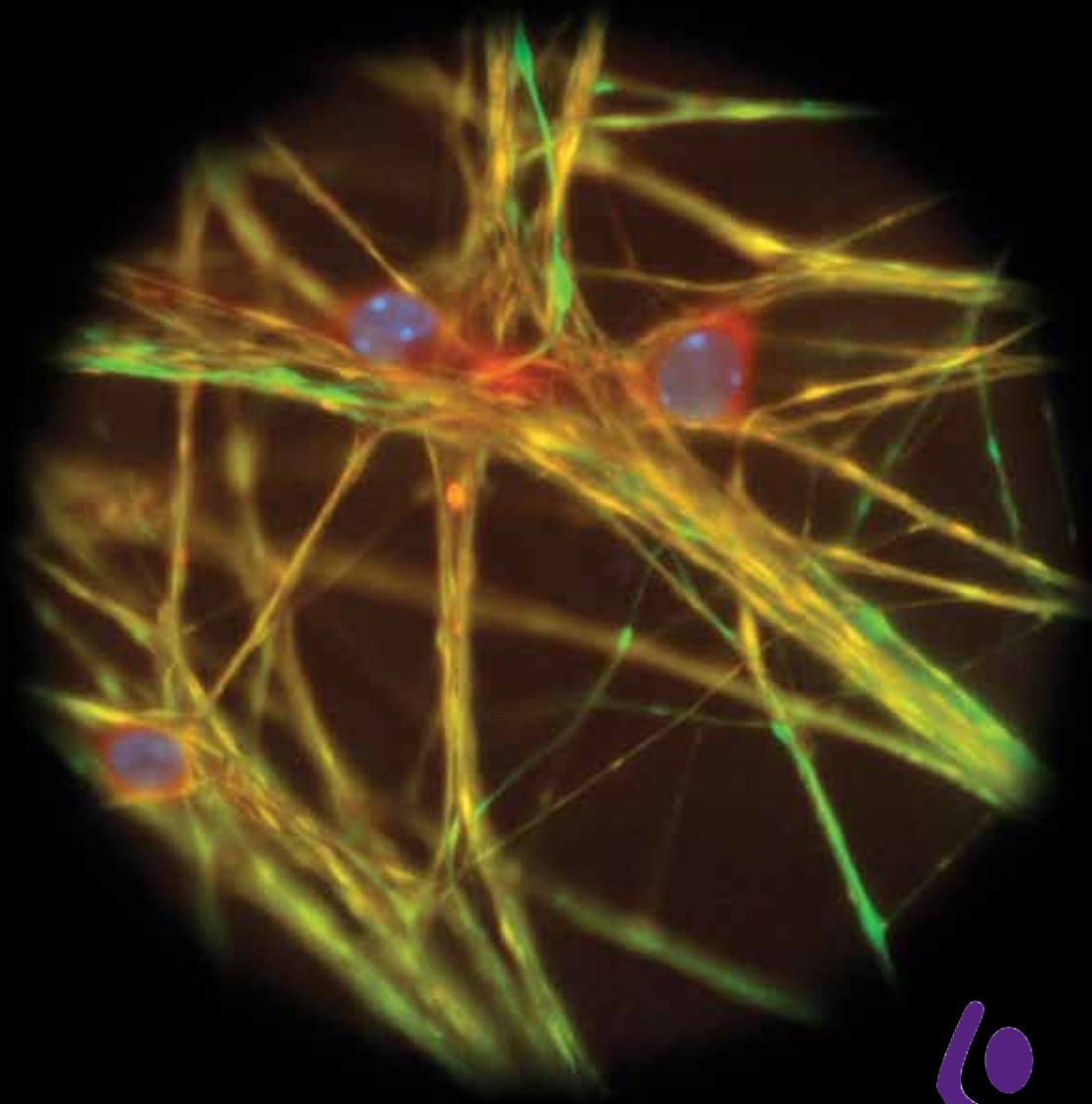


the Northern Neuron



ALS Research in Canada

Spring 2010 • Volume 4 • Issue 1

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the Northern Neuron

Spring 2010 — Volume 4, Issue 1

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the Northern Neuron is a publication of the ALS Society of Canada, highlighting ALS research in Canada to inform readers of the promising directions in ALS research toward treatment and a cure for this devastating disease.

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the Northern Neuron is also available online at www.als.ca/research.

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On the cover:

The photograph depicts motor neurons from dissociated mouse spinal cord culture. Staining illustrates motor neuron nuclei (blue/Hoechst stain); cyclin G1 mRNA in the motor neuronal soma (red/FISH); and neurofilaments in distal neuronal processes (green/ICC). Both neurofilaments and cyclin G1 mRNA are localized to the juxta-perikaryal neuronal processes (yellow).

Image provided by D.A. Figlewicz and M. Mlodzienski.

Message from the Chair

THE FACTS ABOUT ALS tell a grim story. ALS is a devastating neurological disease that affects between 2,500 and 3,000 Canadians. Eighty per cent of these people will die within two to five years of diagnosis. ALS affects both men and women of all ethnic and socio-economic groups. Most people with ALS are between the ages of 40 and 70, but it may strike individuals in any age group. Less than 10 per cent of cases are hereditary, meaning that ALS generally attacks people with no family history of the disease.

But ALS affects more than just those living with the disease. It has a huge impact—emotionally, physically and financially—on the family and friends who generally serve as their primary caregivers. The ALS Society of Canada is supporting research towards a cure for ALS. As yet there is no cure and no effective treatment. We believe that research will lead the way.

Canadian ALS researchers are on the cutting edge of worldwide research that will one day lead to a cure for this disease. We believe that successful research will be achieved by maintaining and developing research partnerships, funding excellent and relevant research, cultivating relationships within the ALS communities, both nationally and internationally, and encouraging a new generation of scientists to study ALS.

We are encouraged by the fact that our donors have continued to support our cause through these troubled economic times. Equally heartening is that the Government of Canada committed \$15 million in 2009 to fund a national study on the prevalence and impact of neurological diseases, such as ALS, in Canada. Research funding is crucial in the battle against this devastating disease.

ALS Canada's research mandate

Founded in 1977, ALS Canada is the only national voluntary health organization dedicated solely to the fight against ALS and support for those with the disease. ALS Canada's vision is to find a cure for ALS. The board, researchers, volunteers and staff perform their day-to-day work with this vision in mind. ALS Canada's mission is to fund research that will lead to a cure.

To help fulfill its mission, Denise Figlewicz, PhD, vice president research at ALS Canada, leads the development and implementation of a national ALS research strategy, represents ALS Canada in scientific communities, and acts as an advisor to bodies in government and industry seeking advice in the field of ALS research. Over the last three years, she has greatly improved the status of ALS Canada as a research funding agency.

Research forum

In May 2009, Canadian ALS researchers and clinicians, post-doctoral fellows and graduate students met for ALS

Canada's Fifth Annual ALS Research Forum. Researchers converged on Toronto to present the results of recent innovative work, to meet newcomers in the field of ALS research, and to hear about the latest research taking place elsewhere in the world. To stimulate interest in ALS research among a new generation of scientists, we again invited Canadian researchers to bring along their most committed young researchers. We had a record number of 44 senior investigators, including four speakers from the United States, and 53 junior investigators. The ambitious program, which took place over the course of three days, included platform talks by the Canadian and American researchers and poster presentations arranged by the young investigators. The research forum is funded by ALS Canada. We firmly believe that such a forum, with its combination of formal presentations and informal discussion opportunities, is a window to tomorrow's research breakthroughs.

At the research forum, researchers and clinicians received an update on two ALS clinical trials, both receiving funding from ALS Canada. Sanjay Kalra, MD, of the University of Alberta, described the trial of memantine that is taking place at ALS centres in Edmonton and Calgary. Kalra and his team are investigating whether memantine, a drug normally used to treat Alzheimer's disease, will have a beneficial effect on patients with ALS.

Lorne Zinman, MD, medical director of the ALS Clinic at the Sunnybrook Health Sciences Centre in Toronto, provided an update on the lithium trial, which was ongoing in Canada and the U.S. at that time. The ALS community was subsequently saddened and disappointed to learn in September 2009 that the lithium trial had been stopped. An interim analysis showed that lithium, a drug commonly used to treat bipolar disorder, used in conjunction with the ALS drug riluzole, did not have the same beneficial effects on the progression of ALS as had been found in a previous Italian pilot study.

Despite our disappointment, we would like to emphasize two very positive aspects of this trial. First, the trial was launched under the auspices of the newly formed Canadian ALS Clinical Trials and Research Network (CALN), a consortium of 15 ALS clinics across the country that



Mike Gardner, Chair

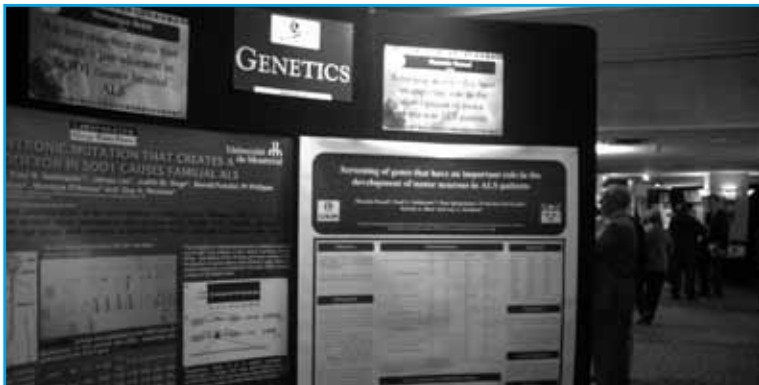
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have incorporated to develop and deliver groundbreaking treatments and scientific discoveries. CALS will continue to develop and promote multicentre ALS research projects in Canada, including clinical trials. Historically, few clinical trials have been conducted in Canada. This model will remain in place for future trials, allowing the participation of Canadians with ALS from across the country, including those who do not live in large centres.

Second, the lithium trial was the first joint effort between CALS and the Northeastern ALS Consortium (NEALS), which includes internationally renowned ALS expert neurologists from reputable university-based ALS centers in the U.S. This good working relationship has set the scene for future collaborations between these two groups.

Research commitments

ALS Canada understands the importance of investing in talented Canadian ALS researchers. Our research budget commitment continues to grow: for the upcoming year, our



*ALS Canada's Fifth Annual Research Forum, May 2009
Above: Poster presentation Below: Young researchers*

research grants budget has increased by \$380,000 (23 per cent) to \$2,033,000, the largest in the society's history.

By contributing to research programs, ALS Canada is determined to develop effective therapies and find a cure. One of the cornerstones of our research program remains the Neuromuscular Research Partnership (NRP), a collaboration set up in 1999 between ALS Canada, Muscular Dystrophy Canada (MDC) and the Institute of Genetics, Institute of Musculoskeletal Health and Arthritis and Institute of Neurosciences, Mental Health and Addiction of the Canadian Institutes of Health (CIHR). To date, the NRP has invested more than \$29 million to fund Canadian researchers investigating neuromuscular diseases.

In addition to our commitment to the NRP, ALS Canada funds the Doctoral Research Awards, in partnership with the CIHR's Institute of Neurosciences, Mental Health and Addiction (INMHA) and the Tim E. Noël Fellowship in ALS Research. Thanks to the generosity of our donors, ALS Canada has also created the Bernice Ramsay Clinical Research Fellowship and the Bernice Ramsay Discovery Grants programs, as well as the Betty Norman Clinical Fellowship in ALS Research.

International partnerships are another focus of ALS Canada's research funding. We have traditionally partnered with The ALS Association (ALSA) in the U.S. to fund researchers. A new initiative in 2009 is a joint-funding project with ALSA and the Motor Neurone Disease Association (MND) of the United Kingdom for the operation of an online gene and protein mutation database.

Particularly exciting this year has been the development of new initiatives in ALS research. In conjunction with Canadian researchers, ALS Quebec and representatives of the André-Delambre Foundation, ALS Canada is moving ahead with the establishment of resources to create and bank induced pluripotent stem (iPS) cells from skin or related tissue of ALS patients and controls. Another new initiative created by ALS Canada, to be run in partnership with the CIHR's INMHA, is the Ronald Peter Griggs Memorial Postdoctoral Fellowship in ALS Research, which, starting from 2010, will be awarded to a recent PhD graduate who chooses ALS research as his or her field of study.

In conclusion

By maintaining our relationships with the international research community and providing research funding on a national and international basis, ALS Canada remains committed to supporting experts in the field of ALS research and to attracting new researchers. Finding a cure for this

We desperately need a series of breakthroughs in ALS research. The only way to do that is to secure more funding. Every donation gets us closer to finding effective treatments, and, ultimately, a cure.

— David Cameron, President and CEO, ALS Canada

devastating disease will require a new generation of researchers determined to make scientific breakthroughs.

New discoveries and treatments will improve the lives of those living with ALS and their families.

Through the efforts of volunteers, donors, researchers, scientists and staff, ALS Canada will continue its work towards finding a cure for this devastating disease. We will continue to fight ALS, but we cannot do it alone. The ongoing support of our donors is crucial. You can help by

contributing online at als.ca/_donate, or by calling 1-800-267-4257 ext. 204. Thank you.



Mike Gardner

Volunteer Chair of the Board of Directors,
ALS Society of Canada

ALS Society of Canada: Leading the Way Through Research

ALS, ALSO KNOWN as Lou Gehrig's disease, kills motor neurons and causes paralysis and death, often within two to five years of diagnosis. The causes of ALS remain little understood. As yet there is no cure and no effective therapy. Research is our only hope for the future.

The ALS Society of Canada funds research and supports its provincial partners in the provision of quality care for those living with ALS. Its vision is to find a cure for this devastating disease.

"Through the efforts of leading researchers in Canada and around the world, we are working towards developing effective treatments and finding a cure. By funding research, we provide hope for those living with ALS," says ALS Society of Canada's President & CEO David Cameron.

ALS Canada is happy to have Denise Figlewicz, PhD, as its vice president research. A highly respected researcher in the international scientific community, Figlewicz has been involved in the ALS research field since the 1980s. In 1993, Figlewicz was part of the international consortium that discovered the first gene responsible for the familial form of ALS. This breakthrough opened the door for the creation of cellular and animal models based on the first known cause of ALS, and for research into developing a cure for the hereditary form of the disease.

Research focus

Canadian ALS researchers funded by ALS Canada are currently exploring the following avenues of research:

- Cell mechanisms and pathways — protein changes including the molecules responsible for their synthesis (mRNAs), and cell pathways, including stress handling and cell death
- Genetics — genes and their roles in disease predisposition and development
- Environmental toxins — potentially harmful factors in the environment that may increase one's likelihood of developing ALS

- Immunology — harmful inflammatory responses that are believed to contribute to the degeneration and death of motor neurons, and the development of ALS immunization or vaccination
- Biomarkers — biological indicators to help diagnose or monitor the progression of ALS

Research programs

ALS Canada funds a variety of research programs and, with the launch of new programs under the leadership of Figlewicz, continues to attract some of the best and brightest scientists to the field of ALS research. ALS Canada is proud to be associated with the following national and international research initiatives:

[The Neuromuscular Research Partnership \(NRP\):](#)

The NRP, a key element of ALS Canada's research programs, funds health research by providing operating grants for research into ALS and other neuromuscular diseases. The CIHR oversees the peer review of all grant proposals.

In 2009, four Canadian researchers received grants from the NRP: Blair Leavitt, MD, of the University of British Columbia, Charles Krieger, MD, PhD, of Simon Fraser University, Janice Robertson, PhD, of the University of Toronto and Guy Rouleau, MD, PhD, of the University of Montreal.

"This is great news for neuromuscular research. Each researcher helps us to find a piece of the ALS puzzle," says Figlewicz. "It is long-term funding commitments like the NRP's that will help us piece together that puzzle and find a cure."

[ALS Society of Canada Senior Investigator Bridge Funding:](#)

New in 2009, an award of \$100,000 was created by ALS Canada to support meritorious research for one year, allowing the investigator to be well positioned for full funding by the

Continued on page 4

CIHR. The recipient was Michael Strong, MD, chief of neurology and co-chair of the department of clinical neurological sciences at the University of Western Ontario and research scientist at the Robarts Research Institute in London, Ontario.

Doctoral Research Award:

To encourage cutting-edge research, ALS Canada, in partnership with the CIHR's Institute of Neurosciences, Mental Health and Addiction (INMHA), created the Doctoral Research Award to fund PhD students conducting ALS-related research for up to three years, to a maximum of \$22,000 per year. The recipient of the 2009 award was Jason Hamlin, a PhD candidate in neurological sciences at McGill University.

Tim E. Noël Fellowship in ALS Research:

ALS Canada established this fellowship in 2006 in honour of Tim E. Noël, former deputy governor of the Bank of Canada, who died of ALS in 2001. The fellowship is a partnership between ALS Canada and the CIHR's INMHA; it recognizes and supports post-doctoral ALS researchers by awarding each recipient up to \$55,000 annually for a maximum of three years. The aim of the fellowship is to encourage young scientists to pursue ALS research. The 2009 recipient was Beibei Zhao, a post-doctoral fellow at the University of Toronto.

Betty Norman Clinical Fellowship in ALS Research:

Created in 2007 to focus on training for ALS clinical care and research, the two-year fellowship in honour of Betty Norman, a Calgarian who died of ALS in 1997, emphasizes clinical expertise, leadership, teaching and research. Kerri Schellenberg, MD, of the University of Alberta, was the first recipient of this fellowship in 2008.

Bernice Ramsay Discovery Grants:

Beginning in 2008, this five-year initiative funds two projects per year (each up to \$100,000) for the pursuit of new and promising directions in ALS research. Made possible by the generous estate of Bernice Ramsay, which donated \$2.28 million to ALS Canada in 2006, each recipient receives a one-time grant to pursue new and promising avenues in ALS research. The 2009 recipients were Victor Rafuse, PhD, of Dalhousie University; and Lorne Zinman, MD, and Yana Yunusova, PhD, of the University of Toronto.

Bernice Ramsay Clinical Research Fellowship:

A new initiative beginning in 2009, the program will support specialized training in clinical care and research skills related to ALS. The program awards a researcher \$100,000 per year for two years.

"The Bernice Ramsay Clinical Research Fellowship will encourage newly trained neurologists wishing to specialize in ALS to conduct clinical research and clinical trials in Canada, bringing us closer to a cure," says Figlewicz.

The 2009 recipient is Vincenzo Basile, MD, from Sunnybrook Health Sciences Centre and the University of Toronto.

ALS Canada Discovery Grants:

This ALS Canada grant program was initiated in 2009 to fund what Figlewicz calls "high-novelty, high-risk and potential high-impact research proposals." The award for each grant is \$100,000, to be used within 24 months. The recipients in 2009 were Kelvin Jones, PhD, of the University of Alberta and Christopher Shaw, PhD, of the University of British Columbia.

Ronald Peter Griggs Memorial Postdoctoral Fellowship:

This is another new initiative from ALS Canada, being run in partnership with the CIHR. The Griggs Fellowship provides three years of funding at \$55,000 per year. It will be awarded in 2010, 2013 and 2016 to recent PhD graduates for ALS-related studies. This fellowship has been made possible through the generous support of Harvey and Sue Griggs of Toronto. Their \$500,000 investment in ALS research represents one of the largest gifts that ALS Canada has ever received. The creation of this fellowship will attract the upcoming generation of scientists, whose work will be pivotal in developing treatments and ultimately finding a cure for ALS.

Joint Funding with The ALS Association (ALSA):

Since 2004, ALS Canada and ALSA have jointly funded prominent Canadian ALS researchers. The recipients of the 2009 grant were co-investigators Charles Krieger, MD, PhD, and Fabio Rossi, MD, PhD, of Simon Fraser University.

Joint Funding project with The ALS Association and the Motor Neuron Disease Association in the U.K.:

In a new partnership with its two sister organizations, ALS Canada has agreed to provide partial funding for the operation of the online Cu/Zn superoxide dismutase mutation database, which was established more than 10 years ago. Researchers have unrestricted access to information about the first gene and protein in which mutations leading to familial ALS were identified. The principal investigator is Ammar Al-Chalabi, ChB, PhD, based at the University College, London, U.K.

Looking ahead

ALS Canada will continue to fund research into ALS to further its central mission of finding a cure for ALS. Our research budget commitment continues to grow and for the upcoming year; our research grants budget has increased by \$380,000 (23 per cent) to \$2,033,000, the largest in the society's history.

ALS Canada believes that through continued efforts to maintain and develop research partnerships, provide funding on a national and international basis, and attract new researchers to the field of ALS research, we will achieve the scientific breakthroughs necessary to find a cure for this devastating disease. 

Doctoral Award Winner Studies Protein Transportation

ATTRACTING BRIGHT YOUNG investigators to ALS research is not an easy task, but through the funding of generous research awards, promising scientists are joining the fight against this devastating disease. To encourage cutting-edge research, the ALS Society of Canada, together with the Institute of Neurosciences, Mental Health and Addiction of the Canadian Institutes of Health Research (CIHR), created the Doctoral Research Award to fund PhD students conducting ALS-related research for up to three years.

Jason Hamlin, a PhD candidate in neurological sciences at McGill University, is the recipient of the 2009 doctoral award. Hamlin, who obtained his BSc and MSc in microbiology from the University of Manitoba, now works in the laboratory of Peter S. McPherson, PhD, at McGill's Montreal Neurological Institute. Hamlin's research will investigate how Scyl1, a protein recently identified by the laboratory, affects the transportation or trafficking of proteins within cells and relates to motor neuron degeneration.

Hamlin explains that cells need to continuously produce new proteins, which are then transported throughout the cell. This process is especially important in motor neurons or nerve cells, which convey impulses from the central nervous system to muscles or glands. In most cases, proteins are transported in small packets—small, membrane-enclosed sacs—called vesicles, which, says Hamlin, “are the equivalent of transport trucks for proteins.” Because the proteins in motor neurons have to be transported over considerable distances, sometimes as far as three feet, it is important for all the transport machinery to be in good working order.

Many newly synthesized proteins are initially sent to the endoplasmic reticulum (ER), a membrane network within the cytoplasm of cells. “The ER functions as a quality control area and ensures that proteins are properly folded before being released to the rest of the cell. Once a protein has passed quality control, it exits the ER in vesicles and is transported to a structure called the Golgi apparatus, where the proteins are further modified and then sorted to their final cellular destination,” says Hamlin.

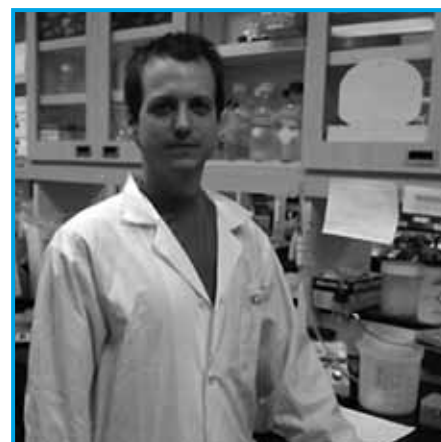
However, sometimes proteins that ought to remain in the ER get transported to the Golgi apparatus. The cell then has to decide whether to move them forward or send them back to the ER. Scyl1 is a recently characterized protein that impacts membrane trafficking. A loss of Scyl1 from a cell disrupts the vesicle transportation of proteins from the Golgi apparatus back to the ER.

“Amazingly,” says Hamlin, “it has been recently demonstrated that loss of Scyl1 is responsible for the mouse neurodegenerative disease model known as ‘mdf.’” Mdf or muscle-deficient mice have a mutation in the Scyl1 gene. At five to six weeks of age, these mice appear slightly smaller than normal and have a waddling gait. They then develop hindlimb paralysis. This discovery regarding Scyl1 provides the first known link between neurodegeneration and the transportation of proteins from the Golgi apparatus to the ER.

The reason why neurons, as opposed to other cells, die when Scyl1 is lost in mdf mice and the mechanisms by which Scyl1 regulates traffic between the Golgi apparatus and the ER have not yet been established.

Hamlin says, “The results from our research will provide information for other scientists studying motor neuron degeneration. It is, of course, funding from ALS Canada that has provided me with the opportunity to focus on this work. This type of basic research is critical to shed light on the many unknown factors that may be underlying the dysfunction in motor neuron diseases such as ALS.”

Promising research like this is opening up new avenues of hope for people living with ALS. [NN](#)



Jason Hamlin

ALS Society of Canada Mission:

The ALS Society of Canada funds research towards a cure for ALS and supports our provincial partners in the provision of quality care for those living with ALS.

ALS Society of Canada Vision:

The vision of the ALS Society of Canada is to find a cure for ALS.

2009 Bernice Ramsay and ALS Canada Discovery Grants



Victor Rafuse, PhD

IN DECEMBER 2009, the ALS Society of Canada awarded the second annual Bernice Ramsay Discovery Grants to two Canadian research projects. ALS Canada also awarded its first ever ALS Canada Discovery Grants to two additional research projects. Each award is a one-time \$100,000 grant for pursuing new and promising directions in ALS research.

The Bernice Ramsay Discovery Grants were awarded to Victor Rafuse, PhD, associate professor at Dalhousie University; and co-investigators Lorne Zinman, MD, medical director of the ALS Clinic at the Sunnybrook Health Sciences Centre and Yana Yunusova,



Lorne Zinman, MD

PhD, associate scientist at Sunnybrook Health Sciences Centre, both assistant professors at the University of Toronto. The ALS Canada Discovery Grants were awarded to Kelvin Jones, PhD, assistant professor at the University of Alberta; and Christopher Shaw, PhD, professor at the University of British Columbia.

The Discovery Grants program was established to support breakthrough research focused on identifying causes of or treatments for ALS. “The spirit of this program is to encourage promising novel research approaches that may be risky in terms of feasibility, but have potential high impact if successful,” says Denise Figlewicz, PhD, vice president research at ALS Canada. “We are excited

to support these groundbreaking projects and are encouraged our research program continues to expand.”

New model for studying sporadic ALS

Rafuse’s research focus is whether induced pluripotent stem (iPS) cells are a viable model system for studying sporadic ALS (SALS) and if they could be used to treat ALS patients in a regenerative medicine approach. Research on SALS is difficult because an appropriate model system is unavailable. The recent discovery that iPS cells can be generated from live biopsies from patients with sporadic ALS, though, offers a possible model, as iPS cells can be differentiated into cell lines having many characteristics of motor neurons. It is possible that this new source of cells could be used to support ALS-damaged neurons.

“This study will examine for the first time whether iPS cell-derived motoneurons develop the same physiological characteristics as embryo-derived motoneurons,” explains Rafuse.

Rafuse will use a series of *in vitro* and *in vivo* studies to critically examine the physiological properties of mouse iPS cell-derived motor neurons compared to mouse embryo-derived motor neurons. He will test whether these derived motor neurons are capable of innervating muscle fibres when transplanted into mice with ALS and whether this capacity is sufficient to recover muscle and motor unit function. He will also test iPS cell-derived motor neurons generated from mice that express mutant superoxide dismutase (mSOD1)—a gene that underlies some cases of familial rather than sporadic ALS, to determine if cells from this mouse model of ALS retain the same susceptibility to SOD1 toxicity as the embryo-derived motor neurons.

New battery of tests may improve our understanding of ALS

Using a newly developed computerized test, Zinman and Yunusova hope to improve our understanding of the cognitive, speech and brain changes that occur in patients with ALS. The ALS Computerized Frontal Battery (ALS-CFB) will probe areas of the brain that have never before been evaluated in subjects with ALS.

The ALS-CFB is short in duration, requires only minimal motor response during testing and can be performed at all phases of the disease. It was developed to measure cognitive function in previously untapped regions of the frontal lobes. Because of its unique design, ALS-CFB has potential to become a new standard for the assessment of cognitive functions in patients with ALS.

Zinman and Yunusova’s study will be the first to examine the link between the profiles of cognitive frontotempo-

ral impairment and correlating neuro-anatomical changes using the ALS-CFB and various imaging techniques. The study also examines the relationship between cognitive deficit and bulbar motor deficit, which have been associated in the past, though the nature of this association remains open.

“I believe it will have a significant impact on our understanding of the neural bases of the changes we see in the motor and cognitive systems of patients with ALS,” says Yunusova. “With new technologies and analysis methods becoming available, we should not miss the opportunity to make a careful examination of the foundations of functional changes with disease progression. Understanding the disease is the first step in conquering it in the future.”

Does exercise slow the progression of familial ALS?



Kelvin Jones, PhD

Jones and his colleagues will study the effects of exercise in slowing the degenerative processes of ALS. The biochemical properties of muscle tissue and the connecting nerves adapt based on changing levels and types of activity.

With Tessa Gor-

don, PhD, professor emeritus at the University of Alberta, Jones discovered in a mouse model of familial ALS (FALS) that motor neurons which innervate slow endurance muscle degrade at a slower rate than motor neurons innervating fast-fatigue resistant and fast-fatigable muscle. Based on this, they will test whether exercise that promotes conversion of muscle to the slow endurance phenotype will have a neuroprotective effect; that is, slow the rate of degradation of motor neurons innervating muscles that are normally fast-fatigable. This work is being done in FALS mice and uses electrical stimulation to exercise the muscle rather than behavioural training such as treadmill running.

Slow motor neurons are used to generate steady forces over long periods of time and are predominant in marathon runners. Conversely, the fast motor neurons predominant in sprinters generate explosive fast and strong forces

over a short period of time. In a mouse model of FALS, the fast-type motor units are the first to be denervated in the presymptomatic phase of the disease, while slow-type motor units become denervated later, coinciding with the onset of overt symptoms. It is hypothesized that if the fast-type motor units can be converted to slow-type, then the progression of degeneration will be slowed. Jones and Gordon will develop an implantable neuromuscular stimulator for the mice and will stimulate the mice for 50 days.

“We predict that the stimulated muscles and their nerves will remain healthy for a longer duration because the exercise will slow the degenerative processes of ALS,” say Jones and Gordon. “This work in mice with inherited ALS will give clinicians and physiologists evidence to inform decisions and serve as the basis for clinical trials of exercise with ALS patients.”

The potential of progranulin

Shaw received an ALS Canada Discovery Grant for his study on progranulin, a neuronal growth factor that may provide a means to treat ALS patients before motor neuron loss becomes irretrievable. Mutations in the gene coding for progranulin, a secreted factor that regulates nerve cell growth, have been shown to cause some neurological diseases. *In vitro* studies have shown that progranulin can prevent neuron cell death following exposure to toxins.

Shaw is testing whether progranulin upregulation can prevent motor neuron death in animal models of ALS.

The study will test progranulin in two animal models. In the first model, a dietary neurotoxin causes a gradual, progressive loss of motor neurons and motor function similar to ALS generally; the second model is a more conventional mSOD1 model.

Progranulin will be administered via a lentivirus delivery system through injections into the gastrocnemius muscle in the legs of the mice.

“Successful outcomes,” Shaw says, “would have major implications for ALS therapy in that they would have provided a means to protect neurons that was both highly selective and yet minimally invasive.” [NN](#)



Chris Shaw, PhD

I am living with ALS not dying of ALS. Remember, life itself is terminal.

— Derek Walton, living with ALS, member of the ALS Canada advocacy committee

First Betty Norman Clinical Fellowship in ALS Research



Kerri Schellenberg, MD

IN 2008, KERRI Schellenberg, MD, was awarded the first Betty Norman Clinical Fellowship in ALS Research. The two-year fellowship focuses on three areas: clinical expertise and leadership, teaching and research.

“There is a serious shortage of ALS specialists in Canada. This fellowship will help ensure doctors are adequately trained to handle the special needs of people with

ALS,” says Denise Figlewicz, PhD, vice president research at the ALS Society of Canada.

The University of Alberta’s ALS multidisciplinary clinic program was established in 2001. The greater part of Schellenberg’s training is taking place at the University of Alberta under Wendy Johnston, MD, a neurologist and associate

professor of neurology. Schellenberg is also participating in monthly clinics at two outreach sites, Misericordia Hospital and Glenrose Rehabilitation Hospital, both in Edmonton.

Schellenberg, a graduate of the University of Saskatchewan’s college of medicine who completed a residency program in neurology at the University of Alberta, expressed her delight on being awarded the fellowship: “Having spent the last six years completing my residency here, I know I will continue to receive high-calibre instruction from Dr. Johnston and her colleagues. I’m looking forward to learning how to provide quality care to people suffering the devastating effects of ALS.”

The fellowship is named for Betty Norman, an active 58-year-old Calgarian diagnosed with ALS in August 1996. Despite her diagnosis, Norman, frustrated by the lack of ALS awareness at that time, was determined to make a difference. Along with family and friends, she organized the first Betty’s Run/Walk for ALS which took place in June 1997—just two weeks before she passed away. Betty’s Run for ALS celebrates, promotes and channels hope for those affected with ALS, their families and their friends. The event has raised more than \$3 million for ALS client services, equipment and research since its inception. [NN](#)

Health Charities Applaud Government of Canada’s Research Investment in Neurological Conditions



Federal Health Minister Leona Aglukkaq with members of the 17 organizations that make up Neurological Health Charities Canada.

IN JUNE 2009, the Government of Canada announced it would provide \$15 million in funding for the first-ever national study on the prevalence and impact of neurological diseases in Canada. This announcement was welcomed by Canadians with

neurological conditions, caregivers and representatives from the Neurological Health Charities Canada (NHCC).

The NHCC, of which ALS Canada is a founding member, is a collection of organizations formed in 2008 to represent Canadians with chronic, often progressive, neurological and neuromuscular diseases, disorders, conditions and injuries.

Experts estimate that more than three million Canadians are currently living with life-altering, and sometimes life-threatening, neurological conditions. These conditions include a group of more than 1,000 diseases, disorders and injuries that affect the brain, spinal cord and nervous system. While there are therapies available for some of these conditions, many of them are progressive and degenerative, with no known cure.

“This boost in funding provides hope to those living with a neurological disorder that research will one day improve their quality of life,” says ALS Canada’s President and CEO David Cameron. “For a disease like ALS that has no effective therapy and no cure, research is our only hope. We are pleased the government is committed to funding this critical research.” [NN](#)

Lithium Trial: Q&A

IN FEBRUARY 2008, Francesco Fornai, PhD, and colleagues at the University of Pisa, Italy, reported in a pilot study that lithium carbonate, used to treat bipolar disorder, taken together with riluzole, showed a positive effect in people with ALS, delaying progression in the early stages of the disease.

To further investigate lithium as a possible treatment for ALS, a randomized, blinded, multicentre trial of lithium with riluzole versus placebo with riluzole was conducted in people with ALS in Canada and the United States. The study used similar dosing to the Italian study.

The ALS Society of Canada played a role in the incorporation of ALS clinicians from 15 centres across the country to form a Canadian consortium for ALS clinical research and trials, the Canadian ALS Clinical Trials and Research Network (CALS). The lithium trial was the first joint effort of CALS and the Northeastern ALS Consortium (NEALS) in the U.S. It was sponsored by ALS Canada, The ALS Association and the National Institute of Neurological Disorders and Stroke (NINDS) of the National Institutes of Health.

An interim analysis was conducted after the enrolment of the 84th subject and presented to the NINDS Data and Safety Monitoring Board in September 2009. Based on the interim analysis, the trial was stopped for futility. This study did not show the same beneficial effect of lithium carbonate on the progression of ALS as the pilot study conducted in Italy had shown.

After the discontinuation of the lithium trial, ALS Canada responded to some frequently asked questions about the trial.

When did the lithium trial start?

The first patients were enrolled in January 2009. There were to be 250 in total: 125 from Canada and 125 from the U.S. The trial was a double-blind, placebo-controlled trial, split into two phases, the first of which was with 84 patients, randomized to take either lithium or placebo. A review of data was to occur after the 84th person was enrolled, and then a decision was to be made on whether to expand to 250 patients. It was after the 84th person was enrolled that the clinical trial was stopped. The data analysis on the interim results of the trial definitively established that we could not replicate the Italian pilot study results.

What were the criteria for enrolling patients?

Patients were included if they were within three years of their diagnosis and not already taking lithium. Treatment was anticipated for up to one year, with disease course and safety assessments measured at regular intervals over that time.

Was the same lithium used in Canada and the U.S. as that used in the original study in Italy?

Yes.

Why did the trial stop?

The drug did not demonstrate the anticipated results at the planned review point.

How were patients told about this?

They received calls from their local ALS clinic. In October 2009, there was a patient teleconference, for patients only, in English and French. This was an opportunity for patients to ask the doctors questions. Also, an announcement about the trial was posted on the ALS Canada and The ALS Association web sites.

Why didn't the drug work?

We do not know this and will not know until the data is available.

Is this a total failure?

This is a disappointment, but we are strongly committed to finding ways to fight this devastating disease.

What can we be proud of?

ALS Canada helped ALS doctors across Canada incorporate into a group of clinical trials specialists—CALC. The trial design was novel and was developed under the leadership of Lorne Zinman, MD, medical director of the ALS Clinic at the Sunnybrook Health Sciences Centre in Toronto. The clinics were able to enrol patients within one year of the first CALC meeting. Also, our patients/clients know that we are responding to their requests to have access to clinical trials. In the past they had to travel to the U.S.

What do we do now? What are the next steps?

CALS is ready to conduct more trials in Canada with other promising drugs.

What is the good news?

While the outcome is extremely disappointing, we now have a strong clinical trials network in CALC, and it has proven its effectiveness and efficiency in arriving at a clinical determination over a short period of time.

Denise Figlewicz, vice president research at ALS Canada, explains further: “We welcomed this opportunity to work together with our American colleagues. This collaborative approach between Canada and the United States will serve as a model for subsequent clinical trials.” [NM](#)

Milton Safenowitz Fellowship Awarded to University of Toronto Researcher

IN 2008, TERESA Sanelli, PhD, received The ALS Association's Milton Safenowitz post-doctoral fellowship (awarded



Teresa Sanelli, PhD

over two years) for ALS Research, the only one of its kind in the United States. The fellowship is awarded annually to encourage young researchers to pursue ALS-related research. Established in 2004 in memory of Milton Safenowitz, who died of ALS in 1998, the award gives post-doctoral students the opportunity to work closely with a principal researcher in the field of ALS research.

Sanelli is a researcher at the Centre for Research in Neurodegenerative Diseases at the University of Toronto (U of T). Under the guidance of Janice Robertson, PhD, who holds the Canada Research Chair in Molecular Mechanisms of ALS

and is an associate professor at U of T, Sanelli is investigating the role of TDP-43 in ALS. She is also working with ALS experts such as Lorne Zinman, MD, medical director of the ALS Clinic at the Sunnybrook Health Sciences Centre in Toronto.

TDP-43 is a protein normally found in motor neurons (cells in the brain and spinal cord that control muscle movements through electrical impulses). Protein inclusions, also referred to as aggregates or clumps, are known to be present in the motor neurons of people with both the sporadic and familial forms of ALS (SALS and FALS). Researchers have confirmed that TDP-43 is a major component of these inclusions and that it is linked to both SALS and FALS.

Sanelli's study is investigating what the normal function of TDP-43 is, and why it forms inclusions. Apart from the recent discovery of mutations in the gene encoding TDP-43, the protein's relationship to ALS is little understood.

Sanelli welcomes the opportunity to "tackle the role of TDP-43, which was recently discovered as a potentially important protein in ALS, in motor neurons" and believes her study may make "very important strides towards a cure for the majority of people with ALS—those with the sporadic form." [NN](#)

WALK for ALS



The WALK for ALS is the ALS Society of Canada's national signature fundraising and public awareness event. Each Walk is organized by local volunteers; in 2009, this annual event was supported by more than 16,000 participants with another 155,000 individual pledges. Most of the Walks take place in June, to coincide with ALS awareness month.

The Walk is an important source of funding for research into ALS and for the provision of quality care for people living with ALS. The Walks have generated more than \$15 million since they began in 2001 in eight locations. In 2009, the 70 Walks across the country raised more than \$2.5 million.



Tim E. Noël Fellowship: Recipient Hopes for Earlier Diagnosis and Treatment of ALS

TIM E. NOËL was deputy governor of the Bank of Canada when he was diagnosed with ALS in 1999. His philosophy of life was simple: “Today is a gift. That’s why they call it the present. And you should never let the thieves of yesterday or tomorrow rob you of that gift.” And Noël applied this philosophy to his own life. He continued to work full time, coming in each day with a ventilator and wheelchair, until his death in 2001.

To honour Noël, the ALS Society of Canada established the Tim E. Noël Fellowship in ALS Research in 2006. The fellowship is a partnership between ALS Canada and the Canadian Institutes of Health Research’s Institute of Neurosciences, Mental Health and Addiction; it recognizes and supports post-doctoral ALS researchers by awarding each recipient up to \$55,000 annually for a maximum of three years.

The aim of the fellowship is to encourage young scientists to pursue ALS research. With the award recipients’ passion and commitment to ALS research, our knowledge of ALS is significantly advancing.

Beibei Zhao is the recipient of the 2009 Tim E. Noël Fellowship. A post-doctoral fellow at the University of Toronto working in the laboratory of Janice Robertson, PhD, Zhao is investigating the types and amounts of protein created by motor neuron cells. This information is called a “translational profile.”

Zhao’s project will use new technology called translating ribosome affinity purification (TRAP), along with techniques such as microarray and bioinformatics, to reveal the specific translational changes occurring in the spinal cord motor neurons of SOD1 mice. TRAP isolates genetic messages as they pass through ribosomes, the parts of the cell that produce protein.

Zhao hopes that her work will lead to the discovery of more biomarkers for ALS, allowing for earlier diagnosis and treatment.

“There is an average 12-month delay of diagnosis from onset of symptoms. This means that a large proportion of motor neurons have already died by the time symptoms are evident and diagnosis is confirmed,” says Zhao.

Stimulated by the process of developing ideas, designing experiments and finding answers, Zhao loves the freedom and excitement of exploration through research.

“Everyone loves the feeling of getting good results and discovering something new, especially after trying for a long time,” says Zhao.

As a researcher she is encouraged by the support she is receiving from the Tim E. Noël Fellowship. She also appreciates that fellowships like these help to provide hope for those living with this devastating disease.

“The Tim E. Noël Fellowship has strengthened my decision to pursue research in ALS over other fields in neuroscience as my career,” says Zhao. “For those who are living with ALS, knowing that there are people making an effort to help them is tremendous mental and spiritual support.” [NN](#)



Beibei Zhao, PhD

What is ALS?

Amyotrophic Lateral Sclerosis, or ALS, is a devastating neuromuscular disease. ALS is sometimes called Lou Gehrig’s disease after the legendary New York Yankees first baseman, Lou Gehrig, who lost his battle with ALS at age 38. Death typically occurs from respiratory failure within two to five years of diagnosis. To date, there is no known cure yet. Research is our only hope.

ALS Facts

- Approximately 2,500–3,000 Canadians over the age of 18 currently live with ALS
- ALS affects the entire family
- ALS is a costly disease — emotionally, physically and financially
- ALS can strike anyone at any time, regardless of age, sex or ethnic origin

Dementia in ALS: Progress Reported on Many Fronts

ONCE THOUGHT TO be a disease of movement, ALS is now also recognized as having cognitive and behavioural effects.

One in two ALS patients exhibits cognitive change, ranging from mild impairment to full-on frontotemporal dementia (FTD), fundamentally impacting how clinicians diagnose and treat the disease, and how researchers study its pathology.

The Third International Research Workshop on Frontotemporal Dementia in ALS, which ran from June 21 to 25, 2009, in London, Ontario, was the forum for discussions surrounding two important questions: How can clinicians reliably detect cognitive change in patients with limited motor function; and how can scientists exploit the overlap of ALS and FTD to understand the underlying biology?

The meeting opened with a backward look. Thomas Bak, MD, of the University of Edinburgh offered the audience some historical perspective with a reminder that reports from as early as 1892 noted cognitive, emotional and personality changes associated with ALS. His review emphasized the point that FTD in ALS is not a new phenomenon, but, rather, one that is difficult for experts in motor neuron disease to detect.

In today's clinics, better detection comes down to better tools.

The first half of the meeting was dedicated to identifying clinical measures of cognitive and behavioural change in ALS.

In her presentation on speech and language deficits in ALS patients with cognitive impairment, Argye Hillis, MD, a neurologist at Johns Hopkins Hospital, reported an association between ALS and "non-fluent primary progressive aphasia," a type of dementia in which patients have difficulty with verb naming and comprehension.

Jonathan Katz, MD, a neurologist at the California Pacific Medical Center, presented ongoing research into the detection of eye movement abnormalities in ALS patients with cognitive deficits. In his study, "ocular apraxia," marked by the inability to maintain smooth pursuit of an object with the eyes, was associated with bulbar onset and cognitive and behavioural change.

Efforts to develop sensitive, reliable screening tests for cognitive change that are fast and easy to administer during a routine visit to the clinic were also reported.

The clinical session wrapped up with a discussion of the consequences of cognitive change in ALS for both patients and caretakers.

"It's another aspect of the disease that affects how patients interact with their spouses and caretakers," said Michael Strong, MD, meeting organizer and clinician-scientist at University Hospital in London, Ontario. "We have to be able to explain that to people."

Moving from the bedside to the laboratory bench, the second half of the meeting focused on genes and proteins.

The identification of TAR DNA-binding protein of 43 kilodaltons (TDP-43) in neuronal aggregates in frontotemporal lobar degeneration (FTLD) and ALS by Virginia Lee, PhD, and her colleagues at the University of Pennsylvania in 2006, was a major breakthrough in ALS research. Since then, mutations in the genes encoding TDP-43 and a similar protein, fused in sarcoma (FUS), have been identified in ALS patients.

The importance of TDP-43 in a variety of cellular functions was discussed in a session dedicated to experimental modelling.

David Morton, PhD, of Queen's University in Kingston, Ontario, used a fruit fly model to show that eliminating TDP-43 expression is lethal in the pupal stage, suggesting that the protein plays an important role in development. But he went on to show that too much TDP-43 expression can be just as deadly.

In species ranging from worms to rats, TDP-43 mutations led to neuronal aggregate formation, neurodegeneration, motor dysfunction and death.

Strong and Kathryn Volkening, PhD, of the Robarts Research Institute in London, Ontario, offered a possible role for TDP-43 in the neurodegenerative process relating to its ability to interact with genes regulating the expression of other cellular proteins. FUS has a similar ability.

Ian Mackenzie, MD, a neuropathologist at the University of British Columbia, presented new histological evidence for FUS pathology in FTLD with ubiquitin-positive inclusions (FTLP-U).

The observation of abnormal TDP-43 and FUS protein aggregates in cases of both ALS and FTD suggests that the two diseases are closely linked. Strong is optimistic that there is shared biology between them and that uncovering those shared pathways could really move research forward.

"Because ALS is so rare, if you can find another disease that overlaps, one where the biology is more understood, then you're ahead of the game," Strong said.

The co-existence of motor neuron degeneration in the cortex of the brain, and cognitive decline, raises another interesting question: Could the degenerative process in ALS spread from the part of the brain controlling movement to adjacent parts involved in cognition and behaviour?

John Ravits, MD, neurologist at the Benaroya Research Institute at the Virginia Mason Medical Center in Seattle, Washington, provided evidence for disease spread between cortical motor neurons in the brain's motor cortex and motor

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Kinga Njilas: Fighting the War against ALS on Two Fronts

The war against ALS is being fought on two fronts: the first is on the home front where families, caregivers and health-care personnel battle to provide care for those stricken with the devastating disease; the other is in the laboratory where researchers use every weapon in their armoury to find a cure for the disease that claims the lives of more than 2,500 Canadians each year.

Kinga Njilas, 25, of Guelph, Ontario, has been fighting this war since 2006, when her mother, Erika Njilas Lincmajer, was diagnosed with ALS. The war started quietly enough, as such struggles often do. So quietly, in fact, that Njilas did not even know it had started.

"I was sitting in the computer lab watching the clock slowly tick away. My mom finally messaged me and told me they know what's wrong. She has ALS. The first thoughts that came to my mind were: 'Thank God, finally they know what's wrong and now they can fix her.'"

Njilas was not left in the dark for long: "Not knowing what ALS was, I decided to Google it right there and then. That probably was the worst thing I could have done. I went on the ALS Society web site and found out that ALS is a terminal disease with most patients living to a maximum of two to five years. The tears started to well up in my eyes, I was absolutely devastated."

And so the battle began. It quickly escalated, and Njilas realized that the fight was not her mother's alone. "When my mom was first diagnosed with ALS," says Njilas, "it was okay to leave her alone at night. But as ALS slowly started taking over her body, my mom's life changed. And so did mine. I went from getting eight hours of sleep in my own bed to four, maybe six hours, in my parents' bed beside my mom. ALS is a disease that not only affects the person with it. If it sounds like a lot of work, it is."

Njilas, like other Canadians whose loved ones have been stricken with this horrific disease, turned to the ALS Society of Canada for information and assistance. One of the aims of ALS Canada is to serve as a resource for the general public. In addition, the national body supports the provincial societies in their provision of quality care to persons living with ALS. The other — critical — part of their mission is to support research, to help find a cure for ALS.

On the research front of the war against ALS, ALS Canada plays a vital role. "Finding a cure is the only way to stop the pain and devastation caused by ALS," says David Cameron, president and CEO of ALS Canada. "Our comprehensive research programs and collaborations with national and international partners position us well for realizing this vision."

The research programs of ALS Canada focus on identifying disease pathways and exploring the causes and treatment of ALS. Canadian scientists funded by ALS Canada are currently studying cell mechanisms and pathways; genetic, environmental and immunological factors in disease; therapeutic proteins; and biomarkers or biological indicators that may help to diagnose or monitor the progression of the disease.

Njilas soon realized the importance of ALS Canada's mission. "ALS Canada is doing everything it can to fight the disease that's taking away my mother. The Society is funding research that I hope will one day make ALS disappear. Research also gives hope to patients and their loved ones — hope that one day, people will be able to live with ALS and have a great quality of life.

With research comes this glimmering light at the end of the tunnel!"

From 2006, Njilas channelled her energies into raising public awareness for ALS and funds for ALS research. Her efforts have included participating in the annual WALK for ALS and, for the past two years, arranging a soccer tournament in Guelph, called Cup for a Cure, in honour of her mother. Additionally, her inspirational story "My life with ALS" was used by ALS Canada in its direct mail program. So far, this initiative has raised more than \$40,000.

In 2009, Njilas went one step further: she joined the battle being waged in the research laboratories. After finishing her post-graduate studies in clinical research at Humber College in Toronto, she started working as research co-ordinator at the Bulbar Function Laboratory of Sunnybrook Health Sciences Centre in Toronto. She is participating in a study focusing on bulbar function, that is, speech and swallowing, in ALS patients.

"In Flanders fields the poppies blow between the crosses row on row." Since Canadian poet and military doctor John McCrae penned those iconic words more than 90 years ago, the poppy has become the symbol of remembrance for the lives lost in conflicts around the world. In the more private war against ALS, the blue cornflower stands as the emblem of the courage and tenacity of those suffering from ALS, those who care for them and those who fight for them. And when the final chapter is written, it will be shown that it was in the laboratory, through the efforts of researchers dedicated to the cause, that the war was won.

Erika Njilas Lincmajer passed away on January 29, 2010.



Kinga Njilas, with her mom, Erika

neurons in the spinal cord's ventral horn—a disease feature that, he said, provides a unique research opportunity.

“Cutting-edge molecular and genomics approaches can be directed to less affected regions to study early to moderately advanced stages of degeneration,” Ravits reported in the manuscript he presented at the meeting, which was recently published in *Neurology*.

The cortical motor neurons degenerating in ALS are close neighbours to those that become sick in FTD.

Denise Figlewicz, PhD, vice president research at the ALS Society of Canada, stressed the importance of studying the cortex—an area of the nervous system that, she said, has largely been ignored because of technical challenges.

“Mechanistically, I would like to know if the ‘spread’ of degeneration from the primary motor strip to adjacent cortical areas is based on the same process [such as inflammation] which is now being aggressively studied in the spinal cord,” Figlewicz said.

Efforts to identify disease biomarkers in the cerebrospinal fluid and saliva were also discussed.

Since its debut in 2005, the biennial meeting has become the forum for clinicians and scientists to discuss their newest data on ALS and FTD. This year's meeting of nearly 100 attendees boasted a record 49 international speakers hailing from eight countries around the world.

But the importance of the workshop is summed up in a different number—more than 50 per cent of patients show signs of cognitive change.

Despite the challenges faced by ALS patients with cognitive decline and their caregivers, there is hope that the unfortunate overlap of ALS and FTD will provide important biological clues that will enhance the understanding of disease processes.

The meeting was sponsored by ALS Canada, Windsor-Essex County ALS Society, The ALS Association through the support of the Linden Foundation in memory of Suzanne V.A. Kelsey, and the Michael Halls Endowment Fund. The next workshop will be held in June 2011. [NN](#)

The more people we can reach, the better off we are. [We need to] raise the flag of ALS.

— Ray Turner (deceased June 2009), presented with the Myra Rosenfeld Volunteer Award in 2009 for his contributions to ALS Canada.

Adult Stem Cell Research Showing Promise

RESEARCHERS ACROSS CANADA are forging ahead with work on stem cells in the quest to learn more about ALS and to develop new treatments. At the 2009 ALS Research Forum, researchers described progress in work using stem cells, ranging from the participation of ALS researchers in a nation-wide stem cell network to the development of new culture-based models that can be used to study motor neurons in a more intimate fashion. Scientists and clinicians partici-



Jean-Pierre Julien, PhD

pated in a general discussion about research opportunities and directions.

Of interest to several researchers at the conference is the use of induced pluripotent stem (iPS) cells. These are a type of stem cell developed from adult tissue samples. They are important in stem cell research because they have many of the same behaviours as embryonic stem cells and can be derived from adults, according to Jean-Pierre Julien, PhD, professor of anatomy and physiology at the Centre hospitalier de l'Université Laval (CHUL) Research Centre, Québec.

Stem cells are non-specialized cells that can divide and turn into specialized cells such as bone, nerve or skin cells. They occur in different areas in the human body, such as the skin, bone marrow, brain and eyes, and are present to help with tissue repair. Stem cells in adults generally only turn into certain types of cells—for instance stem cells

Consortia and Networks

Efforts are being made to create a consortium for research on induced pluripotent stem (iPS) cells from ALS patients. The idea is to allow researchers from multiple laboratories to share ideas and scientific information in this exciting new field, said Jean-Pierre Julien, PhD, of Laval University.

Currently, three laboratories at Laval University have agreed to participate in a consortium, and initial funding has come from the Fondation André-Delambre. Eventually the project will grow to include the participation of laboratories across Canada.

The Stem Cell Network (SCN) promotes and supports stem cell research in Canada, with work on iPS cells being one of the many areas included in its mandate. The SCN supports projects from researchers who are eligible to receive grants from the Canadian Institutes of Health Research, the Natural Sciences and Engineering Research Council of Canada and the Social Sciences and Humanities Research Council. The network is also welcoming partnership proposals from

other potential sponsors, according to Knut Woltjen, PhD, of Mt. Sinai Hospital.

Then there is the newly formed Ontario iPS Cell Facility. William Stanford, PhD, of the University of Toronto, is co-scientific director of the facility. He explained that one of the goals is to establish a bank of patient or disease-specific primary fibroblast cell lines. The cell lines would be available to researchers across the country. The facility also plans to foster research focusing on ways to develop iPS cell lines as safely as possible, and to develop technologies and skills in the field.

At the 2009 ALS Research Forum, all these parties were brought together and the discussion of research opportunities and directions was thrown open to the community of ALS scientists and clinicians. The ALS Society of Canada believes that it may play a role in partnerships with the groups described here and with others that are interested in this particular research approach.

from the bone marrow turn into either bone, cartilage, fat, fibrous tissue or blood cells.

Traditionally, embryonic stem cells have been of interest because they are not limited like adult stem cells and are capable of turning into any type of cell. However, in 2007, researchers discovered a way to turn adult cells into a pluripotent form (iPS) that has many of the same features as embryonic cells.

Julien is at the forefront of researchers who are using iPS cells to help unravel the causes of ALS and the mechanics of how motor neurons become susceptible to the disease. There is an opportunity to use iPS technology to investigate the biological defects associated with ALS, especially the sporadic form (SALS) where there are so many unknowns, he said.

Researchers are planning to take samples of skin tissue from people with ALS, use the skin cells to create iPS cell lines and then nudge those cells to turn into motor neurons. “Studies on iPS cell-derived motor neurons might lead to the discovery of biological defects associated with ALS,” said Julien.

Researchers will study genetic components of the motor neurons in culture and compare them to iPS cell-derived motor neurons from people without ALS. Cells derived from people with SALS may reveal certain characteristics, possibly even genetic, to explain why the disease occurs.

It is not known exactly what results will be obtained from the research. “If we don’t do the experiments, we’ll never know,” Julien said.

The mechanics of how skin cells (specifically fibroblasts) can be turned, or reprogrammed, into iPS cells was described by Ana Sofia Correia, PhD, post-doctoral fellow at CHUL. The work, she noted, was carried out in conjunction with two other laboratories at Laval.

Correia explained that there are four genes, *c-Myc*, *KLF4*, *Oct4*, and *SOX2*, which are known to trigger reprogramming in fibroblasts. In upcoming research, the four genes will be delivered into fibroblasts using small DNA vectors called plasmids that will carry the genes into the fibroblasts. Along with the genes will be a marker to allow researchers to track what is going on inside the cells.

Once fibroblasts have been reprogrammed to turn into iPS cell lines, researchers will follow protocols known to trigger iPS cell lines to turn into motor neurons. Studying the development of motor neurons derived from people with SALS and healthy controls could reveal new ways of understanding the disease, Correia said.

François Berthod, PhD, associate provost of research at Laval University, also spoke at the conference. Work in his laboratory shows that neural precursors derived from the skin can not only be turned into working neurons, but they can also differentiate into Schwann cells—a type of cell that helps maintain the health of nerve fibres.

“We can generate motor neurons and Schwann cells from the same skin biopsy,” he said. The work shows

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François Berthod, PhD

promise for the development of an *in vitro* model of human disease using a patient's own cells. It can also help with the development of new therapeutic approaches to repair the nervous system, he said.

Embryonic cells

Because iPS technology is so new, there are still uncertainties about what it can do. For one thing, it is not known at this

point whether motor neurons generated from this technology are a specific type or some sort of generic or basic motor neuron, said Victor Rafuse, PhD, associate professor of anatomy and neurobiology at Dalhousie University. In fact, while it has been proven that motor neurons can be developed from embryonic stem (ES) cells, it is not known if they are a specific type or not.

"Not all motor neurons are created equal," said Rafuse. There are subtle differences in the activities of motor neurons in different parts of the body. For instance, motor

neurons that innervate, or supply with nerves, big muscles in the leg have a different regional environment and have certain characteristics that differ from those which innervate the chest or arms.

Rafuse and colleagues performed a series of animal studies on mice and unhatched chicks to learn more about the features of motor neurons that are developed in the laboratory from embryonic cells. In one series of studies, researchers directed ES cells to become motor neurons.

The neurons were injected into the spine of developing chicken embryos to see what types of cells they would grow into as the chick developed. To observe this, the researchers also attached a chemical marker to the motor neurons so they would be able to track where the cells went.

The ES-cell-derived motor neurons all moved to the axial muscles—the muscles closest to the skeleton. The research "suggests very strongly that we're making a very specific sub-type of motoneurons; it's not just generic motoneurons," Rafuse said. More recent research in his laboratory has found specific chemical signals that cause the ES motor neurons to travel and grow in the axial muscle locations.

"In my opinion, the embryonic stem cell motor neurons have molecular and cellular mechanisms that are very similar to the endogenous phenotype," said Rafuse, which means that they behave the same way as normal motor neurons.

The next step is to do similar studies with iPS cell lines to confirm that they, in turn, really do have the same characteristics as the ES-cell-derived motor neurons. [NN](#)

Alzheimer Drug Being Tested in ALS



Sanjay Kalra, MD

A TRIAL AT the University of Alberta is investigating the effectiveness of a drug normally used to treat Alzheimer's disease. An update was provided at the 2009 ALS Research Forum by Sanjay Kalra, MD, associate professor of neurology and co-director of the ALS clinic at the University of Alberta. Memantine is a drug known to help reduce the activity of glutamate, an essential brain chemical that, in excess amounts, causes damage to neurons.

It is hoped that memantine will slow functional decline and the speed of motor neuron loss, said Kalra. A

phase-II, randomized, placebo-controlled, dose-ranging trial was launched in spring 2007. The criteria for patients to be included in the study is that they are between 18 and 80 years of age, they have had ALS symptoms (sporadic or familial) for less than three years, and they have a forced vital capacity (FVC) of 60 per cent or greater. They cannot be participating in other trials testing other drugs.

In the study, patients are randomized to one of two doses of memantine: five or 10 milligrams daily, to determine if one dose is better than the other. Researchers wish to enrol a total of 42 patients.

To date, 20 people with ALS have been enrolled, and of these, 12 have completed the study. It is too early to say whether the drug is having a beneficial effect on patients or not, Kalra said. Twelve is too small a number for meaningful statistical analysis. So far, no adverse effects from the drug have been seen, but neither is there any clear evidence of efficacy.

The trial continues. [NN](#)

Awareness of this disease needs to be promoted and research continued. A cure is there somewhere — it just needs to be found.

— Clayton Smailes (deceased July 2009)

Blocking Death Protein Could Slow Motor Neuron Death

RESEARCHERS HAVE FOUND a way to inhibit the death of motor neurons growing in culture by blocking the activity of BNIP3—one of a set of “death proteins” that lead to cell death. But this finding addresses only one small part in the complex series of events that lead to the demise of mo-



Jiming Kong, MD, PhD

tor neurons in ALS, and it is not known how well the finding applies to actual human disease.

However, the finding could lead to a potential target for future therapies, according to Jiming Kong, MD, PhD, associate professor of human anatomy

and cell science at the University of Manitoba. The research contributes to a greater understanding of the cascade of events involved in motor neuron death in ALS.

At the 2009 ALS Research Forum, Kong described various aspects of work in his laboratory investigating the causes of motor neuron death in ALS, including details of new findings related to the BNIP3 protein.

In a healthy cell system, the BNIP3 protein is not detectable in most of the cells, including neurons. When a cell begins to die, the BNIP3 gene is turned on and produces greater amounts of the protein. In fact, in mutant SOD1 mice (which develop ALS as they age), motor neu-

rons take on properties that trigger the production of larger amounts of BNIP3 in the cell.

The BNIP3 protein works by causing damage to a cell’s mitochondria—the part of a cell responsible not only for creating the energy it needs to survive, but also for the regulation of cell death programs.

Previous work in Kong’s laboratory showed that the amount of BNIP3 is unusually high in mouse models of ALS, making it of interest to study.

Indeed, as more degeneration of neurons occurs in mutant SOD1 mice, larger amounts of BNIP3 are produced, contributing to disease progression.

The researchers are now working on ways to block BNIP3 from coming into contact with the mitochondria. If the contact can be blocked, then the function of BNIP3 may be abolished. This would be done by using a compound that can get to the inside of motor neurons where it would interrupt the BNIP3 pathway or activity. Indeed, researchers have found one such compound, necrostatin-1, that has shown some success in this process.

“Eventually, we want something that can be used clinically,” Kong said. Researchers found that if they introduced necrostatin-1 into motor neurons in culture, the compound inhibited BNIP3-mediated cell death and stopped BNIP3 from interacting with the mitochondria. This could be one way of slowing or stopping motor neuron death in ALS.

Other areas of research in Kong’s laboratory include investigating how oxidative stress activates the BNIP3 pathway. Already it is known that oxidative stress contributes to the triggering of BNIP3 protein production. Researchers are still studying the role oxidative stress plays in ALS, and finding ways to alter it. [NM](#)

Coffee and ALS

COFFEE IS A powerful nutritional antioxidant, but can this feature (or anything else about this common beverage) offer any protection against the ravages of ALS? This is the question researchers from York University attempted to answer in a study using G93A transgenic mutant mice — genetically altered mice that are susceptible to developing ALS.

Intriguingly, the answers were mixed. Daily coffee consumption appeared to provide some advantages in male mice, but caffeine supplements were harmful to female mice. Findings were presented at the 2009 ALS Research Forum by Rajini Seevaratnam, MSc, of the school of kinesiology and health science at York University's faculty of health. She is part of a research team in the laboratory of Mazen Hamadeh, PhD. The work was done in collaboration with researchers at McMaster University.

Coffee was of interest because it is known to contain powerful antioxidants, including caffeine and chlorogenic acid. In fact, the beverage has more antioxidant capacity than either cocoa or black, green, or herbal tea, Seevaratnam said. As well, coffee has been shown to have some minor protective effects in Alzheimer's and Parkinson's diseases and other related conditions.

Oxidative stress is one factor known to contribute to motor neuron damage in ALS. Researchers are interested in knowing how dietary factors may contribute to the development and progression of the disease, Seevaratnam said.



Coffee beans

In the study, researchers took 51 G93A mice (G93A is one of the mutations within the SOD1 gene leading to ALS) and put them into four main groups: mice that were given daily doses of coffee; mice that had caffeine added to their diet; mice given chlorogenic acid; and control mice. There were similar numbers of male and female mice in each group, and the coffee-related compounds were added to their food.

The chlorogenic acid and caffeine groups were included to determine whether either of these antioxidant components

alone had an effect on ALS in the mice. Mice in the control group were fed a standard diet of mouse chow and received none of the coffee-derived extracts. The mice given coffee, caffeine or chlorogenic acid supplements



Rajini Seevaratnam, MSc

were given amounts equivalent to what they would have had if they had consumed five to 10 mouse-sized cups of coffee per day, depending on how much they ate.

The mice were given coffee or coffee-related supplements starting from 40 days of age. As they matured, they were observed for signs of ALS and underwent periodic measurements to study function and mobility. At 108 days of age, the mice were sacrificed so researchers could study their brains to better understand disease progression and the amount of oxidative stress on the cells.

Researchers found differences between some of the different groups of mice. Coffee led to less oxidative stress in both male and female mice, which in itself is a good sign, Seevaratnam said. Male mice in the coffee group had increased levels of enzymes that act as antioxidants, decreased inflammation in the brain (which occurs as a result of disease), and a decrease in cell death. These protective effects were not observed in the female mice, which suggested that coffee has a protective effect in males only.

Caffeine in the female mice led to a decrease in the antioxidant enzymes, increased inflammation, and increased cell death. The opposite effects were seen in males, showing that caffeine alone may have a protective effect in males but a detrimental effect in females.

When it came to function and outward signs of ALS, all the mice showed disease progression with age. However, male mice fed coffee had slower disease progression with approximately 8 per cent longer lifespan after disease onset in these mice, she said.

At this point, it is not known whether the effects would translate into a similar story with human subjects, Seevaratnam said. But, she noted, for people who are healthy, drinking coffee should not be a problem. [NW](#)

Diet and Neurotoxins

MICE PREDISPOSED TO ALS that are fed a diet which includes a chemical derived from cycad seed flour develop more severe cases of ALS than mice that are not fed the ingredient. This was the finding in a poster presented at the 2009 ALS Research Forum that investigated the interplay between genetic susceptibility to disease and exposure to a known neurotoxin in food. The poster was presented by Grace Lee, a PhD candidate in experimental medicine at the University of British Columbia (UBC).

Until the 1960s, cycad seed flour was a common part of the diet of a population that lives on the island of Guam in the South Pacific. It is of interest to researchers because the native population in Guam had unusually high rates of ALS or Parkinson dementia complex (PDC). The flour is made from the seeds of the *Cycas micronesica*, a plant that resembles a palm tree, and is used in breads and other foods the people there once ate. A number of neurotoxins have been identified in the flour, making researchers suspect it may actually play a role in the development of ALS in the local population of Guam.

Cycad flour contains steryl glucosides, which have been shown to be toxic to motor neurons. In fact, researchers at UBC had previously found that when steryl glucoside was added to the normal diet of healthy mice, the mice went on to develop an ALS-like disease or a form of Parkinsonism.

In this most recent study, “our goal was to determine whether this environmental component of a dietary ingredient worked in combination with a genetic predisposition to ALS to worsen or somehow change the course of disease,” Lee said.

A study was performed with four groups of mice: two groups of G37-SOD1 mice, which are known to develop ALS (they develop the disease at 11 to 12 months of age), and two groups of wild-type mice, which do not have any of the mutant genes that cause ALS. The two types of mice were divided into two further groups: one that was fed

a normal diet of mouse pellets, and the second that had measured amounts of steryl glucosides added to their food.

The amount of steryl glucoside added to the food was similar, in proportion, to the amount the people of Guam would have had in their normal diet. If one assumes people eat three or four slices of bread a day made from cycad flour as part of their overall diet, that is about the amount, in mouse terms, that the mice ate daily, Lee said. The mice started their experimental diet at approximately six months of age.

As the mice aged, some behavioural changes in the G37-SOD1 mice fed steryl glucosides suggested an accelerated progression of ALS. When histological studies (microscopic studies of cells) of brain and spinal cord tissue were carried out, it was found that the mice fed steryl glucosides had greater signs of disease than SOD1 mice not fed steryl glucosides and wild-type mice, whether they were fed steryl glucosides or not.

These findings suggest that in SOD1 mice, a dietary exposure to this particular neurotoxin can indeed work with a genetic predisposition to make the disease worse, said Lee. The wild-type mice fed steryl glucosides also developed ALS or PDC after approximately 10 weeks of exposure. The researchers will continue their efforts to better understand the interaction between environmental and dietary factors and genetic susceptibility to motor neuron disease. [NN](#)



Grace Lee

I made a conscious decision not to hide my condition, but rather to disclose it to one and all in an attempt to build awareness... It's the one way to give this devastating disease a purpose.

— Sidney Valo (deceased December 2008), former member of the board of directors of ALS Canada. The annual ALS Canada golf tournament is held in his memory.

Doctor Is in the House

RESEARCHERS HAVE DEVELOPED a prototype kit that would allow people with ALS to undergo measurements of strength and mobility at home. At the 2009 ALS Research Forum, details of a prototype home monitoring device and tool kit were described by Chris McGibbon, PhD, professor and research chair of rehabilitation biomechanics at the Institute of Biomedical Engineering (IBME) and the Chronic Illness Research Institute of the University of New Brunswick.

For many people with ALS, simply getting to clinics where measurements are normally performed is a challenge. Some people in remote parts of the country cannot get to far-off facilities, while even those who live in urban centres where there are ALS clinics face obstacles, especially as their mobility decreases. There is no doubt that there is a need for monitoring devices for home use, said McGibbon.

In 2004, IBME received funding from the Atlantic Innovation Fund specifically for the development of wearable technologies with built-in sensors that could help with rehabilitation services.

“What we wanted to do was develop something that patients could use themselves, was inexpensive as it used existing technology, and was able to provide accurate and reliable measurements,” McGibbon said. The information from the tool kit would be used by medical staff to assess a patient’s condition. The project has been dubbed “Doctor is in the House.”



Prototype devices for measuring strength and mobility, from the “Doctor is in the House” kit

A home monitoring device could be used to measure the progress of disease, enable people to participate in clinical trials, and help doctors monitor the effectiveness of therapies.

McGibbon described the various engineering and technical steps involved in designing a home monitoring device, including figuring out what the size and shape of

the device would be and how it would be worn, working out what technology would be used, and determining what could be measured with it. He also described the steps involved in the testing of a prototype in the home and in long-term care environments.

A prototype consisting of several devices was developed. The first device, worn on the user’s arm, measures elbow flexion-extension strength. It uses a plastic orthopaedic immobilization cast for the elbow, with a force-measuring sensor in place of the coupling that normally fixes the cast in position and a wire connecting it to a data-collection device. Another device has numerous built-in fibre-optic sensors that can sense changes in position; it is used to measure the range of motion of the elbow.



Chris McGibbon, PhD

Another part of the kit is a handgrip device that users hold and squeeze to measure grip strength. There is a sleeve and handgrip that transmit data from the sensors to a palm-sized hand-held device that can be plugged into a computer. The accompanying software analyzes data from the devices.

Prototype devices are being tested on both healthy subjects and ALS patients, who were given the kit to use at home for a few months. Tests with healthy subjects will help the researchers further assess and refine the devices, while tests with ALS patients will help determine whether the devices are sensitive enough to detect changes in muscle impairment over the long term.

Researchers will evaluate whether the devices continue to work accurately, whether they are sturdy enough and whether they can be easily used by ALS patients and caregivers. So far, tests look promising, said McGibbon.

“What we think we’ve got here is a way that we can acquire clinically relevant measures of function and impairment in patients’ homes that can give reasonably accurate measurements—we know it’s reliable,” McGibbon said. But there are still some design issues to be worked out, such as finding a material that adheres better to the skin.

The researchers are also starting to work on a device that can be used to measure the function and strength of the knee and leg. [MN](#)

Excess Iron May Lead to Cell Damage in ALS

WORKING WITH A traditional mouse model of ALS, McGill researchers have developed a greater understanding of the role iron plays in motor neuron–related damage in ALS. Generally, iron is essential to life and it plays various roles in maintaining the health of cells throughout the body, said Samuel David, PhD, professor in the department of neurology and neurosurgery at the McGill University Health Centre, at the 2009 ALS Research Forum. But, as with many things, an excess, or simply the wrong form of a substance, can lead to trouble.

Iron often enters the body in a form that is toxic to cells. In healthy individuals this is not a problem because healthy cells have mechanisms to convert the iron into the non-toxic form—ferritin—a stored form of iron found in the cytoplasm of cells. Another form of ferritin is also found in mitochondria, which are present in all cells.

But in ALS, it has been found that there are increased levels of iron in the affected regions of the nervous system. It has been found that mutant SOD1 mice accumulate iron in the spinal cord. “If the iron levels go beyond a cell’s capacity

to buffer it, you get oxidative damage occurring to the cell,” said David.

To investigate whether iron influences the course of disease, researchers treated mutant SOD1 mice with a compound that removes iron from the body—an iron chelator called salicylaldehyde isonicotinoyl hydrazone (SIH). Treatment was started at eight months of age. Mice treated twice a week with the chelator lived an average of five weeks longer than mice given a placebo (no drug).

The finding is an early one, and it is not known at this point if removing excess iron would work as a clinical treatment for patients. [NW](#)



Samuel David, PhD

From Mice to Computers: A New Model for ALS Research

SOMETIMES DOING RESEARCH with mouse models and cells under a microscope is not enough to find answers to the mysteries of the causes of disease. That is why researchers in Chicago have developed a computer model of motor neurons.

Computer models of cells are used to study a wide variety of human disease, but until now there was no realistically detailed model for use in ALS research, according to Sherif Elbasiouny, PhD, of Northwestern University. Elbasiouny is a recipient of the Tim E. Noël Fellowship in ALS Research. In a poster at the 2009 ALS Research Forum, he presented a computer simulation of a motor neuron developed in his laboratory, along with some initial findings from his investigations. Specifically, researchers wanted a model that would help them to better understand changes in the electrical activity of motor neurons in ALS.

“When the cell is active and it fires action potentials for electrical activity, this electrical activity of the motor neuron is translated to muscle activity. The higher the firing activity of the motor neuron, the stronger the contraction of the muscle,” said Elbasiouny. An action potential is a short-term change in the electrical potential on the surface of a cell that leads to the transmission of an electrical impulse.

In ALS, motor neurons appear to become hyperexcitable, meaning they are overly active. When there is high firing or transmission activity, calcium ions enter the cell in large amounts, which can become toxic. This appears to be part of what happens in ALS, said Elbasiouny.

The researchers were interested in looking at the early abnormalities in motor neurons, well before the onset of symptoms. “The question is, What is the bad signal? What happens to the motor neurons that causes them to die? We are trying to go before the onset of disease and study the cascade of events that takes place within the motor neurons and causes them to die,” he said. Changes in electrical activity within cells is one of the clues.

To develop a computer model, researchers needed high quality data derived from actual motor neurons. Elbasiouny and his colleagues obtained the data of a French research group from the Centre national de la recherche scientifique (CNRS). This group had performed detailed three-dimensional imaging studies of motor neurons from transgenic SOD1 mice, as well as of cells from healthy

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wild-type mice. The cells were derived from mice that were only eight to ten days old—young enough for outward disease symptoms not to have yet occurred.

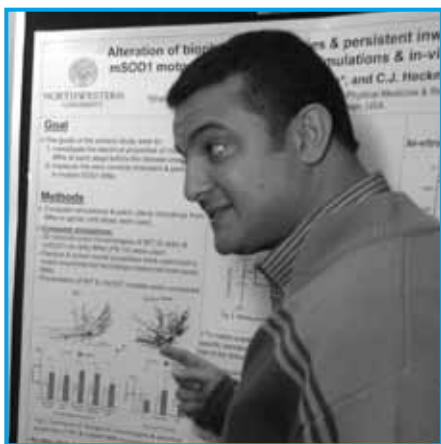
Elbasiouny was able to take the images and convert them to a format that provided an accurate reconstruction of the morphology, structure and size of the cell in three dimensions that could be used in a computer model. “It’s a realistic representation of the cell because you obtain the image from an actual cell, after staining,” he said.

The first thing researchers noted when they looked at their models of motor neurons from SOD1 mice and healthy mice was that those from the SOD1 mice had a noticeably different shape. They were larger and more branchy in appearance. This means that even at an early stage of the disease, the motor neuron is already “changing

its shape and its electrical properties, which means that ALS is starting long before the onset of disease (symptoms),” said Elbasiouny.

Researchers observed that there were differences in the electrical properties of cell membranes between the SOD1 cells and the healthy cells. For one thing, the membrane of the SOD1 cell was more leaky to electrical signals. “The physiological

meaning of this is that this cell will be harder to activate and the muscle will be harder to use. This could explain the weakness and partial paralysis in patients at the early stages of ALS,” he said.



Sherif Elbasiouny, PhD

Further work with the model showed that diseased motor neurons had an increased density of ion channels, a mechanism that is used by cells for firing action potentials. Researchers simulated differing densities of ion channels to see what the effect on a cell’s electrical activity or excitability would be. “We predicted that the excitability of these cells was increased because there is an increase in the density of the different ion channels—mainly sodium and calcium ion channels,” Elbasiouny explained.

In a second part of the study, researchers did a series of studies using actual samples of motor neurons from the spinal cord of both transgenic and healthy mice. The mice ranged in age from one to 12 days when they were sacrificed. A series of studies was performed measuring electrical activity of the motor neurons, and this was charted according to their age and disease stage. Measurements were also taken of the sodium and calcium ion channels. Findings showed there was increased current associated with the sodium and calcium channels, which confirmed what was predicted by the computer model simulations.

The research now provides “evidence that there are early abnormal changes in the electrical properties and ionic currents of the cell in ALS,” said Elbasiouny.

Computer models will not completely replace what can be observed and learned from work with actual cells, but they do complement the work. The two methods are tools for studying different aspects of the same questions. Simulations are a tool for learning even more about motor neurons and their properties, and they “help explain the results you obtained [from cell work],” he said.

This work could assist in identifying early abnormalities in membrane properties of motor neurons in ALS that could be targeted using pharmacological agents to delay or slow down the progression of the disease. Elbasiouny added that “a wider use of simulations in ALS research could help reduce the number of mice needed in studies by better design of experiments.” [NN](#)

Immunization Therapy Might Be Possible in ALS

AN EXPERIMENTAL THERAPY called passive immunization has led to longer survival in transgenic mice destined to develop ALS. The finding could lead to immunization as an approach to slowing disease progression if it proves safe in humans.

There are two types of immunization, according to François Gros-Louis, PhD, a post-doctoral fellow who works in the laboratory of Jean-Pierre Julien, PhD, professor of anatomy and physiology at the Centre hospitalier de l’Université Laval (CHUL) Research Centre, Québec.

The first type, active immunization, refers to immunity produced by the body after exposure to an infectious agent such as a bacteria or virus. Immunity can be acquired naturally after exposure to an infectious agent in the environment, even if symptoms are minor. Immunity to a specific disease can also be attained from vaccination, such as an influenza shot.

Passive immunization works differently, says Gros-Louis. Here, the antibodies themselves are injected and are

already designed to fight a specific target. In the case of research in Julien's laboratory, the antibodies are designed to attack a specific form of the mutated SOD1 protein. They do not affect healthy SOD1 proteins. Gros-Louis notes that passive immunization may be preferable to active immunization in ALS.

There are still a number of challenges when it comes to therapeutic immunization. The goal is to create a vaccine that will induce a desired immune response (such as destroying mutant SOD1 proteins) without having adverse effects. Unfortunately, in other research where attempts were made to use active immunization for Alzheimer's disease, it was found that the vaccine led to the development of meningoencephalitis (inflammatory disease of the brain and central nervous system) in six per cent of subjects.

Because of this type of problem in active therapeutic immunization, "passive immunization approaches would seem more appropriate, and safer, for future immunotherapeutic testing in human ALS patients," says Gros-Louis.

The passive immunization approach using antibodies was tested in two groups of transgenic G93-SOD1 mice. In one group, mice began immunization treatment before they developed clinical signs of the disease. In the second group of mice, immunization began when mice first started showing clinical signs of the disease. Both groups were compared against controls—transgenic mice that were not given any immunization treatment.

The treatment led to both groups of immunized mice living longer than their untreated counterparts. Mice given

the immunization treatment at the start of clinical disease lived an average of six days longer than expected, compared to nine days for the mice treated before symptoms appeared.

Gros-Louis notes that the technique for immunization was not a matter of simply giving the mice a single needle, as is the case with influenza vaccines. For this treatment, the mice had a catheter inserted in the brain. The catheter was connected to a mini-pump, and the mice received a continuous application of the antibody for a full month. Researchers believe that a longer treatment might have had a better effect, he says.

Continuous treatment with drugs is provided for certain human diseases, so it may be possible to extend this approach to ALS, says Gros-Louis. However, much more research needs to be performed before the treatment can be tested in humans. For one thing, the antibodies used in Julien's laboratory were designed for mice—they need to be altered or "humanized" to make them appropriate for human use. As well, it is not known whether the treatment is safe in the long term. [NU](#)



François Gros-Louis, PhD

Learning About ALS Through Population Studies

EPIDEMIOLOGISTS STUDY POPULATIONS to figure out patterns of diseases, their causes, and the influence of environmental factors on the spread of disease. In a talk at the 2009 ALS Research Forum, Marc Weisskopf, PhD, of the Harvard School of Public Health, Boston, described how epidemiological methods can be used to learn more about ALS.

Epidemiology can help provide clues as to whether specific populations are more vulnerable to the disease than others (such as specific racial groups, or people living in certain geographic areas), whether certain groups are more prone to more severe disease, or even whether environment or lifestyle influences when and where disease occurs. Once researchers have these clues, in the form of "associations," they can do further studies to determine the details of what those associations are and whether they are real.

What is already known about ALS is that approximately 30,000 people in the U.S. have ALS and approximately 5,500 new cases are diagnosed each year (the numbers are about one tenth of this in Canada). ALS is slightly more common in men than women, cases peak between the ages of 65 and 75 years, and the median survival is two to three years. About 10 per cent of cases are familial or inherited, while 90 per cent are sporadic.

Overall, the number of ALS cases is small, and with the short life expectancy, it is a difficult disease to study, said Weisskopf. The small numbers make it difficult to "get enough power to statistically see the important associations" that may offer clues about some of the disease's patterns.

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One type of epidemiological study is the case-control study. Here, two groups of people are compared: those with the disease and a similar (control) group of people who do not have the disease. Cases and controls are compared to see if they differ in certain characteristics.

Performing case-control studies can be tricky. One needs to be careful about the populations selected for study. To get the best results, patients and controls should be selected from the same population—for example, people from the same town.

To illustrate how problems can arise when inappropriate controls are used, Weisskopf described a 1981 study in the *New England Journal of Medicine* that looked at coffee consumption and pancreatic cancer. Here, people with pancreatic cancer were compared with controls recruited from hospitals in Boston. The researchers concluded that people who drank coffee were more likely to develop this form of cancer.

However, there was one problem with this study. Many of the controls were patients who suffered from gastrointestinal (GI) problems—and people with GI problems are less likely to drink coffee than the general population. So the association between coffee consumption and increased risk for pancreatic cancer was erroneous because the controls did not have the same sort of coffee-drinking history as the population the cases came from, said Weisskopf.

Another problem may occur in retrospective studies when people are asked to recall exposures. For instance, some studies may ask people for details about the diet they followed in the past.

“The problem is, what people tend to report in questions asked now is very much influenced by what they’re doing now. When you try to report on [eating habits] 10 years ago or 20 years ago, it’s going to be weighted towards what you’re doing right now,” he said.

Prospective cohort study

A better design is the prospective cohort study, where people are asked about their diet now, before any disease has occurred, then followed to observe who becomes ill. They can be asked about diet again in subsequent years, Weisskopf said.

In prospective cohort studies, one starts with a general population that is watched over time. Eventually, cases will develop. “Now you have your cases, you’ve collected data on them before they actually got the disease, and now you can compare your cases to the rest of the population. You don’t have to worry about whether the controls you’re choosing are reflective of your population,” he said.

Another version of this type of study is the nested case-control study. Here, one starts with a defined population, such as people believed to be at risk for developing a spe-

cific disease, then follows that population to see who becomes ill. Controls are selected randomly from that group.

“You’re not comparing your cases to the rest of the entire population, you’re comparing them to a subset of selected controls,” said

Weisskopf. It reduces the problem of control selection bias, (as occurred in the coffee-consumption study).

Historically, ALS has been studied in case-control studies. To go beyond this approach, Weisskopf and colleagues decided

to tap into data from other long-term cohort studies to look for information that might relate to the development of ALS. The researchers turned to the American Cancer Society’s (ACS) Cancer Prevention Study II (CPS-II), which started in 1982 and was designed to study environmental and lifestyle risk factors for cancer.

“Their study is big enough [to give statistical power]. They enrolled 1.2 million men and women from all 50 States,” said Weisskopf. Questionnaires were administered to participants asking about lifestyle, dietary habits, and certain environmental exposures when they entered the study. CPS-II also used the National Death Index (NDI) for information about who died and what the causes of death were.

Military–ALS connection

One question Weisskopf and his colleagues wanted to study was whether people who have served in the military have increased risk of ALS. This was of interest because other studies suggested that Gulf War veterans had an increased incidence of ALS.

To examine this idea, Weisskopf turned to the mortality data from CPS-II and the NDI. Researchers looked at both people who had died and those who had not died of ALS from 1989 through 2002 and whether they had served in the military. Researchers then stratified subjects into groups depending on which war they served in (going back to World War I), which branch of the military they served in, and how many years they had served. Other information was also available, such as smoking, alcohol and vitamin use.

Among the half a million men in CPS-II, about two-thirds had served in the military (women were not asked if they ever served). Out of this number, 513 deaths from ALS were identified.



Marc Weisskopf, PhD

“What we found in the overall picture was that those who reported military service were about 50 per cent more likely to develop ALS,” said Weisskopf. Interestingly, the association of military service with ALS did not depend on how long service was, nor on which war they were in. But the study does not explain everything.

“Epidemiology is by nature an observational science, so practically all of these sorts of studies are going to be limited in some way,” he said. Associations may be found, and that provides fodder for new avenues of study – to discover causes and triggers of disease.

As well, NDI data do not capture all ALS cases. For instance, if an ALS patient dies of a heart attack, the death record will not necessarily mention ALS. It is estimated that such mortality data tends to capture 70 to 90 per cent of ALS deaths. Another limitation related to doing studies based on data gathered for other studies is that not all the questions the researchers may have are answered.

“You don’t have the ability to ask any question you want,” Weisskopf said. For instance, the military data did not include information about whether soldiers had been actually deployed, and only certain exposures were recorded.

As for chemical exposure, some population studies suggested an association between ALS and exposure to

chemicals used in agriculture, while others found no association. However, prospective studies with large populations have not been done on this theme, he said.

In CPS-II, participants were asked in 1982 whether they were ever regularly exposed to several specific chemicals. ALS deaths were identified through to December 2004. Weisskopf adjusted for the sex of the participants, smoking, alcohol intake, military service and other factors.

One chemical that had an association with ALS was formaldehyde, which showed a dose-response trend with the number of years exposed. However, this was the first report of any association with formaldehyde, and overall the numbers are based on a small number of formaldehyde-exposed people who died from ALS. The study was published in the *Journal of Neurology, Neurosurgery & Psychiatry* in 2009. Researchers need to do other studies to better determine the level or frequency of people’s exposure, and whether this link to ALS is true, said Weisskopf.

Studies like this, which look at data pertaining to large populations, demonstrate trends and associations that may lead to specific research directions and more focused questions about the causes of disease. [NN](#)

Nervous System Development: An Inside Look

EVER WONDER HOW motor neurons and the messages they send to muscles are organized and how it all works? This is the sort of thing being studied by Artur Kania, PhD, director of the neural circuit development research unit at the Institut de recherches cliniques de Montréal (IRCM).



Artur Kania, PhD

He is associate researcher in the department of medicine at the Université de Montréal and is also associate member in the division of

experimental medicine and adjunct professor in the department of anatomy and cell biology at McGill University. His

specialty is the study of how neural circuits form — that is, how motor neurons end up where they do, how they communicate with other parts of the body, and how they get their job done.

At the 2009 ALS Research Forum, Kania described the work being done in his laboratory that is providing insights into the complex ways motor neurons are organized and their relationship with muscles in specific parts of the body.

“We are interested in the development of nervous system organization. Specifically, my lab is interested in how spinal cord motor neurons and their nerve organization develop in the embryo,” said Kania. By this he means where the neurons are located with respect to each other, where they are located within the spine, and where the axons that neurons use to communicate with other cells are situated. In neuroscience, this “lay of the land” organization is referred to as myotopy.

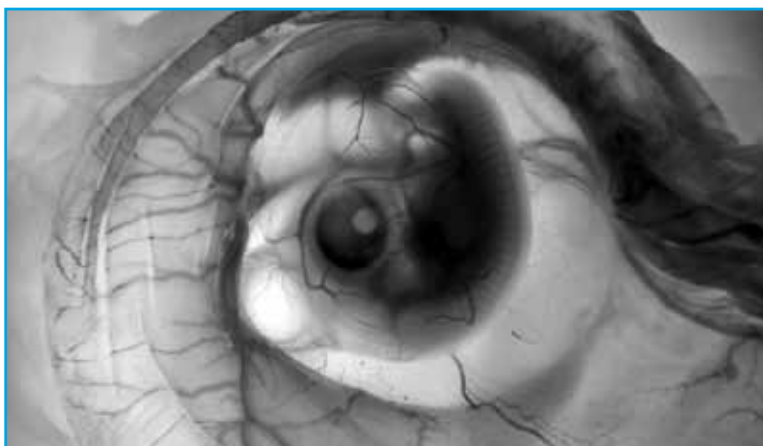
Various studies with developing chicken embryos (still in the egg) have revealed some of the clues about motor

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neurons and their early actions. Motor neurons are located in the spinal cord and are responsible for getting nerve endings to muscles throughout the body by extending long processes called axons. It is the axons that carry the electrical signals to make the muscles flex or twitch.

In the embryonic chicken spine, motor neurons are formed first, then the axons grow and extend to the muscles in different parts of the body. Kania is investigating how the axons get to specific muscles in the limbs.

“These axons leave the spinal cord and travel down a sort of a path all the way to the base of the limb. At the base of the limb, they have a choice of two trajectories,” he said. An axon can travel in a direction that will lead it to innervate, or supply with nerves, either the dorsal part of the limb (where extensor muscles such as the triceps are located), or the ventral part (where flexor muscles such as the biceps are located).



Chicken embryo

What researchers did not understand was what caused axons to go in a dorsal or ventral direction. “It’s like the axon has to make a choice: ‘Am I innervating extensor muscles or flexor muscles; or, triceps or biceps?’” Kania said.

Each axon has one of two receptors, either an EphA receptor or an EphB receptor. Work in Kania’s and other laboratories found that the different sides of the limb

contain one of two corresponding proteins, either ephrinA or ephrinB. These proteins bind to specific receptors on the axon when it gets to the base of the limb. In short, the ephrinA protein binds to axons with EphA receptors, and ephrinB binds to the axons with the EphB receptor.

When this happens, the axons with EphA are repelled by the ventral side, and move up the dorsal side of the limb. In turn, the EphB axons move up the ventral side of the limb. The axons are nudged into travelling dorsally or ventrally through repulsion, not unlike pushing a magnet around with another magnet that has the same magnetic pole.

A second protein called netrin also helps axons get to their correct destination. Netrin occurs only in the dorsal half of the limb, and Kania hypothesizes that it works to attract the EphA axons. Axons with EphB are repelled by netrin, making them less likely to take a wrong turn.

“The whole idea behind these experiments is to figure out these very simple decisions of where to grow, where to send your axon, which muscle to innervate, how is this happening,” Kania said. This sort of knowledge will be useful in the long term when it comes to figuring out new treatments for ALS.

“If therapy for ALS is based on remaking lost neurons, say from stem cells, we need a way of reconnecting those neurons to the muscles. What’s going to be important is to know how this process normally happens,” he said.

Intriguingly, while the axons are extending to the limb they are going to innervate, the motor neuron cells are moving around in the spine. In the end, some of the motor neurons will connect to dorsal muscles, while others will connect to ventral muscles, but these different types of motor neurons start off all being jumbled together. Researchers found that during this time the cells that end up performing similar functions move closer together.

“We’re trying to understand how this sorting of the cells happens,” said Kania. Research in his laboratory has found that a protein called reelin assists in the sorting and movement of these developing motor neurons. But it may be only part of the equation.

“What are the consequences of position? That’s not really clear yet. It’s something we want to study,” Kania said. The work continues. [NU](#)

When my dad was diagnosed, I told my classmates that I could help him by raising money to support the ALS Society, but I needed their help to do it.

— Alec Walker Smith, who raised more than \$10,000 for ALS with his classmates by organizing the Hodgson Senior Public School dance-a-thon in Toronto in May 2009. Alec’s father, who had ALS, died less than a month after the fundraiser

New Approaches: Finding Ways to Help Motor Neurons

WHEN PEOPLE TALK about the possibility of using stem cell therapy to treat ALS, the usual concept is to try and replace ailing motor neurons with cells that will turn into new healthy ones. But another approach to stem cell therapy is to introduce a different type of cell to protect the remaining healthy motor neurons.

At least this is the idea researchers at Johns Hopkins University (JHU) are investigating. In a talk at the 2009 ALS Research Forum, Nicholas Maragakis, MD, an associate professor of neurology at JHU school of medicine, described research that suggests this approach has promise.

The specific type of cell he and his co-workers are investigating is astrocytes. These are star-shaped cells that surround neurons and provide various support functions such as providing nutrients to nerve tissue and helping pass on signals to neurons.

An important feature of astrocytes is that they absorb excess glutamate, the most common neurotransmitter in the brain. Too much glutamate can cause damage to neurons, but astrocytes serve a protective role by picking up excess glutamate so it will not adversely affect neurons.

How does this tie in with ALS? Some studies suggest that in ALS astrocytes undergo changes including a decreased ability to “sop up” excess glutamate, which in turn can contribute to damage to motor neurons.

Researchers in Maragakis’s laboratory have been working with animal models of ALS to learn more about astrocytes and their ability to pick up glutamate. Specifically, they have been doing work with a protein, GLT-1, which is synthesized by astrocytes and is responsible for picking up and removing glutamate. The research included a series of studies using genetically modified SOD1 mice. Here they found that in mice with reduced levels of the GLT-1 protein, the progression of ALS was much faster.

“That would suggest that the loss of the GLT-1 protein could accelerate disease,” Maragakis said. Previous studies have shown that patients with ALS have abnormally high levels of glutamate in their cerebrospinal fluid (CSF).

For researchers, these were pieces of a puzzle that led to the idea of using stem cells to replace the astrocytes that had lost their ability to remove excess glutamate with healthy astrocytes.

In ongoing studies, researchers are working with particular stem cells called glial restricted precursors (GRPs). These are stem cells that are already partially specialized and can be easily nudged into turning into astrocytes. The GRPs the researchers are studying create astrocytes that have GLT-1.

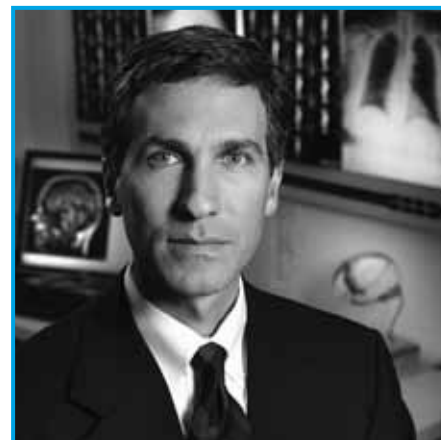
In one study, researchers transplanted GRPs into a restricted region of the spinal cords of SOD1 rats. Most of the GRPs successfully turned into astrocytes, moved to places in

the spinal cord where they were supposed to go, and helped reduce glutamate levels. Even more intriguing was that the SOD1 rats that received the GRP transplants had slightly longer survival rates than SOD1 rats not treated with GRPs.

However, the effect of the GRPs was very localized. In the rats, “there was no change in hind-limb disease onset. So the effect was not a global phenomenon as you might expect with a drug,” said Maragakis. On a more promising note, the progress of the disease appeared to slow down in the animals, suggesting a possibly longer survival.

While the work is promising, there are numerous challenges to be met before the approach can be considered for humans, he said. Researchers need to know if the astrocytes will survive long term, whether they have all the characteristics of naturally developed astrocytes, whether or not they will eventually succumb to the ALS disease process, and even whether the cells might eventually become cancerous (a risk with stem cell transplants).

Maragakis is now working with a company, Q Therapeutics in Salt Lake City, to continue studies to see if the approach can eventually be used for humans.



Nicholas Maragakis, MD

Spinal muscular atrophy—a novel genetic approach to motor neuron disease

Researchers investigating a different but related disease, spinal muscular atrophy (SMA), are testing a totally different approach to the battle against motor neuron disease. Adrian Krainer, PhD, of the Cold Spring Harbor Laboratory, a not-for-profit research centre in New York State, described efforts in his laboratory that are largely focused on how gene transcripts are processed, and how they produce proteins. He is studying ribonucleic acid (RNA) and its role in protein synthesis. RNA occurs in all cells and has various functions, but one of its forms is involved in the generation of proteins.

In SMA, some of the same motor neurons that are affected in ALS, the lower motor neurons, die as a result of mutations in one specific gene. As a result, there is either a

Continued on page 28



Adrian Krainer, PhD

decreased quantity or complete lack of a protein called survival motor neuron (SMN). The less of the protein an individual with SMA has, the more severe the disease. Essentially, the mutant SMN gene does not produce the needed protein.

Intriguingly, there is a second gene in humans, the SMN2 gene. It is capable of producing the SMN protein, but in fairly small amounts. Researchers have discovered that the SMN2 gene can be coaxed to produce larger amounts of functional SMN protein.

Some early studies have been performed in SMA mice and have shown promising results in terms of increased production of the SMN protein. There is still a lot of work to be done and many questions to be answered. Among them, if the findings are translated to a genetic therapy, when is it best to apply it? Is it safe? Will the effects last? Will it affect human disease and survival?

According to Krainer, "If we succeed with all this, and also demonstrate a lack of toxicity in primates, then we can apply for investigational new drug status and move on to phase-I trials. I'm optimistic that this can be done within one year." [NW](#)

The human motor system

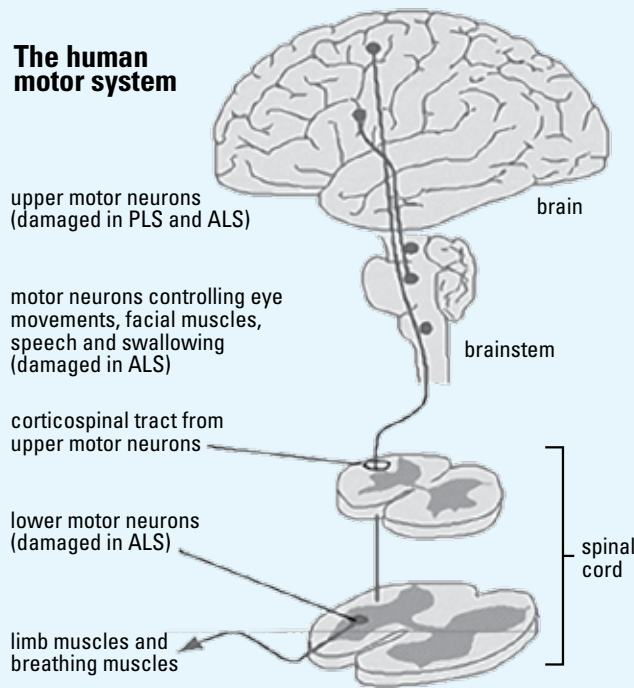


Illustration courtesy of Pamela Shaw, MD, University of Sheffield, U.K.

Of Mice and Zebrafish

STUDIES USING ANIMAL models can provide insight into human disease, especially when it comes to genetic research. The study of genes, their interactions with other genes and proteins, details about their functions, the influences that affect how well they function, and the differences in genes in healthy versus non-healthy animals can lead to an increased understanding of human disease.

Mouse models are especially useful. The mouse genome is 95 per cent identical to the human genome; in addition, mice breed and grow fairly quickly, allowing researchers to observe how disease affects mice as they age and over successive generations.

One researcher who spoke at the 2009 ALS Research Forum has a special advantage when it comes to studies with mouse models—he works at the Jackson Laboratory (JAX), which is not only a research facility but is home to

one of North America's largest non-profit breeding facilities and distributors of laboratory mice. The facility breeds more than two million mice a year.

"About half of my lab focuses on muscular dystrophy models; and the other half does motor neuron disease models," said Gregory Cox, PhD, associate professor at JAX.

With ALS, a focus of his research is investigating genetic factors that may influence the time of onset of disease and the rapidity of its progression. Using specialized strains of mice helps because mice within a strain are genetically identical to each other.

"Within [human] families that have inherited the same ALS predisposing mutation, one can often see a large variation in the age of onset and rate of progression of motor neuron disease, which may be due to particular susceptibility and resistance alleles that they have inherited," said

Cox. An allele is an alternative form of a gene that may have a slightly different function.

With inherited ALS, there are various factors that may influence the clinical parameters of the disease. Environmental factors are one possibility, but there are likely genetic factors too—genes that contribute to the variation of the course of the disease in individuals who inherit the same primary genetic trait, for ALS. It is these influencing or background modifying genetic factors that Cox is studying.

Unlike specific strains of mice, family members are not genetically identical to each other, meaning that individual family members may have genes that cause the disease to behave differently.

Researchers took the G93A–SOD1 mouse (a strain of mouse that carries the human ALS gene and develops the disease) and crossed it with other strains of mice to see



Gregory Cox, PhD

whether the nature of the disease changed. In one particular strain called the ALR mouse, the disease had an early onset and progressed more quickly than it did in the original SOD1 mice. In another strain, the C57/BL/6 strain (B6), the onset of symp-

toms was much later and the mice lived longer.

Crossing the mice was not simply a matter of breeding one pair and studying the immediate offspring. When crossing the ALR mice with the original SOD1 strain, researchers bred the subsequent ten generations of offspring with ALR mice. The idea was to create a mouse that was as genetically identical to the ALR line as possible (greater than 99.9 per cent), except that it would also carry the human SOD1 disease-causing gene. The researchers wanted to breed out other genes that came from the original SOD1 mouse background. They did the same thing with the B6 strain, and the process took close to ten years.

“Even though these other mice carry exactly the same human SOD1 gene, they get sick at different times. That tells us there is something in the genetic background in these two different strains that’s controlling how early the disease occurs in those mice,” Cox said.

The genomes for the ALR and B6 mice are well known, and work has continued to try and identify where in the mouse genomes there are genes that affect the course of ALS.

“We found two regions of the genome that seem to correlate with early versus late onset. On mouse chromosome 17 there is a region that differs between our ALR and B6 mice that seems to be contributing to this process, so one of the modifier genes is likely located there.” Also compelling “is another region on mouse chromosome 4,” said Cox.

“We hope to continue our genetic mapping to identify the genes that are regulating this process so we can determine if the same genes or cellular pathways are regulating the disease process in ALS patients,” he said.

Zebrafish – another model for studying ALS

Another model being used to study ALS is zebrafish. The small fish, part of the minnow family, is commonly used in developmental and genetic studies. Zebrafish are useful in developmental studies because their embryos develop from eggs to larvae in less than three days. Also, the embryos are large and transparent, which makes it easy to observe and study the internal organs and tissues as they grow and develop. Their genetic code has been sequenced and is well understood.

In the past three years, there have been substantial advances in the use of zebrafish as models for neurodegenerative diseases such as Alzheimer’s, Parkinson’s, and Huntington’s disease, said Edor Kabashi, PhD, of the Centre hospitalier de l’Université de Montréal (CHUM).

And now genetically modified zebrafish are also being used in ALS research. By genetically altering fish to carry one of several mutant human genes that cause ALS, researchers can observe the fish’s locomotor development, as well as motor neuron and axon development.

“We are studying zebrafish during their embryonic and larval stages,” Kabashi said. Impressively, the spinal cord is almost fully developed within 48 hours.

ALS2 knock-down (the reduction of gene expression) in zebrafish led to a specific motor phenotype in the early stages of embryonic development. Phenotype refers to the physical features of an organism,



Edor Kabashi, PhD

and ALS2 is the gene responsible for a rare form of familial ALS that affects younger adults. It was also found that the ALS2 knockdown fish’s motor axons did not extend

Continued on page 30

properly, which adversely affected the fish's ability to swim after hatching. The effect was analogous to locomotion problems seen in people with the ALS2 mutation.

"Zebrafish models are very useful to confirm whether a particular gene may cause disease through loss of function [by knocking down their expression levels] or through a gain of function by overexpressing the human mutant RNA," Kabashi said. Most importantly, these questions can be answered very rapidly using these models, which helps

ALS researchers to better understand molecular mechanisms that may trigger this disease.

"If we see a phenotype when we introduce a mutant gene (such as a human ALS gene), then we know there is a gain of function or loss of function that will cause the disease," he said. In other words, when a gene is introduced, researchers can see whether or not the fish ends up being able to move normally or not.

Clearly, an advantage with animal models in general is how quickly certain questions can be answered. [NN](#)

Protein Defects and the RNA Connection

ALS STILL HOLDS many mysteries in terms of what causes the disease and how the cells involved in the disease process interact with each other. Researchers are now looking at ALS, at least in part, as a disease in which ribonucleic acid

(RNA) processing is faulty. This was a topic discussed at the 2009 ALS Research Forum.

One researcher, Michael Strong, MD, asked specifically: Could ALS be a disorder of RNA metabolism? Strong is chief of neurology and co-chair of the department of clinical neurological sciences at the University of Western Ontario, research scientist at the Robarts Research Institute

in London, Ontario, and director of the Motor Neuron Diseases Clinic at the London Health Services Centre.

RNA occurs in all cells and plays several roles. Its structure is similar in many ways to DNA (a molecule which contains the genetic blueprint for a living organism), and its activities in the living cell are highly regulated or controlled. One of RNA's key roles is to help generate proteins. If something in the system that regulates RNA goes wrong, it could either prevent specific proteins from being made or lead to too many proteins of a certain kind being produced.

From a single gene, hundreds of RNA molecules may be made which in turn help synthesize tens of thousands of proteins, Strong said. There is a finely regulated system within each cell where all this protein building takes place that dictates when RNA has finished its job and can be degraded. This regulation is achieved through various special-

ized proteins which attach themselves to the RNA and help control exactly what it does for specific tasks.

"There are proteins that attach themselves to the RNA that shut it down so it just sits quietly. There are other proteins that [signal a] need to be degraded, and there are other proteins that transport the RNA to where it might be needed in the cell," said Strong.

Why focus on RNA when it comes to ALS? For one thing, there are other medical conditions, such as one type of muscular dystrophy, and spinal muscular atrophy, that affect the muscle and motor systems, and "we know the primary defect is RNA," Strong said. As well, there are some clues in ALS suggesting there is RNA instability occurring in neurofilament proteins—but it is too early to say whether this instability is a cause or effect of the disease.

Researchers in Strong's laboratory have identified several of the proteins involved in regulating RNA stability of neurofilaments (NFs), which are string-like structures in neurons that give the cells extra strength. "Interestingly, these proteins are also ones that are critical to ALS," he said. These include TDP-43, mutant SOD1 (found in familial ALS) and 14-3-3 proteins.

Work in his laboratory showed that in ALS, 14-3-3 would bind to human neurofilament messenger RNA and have a destabilizing effect. TDP-43 also binds to RNA, but in ALS it does not function properly. In the end, Strong does not know whether ALS could actually be called a disease of RNA malfunction, but because there are odd things happening with the RNA of NFs, it is an aspect of the disease well worth investigating.

Peripherin defects caused by RNA errors

From another angle, Janice Robertson, PhD, associate professor of laboratory medicine and pathobiology at the Centre for Research in Neurodegenerative Diseases, University of Toronto (U of T), is studying a specific protein, peripherin — a type of intermediate filament (NIF) protein



Michael Strong, MD

that is found only in neuronal cells. It is known to affect the early growth of axons and dendrites from nerve cells (the tendril-like extensions of neurons) as well as the regeneration of axons. Beyond this, the overall function of peripherin is unknown.

There is a peripherin gene that leads to the production of the peripherin protein. However, in ALS there is some sort of disruption that occurs at the RNA-processing level, which leads to the generation of aberrant forms of peripherin protein, said Robertson.

In ALS, the aberrant forms of peripherin are a component of the protein aggregates (or clumps of proteins) that are characteristic of the disease. And because peripherin is common to all types of ALS, it makes it worthy of study. “Whatever is happening to peripherin is a common pathway in both familial and sporadic forms of the disease,” Robertson said.

“Looking at models of peripherin, we know that if we overexpress peripherin in transgenic mice it kills motor neurons. If we overexpress peripherin in motor neurons in culture, the motor neurons die. And, intriguingly, there is an increased expression of peripherin in ALS,” she said.

Robertson described some of the work performed in her laboratory, which has uncovered several versions of malformed peripherin, including Per56, Per58 and Per28.

One example she discussed is Per28, which has been found to induce aggregates inside motor neurons. Researchers found that Per28 can be detected in the spinal

cord fluid of ALS patients, but it is not present in healthy people. This means that there is a chance that Per28 could potentially be used as a way to test for ALS. This is key because to date there is no test for an early diagnosis of ALS.

“If we can get an earlier diagnosis, treatments could be started earlier and there may be a chance of halting the disease,” she said.

Researchers in Robertson’s laboratory are also investigating the effects of stress on peripherin.

In a poster presented at the Research Forum by Jesse McLean, PhD, of the U of T, researchers showed evidence that oxidative stress and inflammation can trigger peripherin to react and form aggregates.

This suggests that “aggregation may serve a physiologically relevant role during oxidative stress,” researchers noted in the poster. This finding emerged from work using cell cultures. The problem now is to confirm whether this is some sort of normal physiological reaction to stress, and, if it is, why aggregates cause problems in motor neuron disease. [NN](#)



Janice Robertson, PhD

The Subtle Workings of Genes

RESEARCHERS IN MONTREAL continue to be among the world’s leaders when it comes to the genetics of ALS. The researchers, now based at the University of Montreal, were



Guy Rouleau, MD, PhD

part of the international team that discovered mutations in the SOD1 gene. They were also part of the team that discovered the TDP-43 gene that is responsible for the disease in some families. And in February 2009, their names again appeared

in connection with the most recent discovery, the fused in sarcoma (FUS) gene.

The FUS gene takes its name from its association with sarcoma, which is one of a group of generally malignant tumours formed in connective tissue. The FUS protein is thought to be involved in DNA repair, as well as regulating the transcription from DNA to RNA; subsequent RNA processing; and movement of RNA from the cell nucleus to the cytoplasm. It performs similar functions to the TDP-43 protein.

The FUS protein is normally found in the nucleus of motor neuron cells. However, FUS protein molecules from mutated FUS genes are more likely to be located in the cytoplasm, where they aggregate or clump together. This type of neuronal cytoplasmic protein aggregation appears to underlie the degeneration of nerve cells in ALS and other motor neuron diseases. Cytoplasmic aggregations are also found in mutations of the TDP-43 gene.

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Guy Rouleau, MD, PhD, professor, department of medicine at the University of Montreal, discussed the significance of these findings at the 2009 ALS Research Forum. Rouleau summarized the state of genetics research—what it means to the understanding of ALS, and where research should be headed.

Familial or inherited forms of ALS (FALS) represent, at most, only 10 per cent of all ALS cases. But such cases have the same symptoms and outcomes for patients as non-familial ALS, also known as sporadic ALS (SALS), and hence may be a key to understanding the disease. Studying the genes which cause ALS and how specific mutations lead to disease can help broaden the understanding of the



Hussein Daoud, PhD

mechanisms of the disease in all forms of ALS, and suggest new treatment strategies, said Rouleau.

Studying families is the key to finding the genes that cause ALS. Geneticists study family trees and look at the pattern of who gets the disease and when. If a disease occurs with a certain frequency in a family, that is a clue that the disease may be caused by an inherited gene.

Over the years, researchers from around the world have identified a number of “suspect” or candidate genes. For the most part, however, these genes did not turn out to be true ALS genes though some were linked to diseases that had some overlapping features with ALS. Right now, said Rouleau, researchers have three true ALS genes to work with—SOD1, TDP-43 and FUS.

Another approach genetics researchers use is whole genome association studies.

This involves comparing the

complete genome (the full genetic blueprint) of healthy people to those of people with ALS. The idea is to try and identify not only genes that cause the disease, but also genes that might confer susceptibility to the disease process. These are difficult studies to perform because in order to be effective, large numbers of both patients and controls need to be involved. Generally, this type of study has not been very useful in ALS research, Rouleau said.



Veronique Belzil

Much can be learned from the study of ALS genes and more research needs to be done, he said. Canada would benefit from developing a high-quality diagnostics laboratory where genetic data derived from ALS patients would be available for study. As well, a system should be created so more laboratories could work together and share information more easily.

To further the quest for more true ALS genes, researchers are screening blood samples from people with FALS and SALS for candidate genes. According to Hussein Daoud, PhD, a post-doctoral fellow in Rouleau’s laboratory, the candidate genes they are looking for are known to be involved in the development of motor neurons in mice. These genes govern the ability of developing brain cells to connect properly to the spinal cord, which may have significant relevance to ALS and other motor neuron diseases. The role of these genes in ALS is the focus of his study.

A total of 80 patients with FALS and 110 with SALS were screened for a total of 29 candidate genes. A total of 190 healthy controls were also screened. Through this project, “we expected we might find novel genes of interest,” Daoud said. And researchers have identified five genes. The research is still at an early stage, and it is not yet known if any of the genes are true ALS genes or exactly what their role might be.

Adding to the excitement are new findings that show some cases of FALS have different mechanisms in terms of how the SOD1 gene can cause the disease. In a poster presented at the ALS Research Forum by Veronique Belzil, a PhD candidate in Rouleau’s research group, details were revealed of how abnormal proteins in motor neurons can result from a non-typical genetic mistake.

Normally, in inherited disease, there is an error in the arrangement of some of the nucleotides that make up a person’s genetic code. Nucleotides are the units that join together in a chain to make up the structure of DNA and RNA. A total of four nucleotides appear in various combinations within DNA and RNA.

But in a study of a family with a large number of ALS cases, researchers were unable to find a typical type of genetic defect (a problem in nucleotide sequences) in the SOD1 gene. They took another approach and looked closely at the RNA of members of the family. RNA has a structure that is similar in many ways to DNA, but one of its key roles is to help generate proteins using instructions provided by DNA.

When investigating the RNA, researchers found an abnormality in a section of the RNA that led to an alteration in both the length and the composition of the resulting protein. There was a segment that is normally not transcribed into the RNA during SOD1 protein-building. As a result the proteins produced by the SOD1 gene ended up being aberrant, said Belzil.

Using this as a clue, researchers went back to the DNA in the SOD1 gene and identified an abnormal nucleo-

tide which led to the problem in the RNA. The incorrect instructions were coded into the RNA; for SOD1 protein, the resulting proteins are too short, malformed, and have a toxic effect on motor neurons. This same sort of mechanism is seen in certain other diseases, but this is the first time it has been identified in ALS pathology related to the SOD1 gene, Belzil said.

As stated above, mutations in two additional genes — TDP-43 and FUS — have been found in ALS patients over the past few years.

Researchers in Rouleau's laboratory have also found that there are some similarities between the TDP-43 and FUS genes, such as the fact that mutations occur in clusters in small areas of the genes. It is not known what this means, how it occurs, or why.

In summary, concluded Rouleau, "there are still a lot of mysteries about SOD1, TDP-43, and FUS." [NW](#)

Mutations in a New Gene Identified as a Cause of Familial ALS

Two separate teams of ALS researchers have revealed that mutations of the fused in sarcoma/translated in liposarcoma (FUS/TLS) gene are implicated in the development of inherited or familial ALS (FALS). Their studies,



Paul Valdmanis, PhD

both published in *Science* in February 2009, reflect international efforts to focus on the search for the genetic causes of ALS.

Canadian investigators Guy Rouleau, MD, PhD, and PhD candidate Paul Valdmanis, both of the

Research Centre of the Centre hospitalier de l'Université de Montréal, were part of an international team that discovered a genetic mutation estimated to cause 5 per cent of FALS cases, similar to the frequency of mutations of the TAR DNA-binding (TARDBP) gene, which encodes the TDP-43 protein.

Valdmanis says: "The discovery that mutations in the FUS gene cause ALS is a tremendously exciting finding and should provide an understanding of some of the mechanisms that cause ALS. It arguably is the most important finding since the discovery of the SOD1 gene."

The FUS protein is normally found in the nucleus of motor neuron cells and is involved in DNA repair as well as regulating the creation, modification and transportation of RNA. It performs similar functions to

TDP-43. Both FUS and TDP-43 move from the nucleus to the cytoplasm of degenerating motor neurons, where they tend to clump together or form aggregates. The implication is that disease may be caused by a decrease of FUS/TDP-43 function in the nucleus, an increase of function in the cytoplasm, or a combination of both these processes.

Following on a lead about mutations in FUS from the team that included Rouleau and Valdmanis, a group of researchers in the U.K. and Australia, led by Christopher E. Shaw, MBChB, MD, and Caroline Vance, PhD, of the department of clinical neuroscience, King's College, London, U.K, confirmed that these mutations were present in approximately 4 per cent of U.K. patients with FALS.

The identification of the FUS gene is likely to give insights into the mechanisms underlying the development of ALS and lead to the creation of new rodent models for the disease. It may ultimately lead to the development of therapies for ALS and other neurodegenerative diseases.

Paul Valdmanis received his PhD in spring 2009 and is now doing post-doctoral studies at Stanford University.



DNA

Time for a New Paradigm for ALS

ALS IS THOUGHT of as a disease that develops later in life, usually in the fifth or sixth decade. But one Canadian researcher asks whether it may be possible that ALS actually starts either *in utero* or in childhood and simply does not manifest symptoms until decades later.

The question was presented to an audience of researchers at the 2009 ALS Research Forum by Andrew Eisen, MD. He is professor emeritus of neurology at the



Andrew Eisen, MD

University of British Columbia and was previously head of the ALS Clinic in Vancouver. Eisen has been studying ALS for more than 30 years.

When it comes to the causes of ALS, Eisen said, “There may be a chance for a paradigm shift.”

It has been assumed that ALS is caused by degeneration of an aging nervous system, with onset occurring shortly

before the actual symptoms. Is it possible that certain individuals are primed at a point much earlier in life to develop ALS? This could explain why there have been so many failures when it comes to treating the disease.

“Failures could be due to the fact that we start treatment long after the horse has bolted the barn,” he said.

Eisen’s position is that ALS is a neurodegenerative condition like Alzheimer’s and Parkinson’s disease and that the diseases share many features. But he points out that research has shown that the disease process in Alzheimer’s begins long before major symptoms appear, making detection of the disease difficult. With Parkinson’s disease, people may experience symptoms that do not appear to be parkinsonian, such as sleep disturbance, loss of smell, and depression, several years before explicit symptoms of the disease manifest.

Parkinson’s disease is a condition in which people suffer from various neurological impairments because of a deficiency of dopamine, a vital brain chemical or neurotransmitter. It is a disease which tends to manifest later in life.

However, there is a hypothesis that the stage is set for Parkinson’s much earlier in life. “The idea is that during the prenatal and perinatal periods, because of genetic and/or environmental issues, you lose dopaminergic and even non-dopaminergic cells to an extent that it doesn’t do you any harm at the time — but it lowers the threshold for

disease,” said Eisen. Dopaminergic means that it is related to the neurotransmitter dopamine—for instance, a synapse is dopaminergic if it uses dopamine.

One study in the medical literature suggests that up to 60 per cent of the dopamine-producing cells may be lost early in life, allowing people to lead normal lives until they are much older. Then the number of cells dips below a critical level and symptoms appear.

Eisen suggested that with many diseases that present in older age, the underlying cause, such as a genetic mutation, has already been passed on. “What we’re doing is accumulating good but also bad genetic material. This translates into diseases of older age. And that implies that in fact these diseases have a much earlier onset than when they really begin [to manifest] clinically,” he said.

Part of it has to do with genetics and evolution. In the process of evolution, genes constantly mutate, with stable mutations being passed on to subsequent generations. Unstable mutations die off. Some of the stable mutations are beneficial, while others may actually lead to disease.

Because of advances in technology and lifestyle, people live long past their reproductive years—much longer than their ancestors did. In the wild, animals and plants tend to reproduce then die off fairly quickly, Eisen said. But nowadays, people live much longer, which means that humanity can accumulate various mutations that lead to diseases of older people. Diseases of the elderly do not necessarily affect younger people so people who are in their reproductive years are able to reproduce and pass on genes that affect older people. The genes stay within the population.

Plus, the global population has increased substantially. “And this is associated with marked evolutionary explosion. In fact, the rate of evolution is exponential... This has resulted in, and allowed, a wide spectrum of genetic diversity to creep in. Most of it is good; some of it is bad,” said Eisen. So it is not surprising that there are various mutations that affect people during their golden years.

When it comes to the motor system in the human body, its development actually takes years. The process of its development “starts prenatally and doesn’t complete until well into teenage years,” he said. It is a complex process, and one that can be prone to error.

There are two periods in particular when the motor system is at its most vulnerable. One is the perinatal period and the other is adolescence, he said. Adolescence is a time when there are changes in the volume of the white and grey matter in the brain, and changes in neural connectivity and neurotransmission. In fact, adolescence is when psychiatric diseases such as schizophrenia and

mood and anxiety disorders become more apparent. It is possible that some change occurs at this point, making a person vulnerable to ALS later on.

If the processes leading to ALS do begin at some point before birth or in childhood, then it opens up opportuni-

ties to look for other sorts of markers of the disease—and find ways to prevent the overt symptoms of ALS from ever occurring.

“At the very least,” said Eisen, “these ideas are something to think about.” [NN](#)

What's New with SOD1

IN A ROUND-TABLE session at the 2009 ALS Research Forum, researchers discussed recent advances in work related to investigations of mutations in the SOD1 gene and new findings about the characteristics of the SOD1 protein. Research ranges from studies contradicting the idea that SOD1 may possibly play a role in all types of ALS to studies showing that SOD1 misfolding may be triggered for a variety of reasons.

Normal forms of SOD1 proteins serve to keep cells safe from metabolic waste. But in some cases of inherited or familial ALS (FALS), the misfolding of SOD1 proteins is associated with an aggregation or clumping of proteins inside motor neurons. Protein aggregates are toxic to the cell and trigger an early cell death. It is therefore important to understand why and how proteins misfold, and what causes them to form aggregates.

Mutations of the SOD1 gene are known to be linked to FALS and account for up to 2 per cent of all ALS cases. SOD1 is the main known cause of ALS. To date, more than 130 mutations of the SOD1 gene are known to exist. Through various pathways, the mutations all lead to the development of forms of the SOD1 protein which have a toxic effect on motor neurons.

In work being conducted at the University of Toronto (U of T), Janice Robertson, PhD, associate professor of laboratory medicine and pathobiology at U of T's Centre for Research in Neurodegenerative Disease, and Avijit Chakrabarty, PhD, senior scientist at the Ontario Cancer Institute of the University Health Network, described findings from their laboratories which suggest that decreased levels of mutant SOD1 could have a beneficial effect on the disease course. They also presented details of work in which a new antibody was developed that may be used to better study the SOD1 protein.

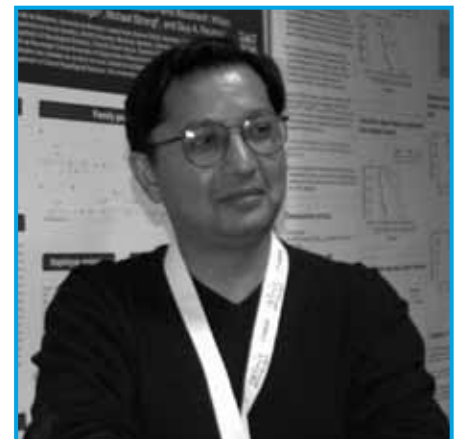
Chakrabarty and Robertson developed and characterized an antibody, the SOD1-exposed-dimer-interface antibody (SEDI), that attaches itself only to misfolded forms of the SOD1 protein. The antibody can be used as a marker to help study how the protein behaves, including the steps the protein takes when it is misfolding or causing aggregates to form.

Robertson presented new data from studies using the SEDI antibody that show there is an absence of misfolded SOD1 in sporadic ALS (SALS). This is important because some research in the medical literature suggested the possibility that SOD1 might be causative of all forms of ALS, not just FALS. It was hoped that misfolded SOD1 might be a viable therapeutic target for all forms of ALS; however, that may not be the case.

Although there may possibly be other forms of SOD1 not detected by use of the SEDI antibody, said Robertson.

However, the good news is that there is a possibility research with SEDI could lead to the development of ways to remove toxic misfolded SOD1 in FALS cases through immunization.

Chakrabarty described work relating to the use of spatially targeted optical micro-proteomics (STOMP) in ALS research. Proteomics is the study of the structure and function of proteins within an organism. It includes how proteins are formed, how they behave, how they interact, and how their formation, structure and interactions may be influenced by differing environments and conditions. STOMP includes a mix of technologies that allows researchers to use relatively small tissue samples, and it will help them study, and even



Avijit Chakrabarty, PhD



Neil Cashman, MD

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discover, new protein biomarkers for the disease. STOMP is only now beginning to be used in ALS research.

Also at the Research Forum was Neil Cashman, MD, professor of neurology at the University of British Columbia and academic director of the ALS Centre at Vancouver Coastal Health (VCH) and VCH Research Institute. He is best known for his research in prion diseases, such as Creutzfeldt-Jakob disease (CJD). A key feature of prion disease is the presence of misfolded prion proteins in the brain. Like ALS, some cases are familial, but most are sporadic. Indeed, the misfolding of various proteins is common to many neurodegenerative diseases, though the specific proteins which misfold may vary in different diseases.

While CJD and ALS involve the folding of different proteins, Cashman asked whether there might be some parallel mechanisms. In prion disease, there is a characteristic domino effect in terms of what happens with the folding of prion protein molecules—once one misfolds, the rest follow suit. He asked whether this is something that might happen with the misfolding of SOD1 proteins.

Cashman's preliminary data showed that a specific form of mutant SOD1 can trigger normal, non-mutated SOD1 proteins to misfold. This idea, if confirmed, could lead to new avenues of research for the development of treatments for ALS.

Looking at another aspect of SOD1-related disease, Miranda Tradewell, PhD, of McGill University, who received an ALS Society of Canada studentship award in 2004 and currently holds the Tony Proudfoot postdoctoral fellowship in Quebec, described the role that elevated levels of calcium play in affecting disease in neuronal cells.

Normally, calcium is needed by nerve cells to trigger electrical activity, and the amount present in cells is highly regulated. Motor neurons have lower levels of proteins that bind to calcium, which leads to increased levels of “free” calcium even in healthy cells. But in addition, the motor axons of ALS patients have been found to have abnormally high levels of calcium.

To learn about the role elevated calcium plays, researchers studied cell cultures which were prepared from spinal cord samples from SOD1 mice (mice that have a mutant SOD1 gene leading to ALS). The idea was to examine what happens with free calcium levels in different compartments of motor neurons over time. The researchers found that calcium became elevated as early as day one in some compartments within the cells and increased in other parts of the cell within a few days.

Researchers also noted that the elevated calcium levels were associated with decreased activity of the proteasome system, a system within cells responsible for breaking

down unneeded, damaged or misfolded proteins. A distinct feature of ALS is the presence of misfolded proteins in motor neurons.

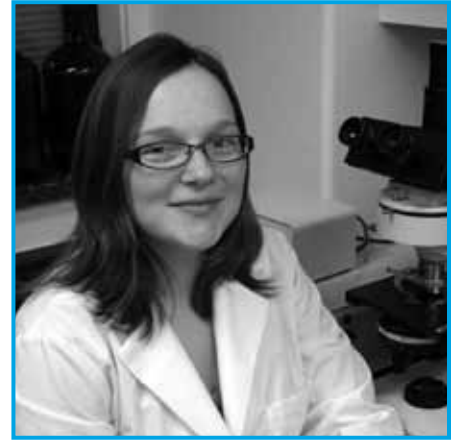
Elizabeth Meiering, PhD, an associate professor in the departments of chemistry and biology at the University of Waterloo, described research in which different types of SOD1 mutations are being studied. The SOD1 protein can occur in various forms in cells and can be susceptible to certain changes as the protein matures. A puzzle about mutant SOD1 is why and how so many different mutations, regardless of where they occur in the SOD1 protein, all lead to protein misfolding in motor neurons. Mutations that cause ALS occur in a variety of sections on the SOD1 protein.

Researchers in Meiering's laboratory developed methods to measure the effects of mutations on folding, unfolding and aggregation activity at different maturation stages

of the protein. They found that mutations have complex and different effects on the different forms of SOD1.

However, all mutations ultimately either increase unfolding or decrease folding of the protein—which increases the aggregation of SOD1. This means different mutant SOD1 proteins can form different kinds of aggregates by a variety of pathways.

“The balance of these pathways may be linked to the different characteristic disease durations that are associated with some specific SOD1 mutations,” said Meiering. Understanding these aggregation processes may eventually help to assess and predict the progression of ALS, and assist in the development of new treatment strategies. [NU](#)



Miranda Tradewell, PhD



Elizabeth Meiering, PhD

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The cornflower, the emblem of the ALS Society of Canada

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