in Syracuse, NY, presented the results from his study

change than the ALS functional rating scale (ALSFRS-R)—a widely used clinical trial outcome measure—and that trials using modified incremental MUNE would require 30 per cent fewer subjects to achieve the same power as ones relying on the ALSFRS-R. ●

Exercise, Metabolism and Nutrition in ALS

The role of environmental factors and lifestyle behaviours in ALS progression is gaining significant research attention. In a session dedicated to exercise, metabolism and nutrition in ALS, past reports were debated and new insights were provided.

Exercise

The controversy over the role of exercise as a predisposing factor in ALS—long a topic of debate but one revisited with strong interest following a 2005 report of a severely increased risk of ALS in Italian soccer players—was highlighted by consecutive speakers presenting arguments for and against the possible link.

Adriano Chio, MD, from the department of neuroscience in Torino, Italy, (and first author of the report “Severely increased risk of amyotrophic lateral sclerosis among Italian professional football players,” published in the March 2005, issue of *Brain*) summarized the evidence from retrospective human studies, which suggests that strenuous exercise may lead to a younger age of onset and a shorter disease duration of ALS. He stressed that the type of exercise may be important—pointing out that the higher incidence noted in soccer and football players was not observed in cyclists or basketball players.

Chio also gave an overview of observations made in animal studies, some of which suggested that moderate exercise might actually be beneficial. In one such study, larger cages with more room to run extended survival in a mouse model of ALS.

However, in a separate study using the same model, endurance training accelerated disease progression.

Chio concluded that the data on exercise and ALS is too sparse and variable to draw any firm conclusions yet. He proposed that future studies investigating exercise and the expression of vascular endothelial growth factor and angiogenin—genes involved in the response to increased oxygen demand that are implicated in ALS risk—may reveal interactions that modulate disease phenotype.

Making the argument against a role for exercise in ALS risk, John Wokke, MD, from the department of neurology at the University Medical Center in Utrecht, the Netherlands, pointed out the increased ALS incidence associated with a variety of non-athletic occupations, such as hairdressing. He also highlighted the limitations of the retrospective human studies, including what he called “methodological flaws.”

Wokke concluded that there is no real evidence for exercise as a risk factor in ALS and stressed that exercise might even be in fact beneficial in a subset of patients.

Many questions about the role of exercise in ALS remain unanswered. An interesting one was raised in the discussion following the presentations: do ALS patients simply tend to be more athletic? Could the disease predispose patients to athleticism rather than vice versa? Both Chio and Wokke acknowledged the need for a properly controlled clinical trial of exercise to objectively assess any risk or treatment potential.

Metabolism

Mice expressing mutant superoxide dismutase 1 (mSOD1—the most widely used animal model of ALS) are hypermetabolic. This means they have a higher-than-normal resting metabolic rate, making them leaner than normal mice even before they get sick. When mSOD1 mice are fed a high-fat diet, they retain a normal level of leanness, get sick later and survive longer.

This observation prompted Luc Dupuis, PhD, from the Institut National de la Santé et de la Recherche Médicale in Strasbourg, France, to investigate the potential link between metabolism and ALS. He noticed that even the muscles of mSOD1 mice were hypermetabolic, meaning they were using more glucose, and therefore spending more energy, than normal.

To determine whether muscle hypermetabolism could specifically contribute to neurodegeneration, Dupuis developed a special mouse that had hypermetabolic muscles but was healthy in every other way. Muscle hypermetabolism led to massive weakness and atrophy of the glycolytic muscles—ones that contract quickly and powerfully, which tend to be more sensitive to ALS progression—with relative sparing of the diaphragm. When he looked microscopically at the nerves, he noticed a separation of motor neurons from their muscle targets that became more pronounced and widespread with age. He also observed a 20 per cent loss of motor neurons and activation of astrocytes—non-neuronal cells which help neurons to function—both of which are features of ALS.

ty of Sheffield, England, presented the results from a study in which he screened samples from 168 ALS patients, including 49 familial cases, for FUS mutations. He concluded that some FUS mutations might underlie a more severe ALS phenotype than

others. His findings suggested that the G507D and R524W mutations were disease-causing, while the G174del mutation was relatively benign.

These studies confirm the rapid progress being made less than a year after the discovery of the third gene known to cause familial ALS. Functional similarities between FUS and

TDP-43 suggest that defects in RNA-binding might be a common disease mechanism. Researchers hope that further studies investigating the consequences of FUS mutations will clarify their role in motor neuron degeneration in ALS. ●

Patient and Caregiver Support

Opening with an overview of the current health care situation in the United States, Steven Ringel, MD, from the University of Colorado, described the need to establish and adhere to guidelines for care, measure care by collecting timely and credible feedback, and track accountability. He commended the ALS community for already having these things in place but stressed that the study of processes associated with care should receive as much consideration as basic research.

A review presented by Robert Miller, MD, from the Forbes Norris ALS Research Center in San Francisco was a perfect example of such a study.

Miller reviewed the American Academy of Neurology's report on managing care for ALS patients, developed to assist practitioners and patients in making informed decisions based on evidence from research studies. Miller described how he and a team of experts systematically reviewed the literature from the past decade, identifying topics relating to informing patients of their diagnosis, slowing disease progression,

managing symptoms, respiratory management, nutrition, communication, cognitive and behavioural impairment, multidisciplinary clinics and palliative care.

By scoring studies based on the strength of the evidence they provided, Miller and his colleagues determined that multidisciplinary clinics are probably effective in encouraging the use of adaptive equipment (such as wheelchairs), prescribing Riluzole (the only FDA-approved drug for the treatment of ALS), promoting non-invasive ventilation (NIV) and percutaneous endoscopic gastrostomy (PEG), and improving the quality of life. They concluded that multidisciplinary clinics should be used.

They also determined that Riluzole should be used for slowly progressing patients, PEG should be used in patients losing weight, botox injections should be used for drooling, NIV should be used at the earliest signs of respiratory decline, and cognitive screening should be considered.

Miller said that PEG and NIV are underused, and multidisciplinary clinics should be more widely used. He

also reported that while tracheostomy and permanent mechanical ventilation can preserve (but not necessarily enhance) quality of life, caregiver depression is a consideration. Therefore tracheostomy is not strongly recommended.

Fears over the use of pain killers such as morphine and midazolam for ALS patients in the last days of life prompted David Oliver, MD, palliative care consultant with Wisdom Hospice in the United Kingdom, to perform a study investigating the purpose and benefit of end-of-life medications. Oliver collected data about the use of such medications in the last 72 hours of life from six palliative care centers. He found that the majority of patients were given morphine, midazolam or anticholinergic medications in the last days of life and died peacefully. He concluded that physicians should feel secure in giving such medications, stressing that the aim is to control symptoms and not to terminally sedate or euthanize. He also said that families and patients should feel reassured that dying can be peaceful when these medications are used.

According to a 2005 study, 80–90 per cent of ALS patients want to die at home but 60 per cent die in hospital. Pauline Callagher, the co-ordinator of the Preston Motor Neuron Disease Care and Research Centre in the United Kingdom, presented the results of her study examining the effectiveness of a Preferred Priorities of Care document—a form outlining preferences for end-of-life care—in achieving the preferred place for end-of-life for ALS patients. Out of 44 patients who completed the form, 39 expressed wishes to die at home, 25 died at home and

9 died in hospital. Out of 45 patients who did not complete the form, 18 died at home and 16 died in hospital. Callagher concluded that patients have a higher chance of achieving their preferred place of end-of-life care if they have made clear instructions about their wishes. This is in agreement with other studies suggesting that end-of-life decisions should be made clearly and early on.

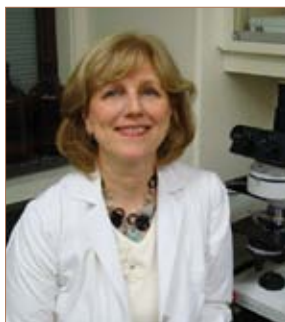
The quality of life for people caring for ALS patients is significantly affected by their caregiving activities. Zachary Simmons, MD, from Penn State

University, sought to identify ways to support caregivers by reviewing literature on the topic, consulting experts and monitoring caregiver focus groups. The team conducting the study concluded that the development of a form assessing risks (such as concerns with tasks and stress) and protective functions (such as optimism and confidence) for clinic use could help to support caregivers. Simmons says that caregiver issues that could benefit from formalized training should be identified. ●

Canadian Research Posters

Along with three days filled with exciting presentations of the latest and greatest basic science and clinical research, more than 340 research posters lined the conference rooms at this year's symposium. Among them were posters presented by Canadian researchers and young investigators detailing the following ongoing and late-breaking studies.

Heat shock proteins (HSPs) can clear out misfolded proteins or help them to fold properly. Heather Durham, PhD, from the Montreal Neurological Institute at McGill University presented the results from her study, which investigated the use of NXD30001—a small molecule HSP 90 inhibitor—in inducing other HSPs. Durham found that NXD30001 induced the dose-dependen-



Heather Durham, PhD

dent expression of HSP 70 and HSP 40 in cultured neurons at concentrations that were not toxic to the cells. She concluded that HSP 90 inhibitors like NXD30001 show promise in cell culture models of motor neuron disease, and that the ability of NXD30001 to permeate into nervous system tissue makes it a good candidate for testing in animal models—the next step before a drug can be considered for human trials.

The death-inducing gene, BNIP3, is upregulated in the motor neurons of mutant superoxide dismutase 1 (SOD1) mouse models of ALS. By injecting short hairpin RNA—a molecule that interferes

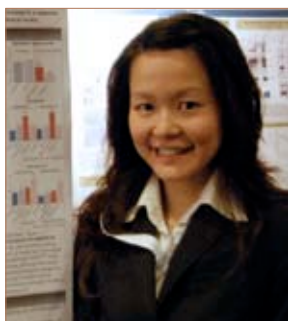


Jiming Kong, MD, PhD

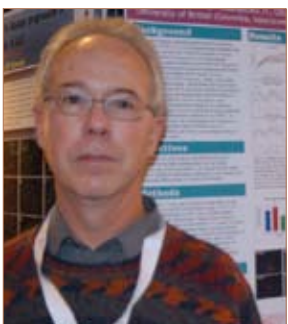
with the BNIP3 RNA preventing it from becoming protein—into the spinal cord of eight-week-old mutant SOD1 mice, Jiming Kong, MD, PhD, from the University of Manitoba was able to inhibit BNIP3 expression and increase the number of neuronal axons coming out of the lumbar spinal cord. Kong concluded that therapies targeting the BNIP3 pathway might be effective in the treatment of ALS.

The dietary intake of steryl glucosides—natural components of flour made from the cycad seed—is thought to contribute to the high incidence of ALS-Parkinsonian Dementia complex on the South Pacific island of Guam. Feeding mice a diet high in

steryl glucosides isolated from cycad flour results in a similar phenotype. Grace Lee, a PhD candidate in the lab of Chris Shaw, PhD, at the University of British Columbia, presented the results of her study, which investigated the effects of dietary steryl glucosides on the disease onset, duration, and progression in mutant SOD1 mice with a slow progressing disease (SOD1 G37R mice). Lee observed an accelerated decline in motor function, increased accumulation of astrocytes — non-neuronal support cells — around motor neurons, and increased neuronal death. While the mechanism of steryl glucoside-mediated neuronal death remains uncertain, Lee was able to conclude that both environmental influences and genetic factors contribute to disease progression in ALS.



Grace Lee



Chris Shaw, PhD

The work of Opiyemi Banjo, MSc, from the same lab, was presented by Chris Shaw, PhD. Banjo studied the effects of dietary steryl glucosides on fetal development by feeding them to pregnant mice and observing the effects on their pups. Banjo found that prenatal exposure to steryl glucosides had significant effects on the central nervous system, which were worsened by exposure persisting into adulthood. Interestingly, Banjo found that female mice were able to recover from fetal exposure unless re-exposed as adults, whereas male mice were not. Banjo concluded

that developmental exposure to cycad-derived steryl glucosides leads to neurodegeneration characterized by programmed cell death and activation of non-neuronal inflammatory cells (both features of ALS), which can be exacerbated by adult exposure. This further supports a role for environmental factors in the progression of ALS, and offers interesting insight into gender differences (the prevalence in men is up to 1.5 times that in premenopausal women according to a 2001 review published in the *Journal of Neurological Sciences*).

The work of Samer Abou Ezzi, PhD, was presented by Jean-Pierre Julien, PhD, from Centre de recherche du Centre hospitalier de l'Université Laval. In light of Julien's finding that mutant SOD1 can be secreted from cells with the help of chromogranins and the identification of chromogranin muta-

tions in a subset of ALS patients, Ezzi developed two lines of double transgenic mice: one expressing the G37R SOD1 mutation and overexpressing chromogranin A; the other expressing the G37R SOD1 mutation but lacking chromogranin A. Ezzi found that chromogranin A overexpression enhanced motor neuron degeneration in mutant SOD1 G37R mice and led to higher levels of misfolded SOD1, whereas the absence of chromogranin A resulted in less neurodegeneration. Ezzi concluded that chromogranins are important in the pathogenesis of mutant SOD1-mediated ALS. This work is of particular interest given the recent report by Julien that variations in the chromogranin B gene confer a risk for ALS.

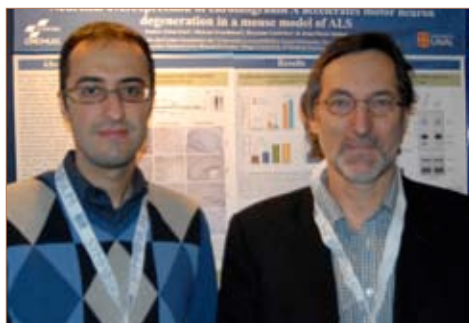
Rodolphe Perrot, PhD, also from Julien's lab and a recipient of the Tim Noël Fellowship in ALS Research in 2008, presented the results from his



Rodolphe Perrot, PhD

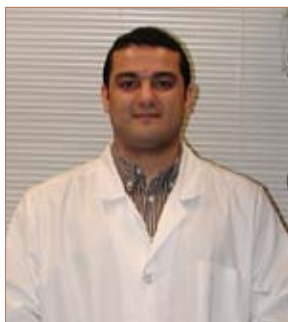
study which used time-lapse imaging to observe axonal transport in mice with altered expression of intermediate filament proteins — the building blocks of the motor neuron cytoskeleton. He found an unexpected net retrograde transport, meaning backwards

towards the cell body, of energy-producing mitochondria in mice overexpressing the intermediate filament peripherin while not expressing the low molecular weight neurofilament protein subunit (NFL). Perrot concluded that intermediate filament disorganization might contribute to neurodegeneration by interfering with the transport of important organelles like mitochondria.

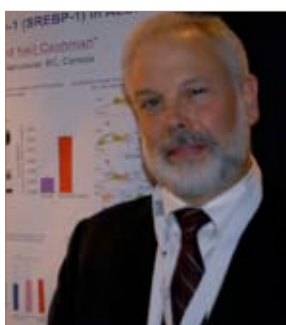


Samer Abou Ezzi, PhD, (left) and Jean-Pierre Julien, PhD

Sherif Elbasiouny, PhD, a postdoctoral fellow at Northwestern University and recipient of the Tim Noël Fellowship in ALS Research in 2007, presented data from his study assessing motor neuron excitability in presymptomatic mutant SOD1 mice (with a G93A mutation), long before disease onset. Using whole-cell patch clamp recording on cultured slices of spinal cord tissue—a technique which enables the current across the membranes of individual cells to be recorded—Elbasiouny found that motor neurons that are vulnerable in ALS have a significantly higher peak inward current than control motor neurons. This means that more calcium gets into the cells when they are excited. These vulnerable motor neurons also have low calcium buffering capacity; meaning that this increased intracellular calcium concentration can cause damage and could lead to their degeneration.



Sherif Elbasiouny, PhD



Neil Cashman, MD

Excitotoxicity caused by over-activation of the Ca⁺⁺-permeable AMPA receptors has long been considered a pathogenic mechanism in ALS. The work of Changiz Taghibiglou, PhD, presented by Neil Cashman, MD, from the University of British Columbia investigated the effects of Indip—a blocker of sterol regulatory binding protein-1—on blocking excitotoxicity in a cell culture model of ALS. Taghibiglou found that Indip protected motor neurons from glutamate-induced cell death and concluded that drugs target-

ing the activation of sterol regulatory binding protein may be neuroprotective in ALS.

The work of Zhong Ping He, PhD, was presented by Michael Strong, MD, from the University of Western Ontario. He has been characterizing the expression profile of micro RNAs (small RNA molecules that bind to complimentary mRNA sequences and prevent them from being translated into protein), which interact with NFL messenger RNA. He hoped to determine whether the profile differed between spinal cord tissue from ALS patients and controls. He identified several micro RNAs that

are differentially expressed in control, sporadic ALS and familial ALS tissue, and concluded that a micro RNA expression profile for ALS spinal cord tissue can be identified. This work adds a new layer of complexity to the network of molecular players in the pathogenesis of ALS and suggests that certain micro RNA molecules might be good targets for ALS treatments.

A second study presented by Strong investigated a novel mutation in the gene encoding lamin A in an individual with ALS. Lamin mutations are usually associated with heart

abnormalities, irregular fat deposition or progeria—a disease resembling rapid aging. The patient described by Strong had signs of lamin abnormalities (trunk obesity and irregular heart rhythms) and ALS (fasciculations and muscle wasting). Based on this rare case, Strong concluded that patients presenting motor neuropathy or ALS and signs of a possible laminopathy should be screened for lamin mutations.

Strong also presented the results from a prospective study characterizing the clinical and pathological features of ALS resulting from mutations in the fused in liposarcoma gene (FUS). Strong found only one case resulting from a FUS mutation (which was familial) out of seven familial ALS cases and 94 total ALS cases. FUS-positive accumulations were present inside the motor neurons from the case, but no TAR DNA-binding protein (TDP-43, another protein implicated in familial ALS) inclusions were noted, suggesting that FUS and TDP-43 may be unrelated but similar contributors to disease mechanisms in ALS.



Michael Strong, MD

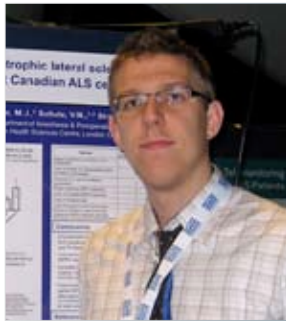
Veronique Belzil, a PhD candidate in the lab of Guy Rouleau, MD, PhD, at the Centre hospitalier de l'Université de Montréal also presented data on FUS mutations. She screened for mutations in a cohort of 200 French and French Canadian patients with familial (80) and sporadic (120) ALS. Belzil identified



Veronique Belzil

three different mutations in four different patients, three of whom had sporadic ALS. She concluded from her study that FUS mutations accounted for 1.25 per cent of familial cases and 2.5 per cent of sporadic cases. Most interestingly, she identified two mutations in patients with sporadic ALS that had been previously reported in familial ALS patients. This strengthens the role for genetic contributors to sporadic disease.

Benjamin Ritsma, a medical student at the University of Western



Benjamin Ritsma

Ontario, presented the results from his study in collaboration with Strong, which investigated the use of non-invasive positive pressure ventilation (NIPPV) in Canada and barriers influencing its use. Ritsma collected data using a survey that he distributed to physicians at each of Canada's multidisciplinary ALS centres. He found that

the prevalence of NIPPV across Canada was 18.3 per cent, compared to only 1.5 per cent of patients choosing tracheostomy and invasive ventilation. NIPPV was initiated at the onset of respiratory symptoms including short-

ness of breath and morning headaches. The primary barriers to NIPPV use were patient intolerance and the lack of access to the required expertise and technology. Ritsma concluded that centres should establish more definitive criteria for NIPPV initiation and ensure that patients have access to the appropriate respiratory care. ●



Canadian researchers in Berlin

Research News Special Report

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