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ALS SLA

the NORTHERN NEURON

AMYOTROPHIC LATERAL SCLEROSIS SOCIETY OF CANADA • SOCIÉTÉ CANADIENNE DE LA SCLÉROSE LATÉRALE AMYOTROPHIQUE

ALS Research in Canada

WALK for ALS is the signature fundraising event of the ALS Society of Canada run in partnership with provincial ALS Societies across the country. WALK for ALS is designed to raise money to support those living with this devastating disease, to raise awareness of the disease, and to raise funds for ALS research. In 2005, 63 walks raised more than $1.7 million. Forty per cent of the proceeds will support the ALS research program and 60 per cent provides local services for those affected by ALS.

The 2005 WALK for ALS was once again supported by McDonald's Restaurants of Canada, national presenting sponsor and Canon Canada, national gold sponsor.

President's Message

Sean McConkey

ALS is a devastating disease that affects thousands of Canadians. This devastation not only affects those living with the disease, but the circle of friends and family that surrounds them. Ninety per cent of care is shouldered by family caregivers and ALS is a costly disease, emotionally, physically and financially.

Funding research for a cure for ALS is the central mission of the ALS Society of Canada. We work toward that goal every day. Our primary research program, the Neuromuscular Research Partnership (NRP), funds health research by providing operating grants in the area of neuromuscular diseases with a mandate to find a cause, treatment options and eventually result in a cure for ALS.

In the second edition of this publication we profile many of the ALS researchers (and their newest projects) whom we fund through the NRP as well as other leading researchers in the neuromuscular field. For the first time, we are profiling many of the new researchers to the ALS community; PhD students who are working closely with experienced clinicians and researchers. In this publication, we are also highlighting the work of clinicians and other ALS researchers who receive funding from a wide variety of sources.

Canadian ALS researchers are on the cutting edge of worldwide research that we believe will one day lead to a cure for this disease. The key to maintaining successful research in the future is to develop new research partnerships, fund excellent and relevant research, cultivate relationships within the ALS community and encourage a new generation of scientists to study ALS.

ALS Canada's research mandate

Our fundraising efforts are geared to finding a cure for this disease. To underline this commitment, we approved, at our national board meeting in the fall of 2004, a new vision statement, which is, "the ALS Society of Canada envisions a cure for ALS."

To ensure our research funds are wisely spent to realize this vision, we have established three guidelines for our funding decisions:

- We invest our research funds where they will have the most impact.
- We fund excellent and relevant peer-reviewed research.
- We fund research that is evaluated at a high level using international evaluation methods adopted by the Canadian Institutes of Health Research (CIHR).

Research forum

In October 2004, we held our first research forum in Toronto to which the top ALS scientists in Canada were invited. Our purpose was to consult with members of the Canadian ALS community to set priorities and direction for our research program. Fifteen researchers attended. We asked each to bring one of their brightest young researchers to the gathering to get them engaged in the research community early on in their career. In this fashion, we seed the future of ALS research. The second forum is planned for the spring of 2006.

Research collaborations

We've expanded our research program and have entered into a number of research collaborations with The ALS Association in the United States. In 2004, we contributed 50 per cent of a grant, led by Dr. Michael Strong, which will investigate cognitive impairment in sporadic ALS. We also contributed funding to the first international research workshop on frontotemporal dementia in ALS, which was held last May in London, Ontario. More than 80 research scientists from around the world attended the workshop.

In 2005, we undertook a second co-funding opportunity with The ALS Association. The recipient is Dr. Jean-Pierre Julien, an ALS Society of Canada board member. The title of his project is "Role of Chromogranin-mediated Secretion of Superoxide Dismutase Mutants in ALS Pathogenesis."

In recognition of the need to "seed the future" with young scientists interested in curing ALS, we have begun to fund promising young scientists with grants of $20,000 per annum for up to three years. Our first two studentships were awarded last fall, and the results of the current competition will be announced soon.

Future directions for research spending

Our commitment is to seed the future with new young researchers. Our next step will be the Doctoral Research Awards which we are undertaking in partnership with CIHR and The Institute of Neurosciences, Mental Health and Addiction. We will fund up to three doctoral research awards in the areas of proteomics, genomics and motor neuron disease relating to ALS. Researchers must be working in Canada to be eligible for this award. The maximum amount awarded will be $21,000 per year per award for up to three years.

We've also compiled a synopsis of ALS and neuromuscular research activity across the country. Listed are the scientist, name of their project, where their funding comes from and a short overview of their research. You can find this new information on the ALS Canada web site at http://www.als.ca/research/researchsynopsis.aspx

The research synopsis is also available in French.

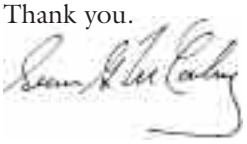
In conclusion

Through the efforts of leading scientists in Canada and around the world, our understanding of ALS has increased dramatically. Combined with the exponential acceleration of advances in neurology and other areas of science, effective therapies and a cure are within reach.

We continue to rely on your help as our battle against ALS gains momentum. You can do this by contributing to the cause though our web site, http://www.als.ca/_donate/ or by calling 1-800-267-4257 ext. 204 to make a donation.

We hope you find this publication helpful. Please feel free to pass it along or request more copies to distribute to friends and family members. Copies of this publication can be downloaded from our web site at http://www.als.ca/research

Thank you.



Sean McConkey, President
ALS Society of Canada

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ALS Society of Canada
265 Yorkland Blvd., Suite 300
Toronto, Ontario M2J 1S5
1-800-267-4257
http://www.als.ca
Email: bg@als.ca

Charitable Registration:
10670 8977 RR0002

Editor:
Bobbi Greenberg
Director of Communications

Writer:
Elvira Orlando

Editorial Review:
Dr. Monique D'Amour
Dr. Chris Shaw

Art Direction/Design/Printing:
Marc Mireault, Haynes Printing

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On the Cover:
An aerial photograph of the Colorado River delta, in the Gulf of California (Aeroservice/SPL/PUBLIPHOTO). Brian Dickie, PhD, Director of Research Development, MND Association, U.K., explains the analogy between the river and ALS (motor neuron disease), "Just as a river has many sources, so motor neuron disease (MND) undoubtedly has many causes. And, just as all the tributaries of a river feed together into one final common pathway, the way a motor neuron dies will have common mechanisms, no matter what the original cause. We are beginning to identify the genetic causes of MND and having this starting point allows us to go into the lab and create new models of the disease, which in turn helps us to follow how motor neurons degenerate from the very earliest stages. As we identify new causes and develop new models, we can begin to cross-reference this information, looking for the points at which these 'degenerative tributaries' meet. If we can identify and understand these pivotal factors, we can potentially develop treatments which will be effective in all forms of the disease."

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An Overview of
ALS RESEARCH

ALS was first discovered by Dr. Jean-Martin Charcot, a French neurologist, in 1869. He gave a talk to his medical students that captures the essence of the ALS Society of Canada. He said "Let us keep searching. It is indeed the best method of finding and perhaps, thanks to our efforts, the verdict we will give such a patient tomorrow will not be the same as we must give this man today." One hundred and thirty-six years later, researchers continue to look for treatment options and ultimately a cure for ALS.

Today, although research is progressing, the disease is still not well understood and there is still no cure. Researchers are investigating several potential causes of ALS. By understanding the mechanisms that trigger this disease, we will ultimately understand ALS and develop desperately needed therapeutic options.

The Neuromuscular Research Partnership

In 1999, The ALS Society of Canada partnered with the Canadian Institutes of Health Research (CIHR), and Muscular Dystrophy Canada (MDC) to create the Neuromuscular Research Partnership (NRP). This partnership was created to collectively fund health research by providing operating grants in the area of neuromuscular diseases with a mandate to find a cause, treatment options and eventually result in a cure for neuromuscular diseases. Each partner contributes an equal share. The benefits of the partnership are significant. By working together, we've been able to lower administration costs, avoid duplication for both researchers and our organizations, and have access to an exemplary peer review process, conducted entirely through the CIHR at no cost to either ALS Canada or MDC.

The NRP allows both ALS Canada and MDC to work as collaborators rather than independents to fund leading-edge research. This partnership is much admired by other voluntary health organizations.

Research granting process

Neuromuscular research grant proposals are submitted to the CIHR, which distributes them to senior researchers. From there the CIHR:

- Assesses and ranks proposals according to criteria for excellence (all identifying information is removed and any researcher submitting an application may not serve on the peer review committee)
- Recommends the funds needed to support research if the application is approved
- Sends a list of proposals to ALS Canada which convenes a panel of experts associated with ALS and MDC

This panel ranks the grants according to relevance to neuromuscular diseases and returns them to CIHR with recommendations.

A final decision about the grants is determined by the CIHR Governing Council and affirmed by our respective boards of directors. The results are then announced and researchers notified. This process ensures the best possible research is being funded.

The CIHR

The CIHR is an arm's-length agency of the government of Canada championing the Canadian research enterprise in four areas: biomedical science, clinical science, health systems and services, and population health. There is a central administration and 13 virtual institutes several of which have close connections to ALS research: Neurosciences, Mental Health and Addiction; Genetics and Musculoskeletal Health and Arthritis. Each institute's task is to develop strategic initiatives to make a difference in its focus area. CIHR oversees the peer review of all grant proposals, ensuring that only those ranking highest in excellence will be funded. This saves our two organizations much time and money and ensures objectivity.

What kind of research is eligible for NRP Funding?

- Basic research
- Focused research
- Applied research

How much is the NRP spending on research?

ALS Canada's joint funding (with MDC and CIHR) through the NRP so far:

2000	$1.20 million
2001	$5.03 million
2002	$3.06 million
2003	$3.98 million
2004	$2.69 million
2005	$3.70 million
Total =	$19.69 million

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How do Canadian researchers link with other ALS research initiatives?

ALS researchers in Canada have always been closely linked. In addition, the International Alliance of ALS/MND Associations (of which ALS Canada is a member) based in the U.K. holds an annual international symposium on ALS research. Many Canadians attend and present research at the symposium. In 2007, Canada will host the 18th annual International Symposium in Toronto. This annual gathering brings together the top scientific and clinical minds in the world to discuss the progress of research towards care and cure for ALS. Having the symposium in Canada will allow us to profile the superb work being done by the Canadian scientific community. And, Canadian ALS researchers and their patients are involved in international drug trials.

What is the future of the NRP funding?

Shortly after the NRP was established, CIHR was reorganized into 13 separate Institutes with an overriding strategic plan and the intention that partnerships and alliances would be formed between health charities and individual institutes. For the last few years, the NRP has been an anomaly in that it has been the only partnership between health charities and CIHR itself. The NRP will continue, though in a new form, through 2006 and beyond. And not just one, but three Institutes are committed to continuing the partnership with us: the Institute of Neurosciences, Mental Health and Addiction, the Institute of Genetics, and the Institute of Musculoskeletal Health and Arthritis.

Electromyography and ALS

Dr. Monique D'Amour

ALS is an extremely difficult disease to diagnose. In the early stages symptoms may mimic other conditions. Because today there is not yet an available ALS-specific diagnostic test, other diseases and conditions have to be ruled out first. However, there are clinical signs that can indicate wasting of motor neurons in either the upper or lower portion of the spine.

In addition to a physical examination, people are often given an electromyography (EMG) test, blood tests, an MRI (magnetic resonance imaging), and other tests to search for the presence of other diseases that look like ALS.

There are numerous ALS clinics across Canada. And, in Montreal, Dr. Monique D'Amour, ALS clinician at the Centre hospitalier de l'Université de Montréal (CHUM) provides medical treatment for people with ALS. She also conducts clinical trials.

"As an electromyographer, I see patients to determine if they have ALS and, if they do, I follow up with them throughout the course of the disease. I became involved with ALS because of the time I spent with people with ALS and the different problems that I witnessed that are related to this disease," explains D'Amour.

EMG, a technique for measuring muscle activity through the electrical signals that muscles produce when stimulated, is often used to confirm an ALS diagnosis following a methodical clinical investigation to rule out other diseases. EMG testing is conducted to confirm lower motor neuron dysfunction in clinically affected regions and to detect evidence of lower motor dysfunction in areas that do not yet appear to be involved. While electromyography is used to diagnose ALS, the diagnosis is based on a process of clinical examination. Some positive findings on electromyography and elimination are used to rule out other diseases.

"We are still looking for an effective treatment for ALS. A curative treatment is needed. We have to continue drug trials for both the cure and the symptoms of the disease," D'Amour explains.

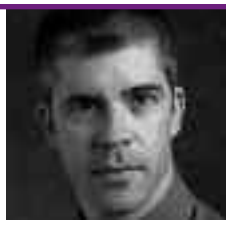
Conducting clinical trials

Frequently, before a new substance can be used in a clinical trial basic research has to be done first on animals, volunteers without ALS, and then in those living with the disease.

D'Amour conducts drug trials at the CHUM clinic. Over the past few years, she has participated with several Canadian physicians in a multicentre Phase IV trial on Riluzole in ALS. The results are being analyzed. Other substances have been and will continue to be studied in this devastating disease.

D'Amour obtained her MD degree from the University of Montreal and studied clinical neurology at the Montreal Neurological Institute, McGill University. In addition to this training, she completed a two-year fellowship in electromyography at Massachusetts General Hospital in Boston. After her return to Montreal in 1976, she began working at the Centre hospitalier de l'Université de Montréal. D'Amour is a member of the ALS Canada board of directors and chairs the Canadian ALS Research Consortium, a group that is comprised of most of the ALS clinicians/researchers in Canada.

Motor Unit Number Estimation



Dr. Tim Doherty

Dr. Tim Doherty and colleagues have developed electromyographic methods that allow them to examine the numbers and health of motor nerves supplying muscles. These methods make it easier for early diagnosis of neuromuscular diseases such as ALS and, in addition, provide a more sensitive examination of therapeutic interventions early on in the disease.

For the past decade, Dr. Tim Doherty of the University of Western Ontario, in London, Ontario has been involved with a group of researchers who use EMG technology to study the numbers and health of motor nerves supplying human muscles. Electromyograms record electrical currents generated in muscles during contraction. This research is relevant to ALS because EMG methods make it possible to assess the number of nerve cells still functioning while the disease progresses.

Doherty is interested in studying ALS because "from a scientific standpoint ALS provides an ideal model for studying how the motor system responds and attempts to adapt to disease."

Doherty and his colleagues have developed a computerized method of Motor Unit Number Estimation (MUNE) that can measure the number of nerve fibers (the threadlike extensions of nerve cells) or motor units in a variety of muscles, ranging from the smaller muscles of the hand to larger muscles such as those of the arm or leg. To develop this method, Doherty has worked closely with Dr. Dan Stashuk, an Associate Professor in Systems Design Engineering at the University of Waterloo. In an initial cross-sectional study, in collaboration with Dr. Michael Strong who is the Chair of Neurology and Director of the ALS clinic at the London Health Sciences Centre, Doherty is testing the applicability of this new method to the examination of the number of motor units in the muscles of people with ALS.

In addition, a longitudinal study is being planned, using the method to monitor disease progression. Doherty's research is also linked to projects with Drs. Richard Lewis in Detroit and Mark Bromberg in Salt Lake City. Cross-sectional studies, also known as "survey" or "prevalence" studies examine a representative sample at one point in time and are designed to provide a broad view of the disease. Longitudinal studies follow the same participants over a period of time.

"To adequately explore new treatments for ALS patients, we need the most sensitive diagnostic and outcome measure possible," says Doherty. "With a quicker and more efficient method of measuring how the motor system responds to ALS, we are better able to provide early diagnosis and measure change in the health of the motor nerves over time. The only way that a treatment can go from merely being an idea to actually being implemented is team work. The image of the scientist working alone in a lab is no longer relevant for many health-related questions," adds Doherty. There are biomedical engineers working on the project, and at any given time five or six graduate students are involved as well.

Doherty has been a faculty member in the Schulich School of Medicine at the University of Western Ontario – where he has a joint appointment in Clinical Neurological Sciences and Rehabilitation Medicine – since 2000. Doherty has had clinical training in rehabilitation medicine, and research training in exercise physiology and neuroscience. He brings a unique perspective and leadership to a research team that includes researchers from clinical and basic neurosciences, rehabilitation, biomedical engineering, and kinesiology. Together, they are working on assessment tools and intervention strategies designed to improve our ability to treat common disorders that effect neuromuscular function.

Doherty's research is partially funded by Compumedics Limited, an Australian-owned manufacturer of medical diagnostic equipment. Additional funding is provided by the CIHR, the Natural Sciences and Engineering Research Council of Canada and the Canada Research Chairs Program.

Doherty awarded Tier 2 Canada Research Chair

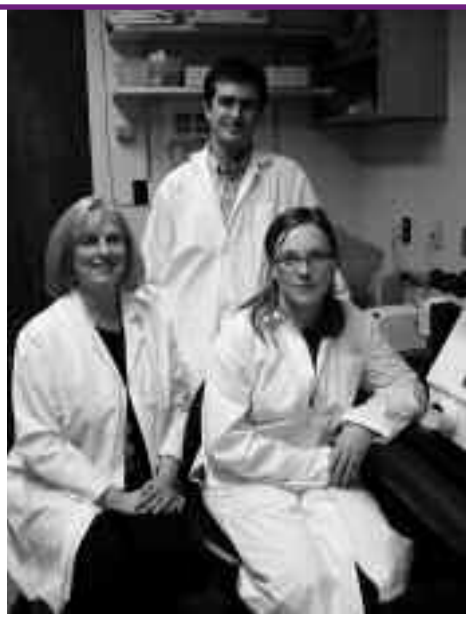
Doherty was recently awarded a Tier 2 Canada Research Chair in Neuromuscular Function in Health, Aging, and Disease, at The University of Western Ontario. His research will lead to the development of new diagnostic tools and therapeutic interventions for those with neuromuscular diseases.

Tier 2 Chairs, tenable for five years and renewable once, are for exceptional emerging researchers, acknowledged by their peers as having the potential to lead in their field. For each Tier 2 Chair, the university receives $100,000 annually for five years.

Canada Research Chairs are awarded to researchers acknowledged as world leaders in their field. The government of Canada program allows researchers to gain access to state of the art research facilities and to mentor and train the next generation of graduate students.

Advancements in ALS Research at the Montreal Neurological Institute, McGill University

Dr. Heather Durham seeks to understand the mechanisms responsible for motor neuron diseases, in particular ALS. The genes responsible for familial forms of motor neuron diseases are being mapped which facilitates the establishment of cell culture and animal models for studying the disease and developing therapies.



Seated left to right: Dr. Heather Durham and Miranda Tradewell. Standing: Edor Kabashi

"ALS research is the primary focus of our laboratory at the Montreal Neurological Institute (MNI), McGill University, Montreal, Quebec," says Dr. Heather Durham. "The overall goal is to understand how cells deal with stresses that contribute to disease and why motor neurons are so vulnerable to damage in ALS and other motor neuron diseases.

Toxic proteins can be generated through genetic mutations or through damage inflicted by the cellular environment, including by free radicals (unstable, very reactive molecules)," Durham explains. These alterations can result in the misfolding of proteins, compromising their function and promoting interactions that cause them to aggregate and even disrupt the function of other proteins. Accumulation of misfolded proteins in cells is a common feature of neurodegenerative diseases, including ALS. After proteins are synthesized, they must take on the right shape in order to function. Cells have specific mechanisms to repair or remove misfolded proteins, but if abnormal proteins escape these controls, they can accumulate, clump together, and poison the cell.

"Two systems are important for eliminating protein garbage: protein chaperones and proteasomes. Chaperones sequester damaged proteins and shuttle them to proteasomes, which chop them up and dispose of them, explains Durham." But motor neurons don't do a good job of increasing the levels of chaperones and proteasomes to meet demand. A proteasome is a large protein complex that helps to destroy other cellular proteins when they are no longer needed or are damaged.

In fact, chaperoning and proteasome activity are compromised in a transgenic mouse model of ALS due to mutation in SOD1 but only in the region of the spinal cord that is most affected in the disease. Instead of making more chaperones and proteasomes to meet the increased load, motor neurons end up having less. The function of these systems also appears compromised in the surrounding cells.

"We need to understand what happens to disease-causing proteins in cells and find drugs that will help cells efficiently dispose of misfolded proteins before they accumulate and wreck their havoc. Protein chaperones and proteasomes are important in many cellular activities. Once their function is compromised, so many pathways are disrupted that intervention is difficult," she explains.

Durham's group is investigating why mutant SOD1 is handled differently in vulnerable tissues compared to those that are resistant to damage and whether similar mechanisms contribute to sporadic ALS. Drugs are being sought that can boost levels of chaperones and proteasomes in cells that need them. Dr. Jeffrey Agar, who is a member of the team (Agar is now Assistant Professor at Brandeis University, but still part of the team) has developed techniques to isolate mutant proteins from small tissue samples and is comparing their structure in vulnerable and resistant tissues using advanced techniques of mass spectrometry. Edor Kabashi, a PhD student, is examining differences in the composition and activity of the proteasome. Another PhD student, David Taylor, is focusing on the mechanisms that control the levels of protein chaperones and proteasomes to determine why they are not being activated in vulnerable tissue.

"How a cell responds to stress may be as important as the stress itself in determining whether it succumbs," Durham explains. "The researchers have developed a model for studying the familial forms of motor neuron disease in tissue culture, and have identified aspects of the normal biology of motor neurons that likely contribute to the course of the disease. In culture, we block neurotransmission to motor neurons and dramatically reduce the toxicity of several mutant proteins." Dysregulation of calcium in motor neurons is a key aspect of the mechanism by which the neurotransmitter – glutamate – promotes protein toxicity.

When glutamate binds to glutamate receptors on motor neurons, there is an influx of calcium ions into the cell. "Motor neurons express a particular type of glutamate receptor. These receptors lack a particular component called 'GluR2.' The result is that calcium ions enter the cell through these receptors during neurotransmission. Also, motor neurons are deficient in certain proteins (calbindin, parvalbumin) that mop up extra calcium. These cells live on the edge, where calcium is concerned," says Durham.

Giving cultured motor neurons proteins that reduce calcium entry or mop up calcium protects them from toxicity of mutant proteins, including aggregation and formation of

inclusions. Using advanced imaging techniques, Durham lab members PhD student Miranda Tradewell and Dr. Rebecca Aarons are investigating how neurotransmission and calcium relate to dysfunction of the proteasome and mitochondria (structures in cells that turn nutrients into energy) in motor neurons expressing mutant proteins that may cause disease.

"By getting to know motor neurons and understanding their vulnerabilities," says Durham, "we can devise ways to help them survive in the face of the multiple stresses that cause ALS and contribute to their demise."

Durham's educational background includes an undergraduate and graduate degrees from the University of Western Ontario and a PhD (Pharmacology) from the University of Alberta. She was a post-doctoral fellow in toxicology at the Department of Pharmacology and Therapeutics at McGill University.

The Durham lab receives funding from the NRP, The ALS Association and Muscular Dystrophy Association (U.S.).

Two Studentships Granted

The ALS Society of Canada has created a new program to fund young researchers as they embark on their careers in ALS research. ALS Canada has awarded two recurring annual grants of $20,000 per year (for up to three years) to ensure that the scientific community in ALS will be populated in the coming years. The ALS Society of Canada partnered with the CIHR and Aon Reed Stenhouse for the studentships. CIHR reviewed the students' applications for scientific merit and excellence. ALS Canada and Aon Reed Stenhouse provide funding for the studentships. Co-recipients Edor Kabashi and Miranda Tradewell are working under the direction of Dr. Heather Durham at the MNI.

The Role of Calcium Ions in ALS

PhD candidate Miranda Tradewell received one of the ALS Society of Canada's first two studentship awards for her thesis on "The Role of Calcium in Motor Neuron Disease." Tradewell, who works with Durham at the MNI, has been awarded three years of funding through this new program. The first year of funding for Tradewell's studentship is funded by Aon Reed Stenhouse.

Tradewell's research seeks not only to examine the cause of motor neuron diseases such as ALS, but to understand why, in ALS, motor neurons become vulnerable to the toxicity of mutant proteins. Her particular focus is on the role of calcium ions. Previous studies in Durham's laboratory have shown that a key factor in motor neuron vulnerability is the entry of calcium ions as a result of certain types of neuron-to-neuron communication. While this neurotransmission is not in itself toxic, it appears to accentuate cell death in models of ALS.

Tradewell will be seeking to understand how the activation of glutamate receptors makes motor neurons more sensitive to the toxicity of mutant SOD1, a genetic cause of some forms of ALS, and to determine whether this sensitivity also occurs in other forms of motor neuron disease.

Glutamate is the key neurotransmitter other neurons use to excite motor neurons and other neurons in the nervous system. This small molecule works by binding to receptor proteins on the surface of motor neurons, opening channels through which certain ions flow and initiate an electrical impulse called the action potential. The activation of certain types of glutamate receptors can lead to the entrance of calcium into the cell, which can be toxic to neurons even at low levels under disease conditions. To further examine mechanisms underlying glutamate toxicity in ALS and other motor neuron diseases, Tradewell will be using live cell imaging in culture models of ALS to follow where this calcium goes in motor neurons and how it affects the function of key organelles, such as mitochondria, and the protein-handling machinery.

Her work may also prove important to the study of other neurodegenerative diseases, such as Parkinson's and Alzheimer's, in which neuronal death may be the result of similar processes.

The Durham laboratory, which has developed a unique culture model of familial ALS, is one of the few laboratories in the world where primary motor neuron cultures are studied, providing Tradewell with a unique opportunity to examine motor neurons in vitro.

Tradewell, who completed her undergraduate degree in biochemistry with distinction in 2001 at the University of Victoria, plans to continue her research in neurodegenerative diseases.

Problems with Protein Disposal in ALS

Edor Kabashi would like to contribute to a better understanding of how ALS kills motor neurons so that new therapies can be developed to help people who suffer from this catastrophic disease.

He is currently pursuing a PhD in Neurology and Neurosurgery at McGill University. Kabashi has been awarded two years of funding from the ALS Society of Canada for his research project "Problems with Protein Disposal in ALS."

SOD1 was identified in 1993 as the gene responsible for one of the familial forms of the disease (fALS). The protein encoded by the normal SOD1 gene is important in detoxifying superoxide – a harmful byproduct of the cell's normal metabolism. However, when a mutation in this gene occurs – as in one familial form of ALS – the resulting protein readily misfolds and assumes a new, toxic function that is not completely understood. Kabashi's hypothesis is that the mechanism for disposing of abnormal proteins is compromised in both the motor neurons and in the surrounding spinal cord cells. The result is accumulation of protein aggregates and disruption of cellular function on

continued on page 8...

multiple fronts.

"Cells have a special disposal unit called the proteasome, whose normal function is to rid the cell of damaged or abnormal proteins as well to regulate the turnover of normal proteins," Kabashi explains. "I measured the function of the proteasome in cultured cells and in the spinal cord of transgenic mice expressing the mutant human SOD1 gene. What I found was that there was impairment of proteasome function long before the appearance of ALS-like symptoms in the transgenic mice."

Kabashi's research to date has shown that mutant SOD1 impairs the ability of proteasomes to break down other proteins. In the next phase of his project he will try to determine why proteasome function is compromised and how this contributes to the death of motor neurons. A more thorough understanding of this mechanism of the pathogenesis of ALS could lead to new therapeutic agents to help people with this disease.

Kabashi obtained his B.Sc. (Biology) degree with distinction, in 2001 at McGill and plans to continue his research in neurodegenerative diseases.

A Model Researcher

Marie Gingras

PhD student Marie Gingras first became interested in researching ALS after visiting Dr. Heather Durham's laboratory at the Montreal Neurological Institute, McGill University, to learn more about neurons for her research.

"I am also interested in researching ALS because of the devastating nature of the illness," says Gingras. She is currently working with Dr. François Berthod, a researcher and a professor at Laval University in the Department of Surgery, Faculty of Medicine, on a project that is designed to develop a tissue-engineered model of the spinal cord for ALS research.

"Our lab specializes in tissue engineering," Gingras explains. "We want to study ALS through an in vitro model, using cells from mice, by overexpressing the mutated human SOD1 gene. However, before working with cells from those mice, we first make an in vitro model of a reconstructed spinal cord, using cells from normal mice – this reproduces a highly physiological environment." The biological material used for the models is made from collagen.

The objective of the project is to develop a three-dimensional model of a reconstructed spinal cord so that the interactions between different cell types and motor neurons can be studied.

"With the three-dimensional model, we can insert the spinal cord cells that are implicated in ALS. There is now new insight that healthy cells surrounding motor neurons that express the mutated human SOD1 can prevent them from dying and may stop ALS from developing, so the degeneration in motor neurons seen in ALS may require damage from mutant SOD1 in supporting cells," says Gingras. Using the model that has been developed, researchers will likely be able to discern which type of cells – or which combinations of these cells – are the cause of motor neuron death in ALS.

This type of model may be useful to other neurodegenerative diseases as well. "You would only have to change the type of cell that is being inserted into the tissue, and other diseases can be studied." Gingras explains. She is collaborating with Dr. Heather Durham of the Montreal Neurological Institute on this project.

Gingras co-authored a research paper - "In vitro development of a tissue-engineered model of peripheral nerve regeneration to study neurite growth"- that was published in 2003 in *The Faseb Journal*, a Maryland-based American publication. It explains the process by which Gingras and her fellow researchers developed the first three-dimensional tissue-engineered culture system that supported nerve regeneration.

Gingras has a studentship from the Fonds de la Recherche en Santé, and funding for the project is provided by a research grant from Muscular Dystrophy Association (U.S.). Her undergraduate degree, a B.Sc. (Biochemistry) was completed at the University of Montreal in 2000. Gingras then obtained a Master's degree in Cellular and Molecular Biology at Laval University, where she is currently pursuing her PhD. In the future, she would like to continue working on ALS.

The Role of Schwann Cells in ALS

Dr. Tessa Gordon

Dr. Tessa Gordon studies how nerves regenerate following injury. Her basic research has led to important insights into the pathways and progress rates of ALS.

Dr. Tessa Gordon and her research team at the University of Alberta, located in Edmonton, have been examining the loss of function and cell death in ALS, with particular emphasis on the capacity of the sick neurons to sprout axons (a long, threadlike extension of a nerve cell that carries a message to the next nerve cell) to compensate for the progressive disconnection of motor nerves from their muscles and the consequent paralysis.

Gordon has spent time focusing on Schwann cells and their relationship to ALS. Schwann cells are a type of non-neuronal cell that wrap around nerve fibers in the peripheral nervous system, forming the myelin sheath. Myelin is an insulating layer that forms around nerves. It is made up of protein and fatty substances. The purpose of the myelin sheath is to allow rapid and efficient transmission of impulses along the nerve cells. If the myelin is damaged, the impulses are disrupted.

The cells are named after the German scientist Theodore Schwann (1810-1882), who discovered them.

In the observed ALS models, Gordon and her graduate student, Janka Hegedus, made the exciting finding that many of the Schwann cells in the muscle failed to respond as they normally do in the adult. These Schwann cells, Gordon explains, are essential for the normal compensatory process of axon sprouting where motor nerves reconnect with muscle cells that have lost their own nerves. The sprouting normally occurs during aging as well as after nerve injuries, but does not appear to occur normally in ALS. Gordon outlines how the process of aging is an example of Schwann cells at work as the body ages. Schwann cells guide nerve sprouts to reconnect to muscle cells, thereby preventing muscle wasting. In ALS, where motor neurons progressively die, the remaining motor neurons cannot connect to the muscle cells, explaining the rapid muscle weakness that is seen in people with ALS.

The fact that Schwann cells do not respond normally in ALS partially explains the muscle weakness and other symptoms of the disease. Gordon wants to examine why these cells are abnormal in ALS. Instead of functioning normally to guide axon sprouts to disconnected muscles, the Schwann cells appear to be sluggish in their response. Why? Perhaps the normal communication of these Schwann cells with the motor nerves and the muscle fibers is lost in the milieu of the "sick" motor neurons.

Gordon is a Heritage Medical Scientist at the University of Alberta. She has received funding from the Medical Research Council of Canada, the Canadian Neurotrauma Research Program, the NRP, and the Paralyzed Veterans of America Spinal Cord Research Foundation. Gordon's educational background includes an M.Sc. and PhD in Physiology from The University of Birmingham (U.K.).

Preferential motor neuron loss in ALS

Janka Hegedus

In her PhD thesis, Janka Hegedus characterizes the progressive loss of motor neurons and their muscle fiber connections in ALS. A student in Gordon's laboratory, Hegedus says, "I am especially interested in learning why they die so relentlessly in ALS." Motor neurons normally send their nerves to muscles, where they then branch to supply many individual muscle cells, producing muscle contractions and movement. A process called axonal sprouting can compensate for lost motor-nerve connections. When normal nerve-muscle connections are interrupted, (for example by nerve injuries) the remaining motor neurons with muscle contacts 'sprout' new nerve branches that contact the muscle fibers that have lost their original nerve contacts. By this process, each surviving motor neuron is able to supply many more muscle fibers than normal.

Usually, by the time of definitive diagnosis, a person with ALS has already lost more than half of their spinal cord's motor neurons. Hegedus and her laboratory colleagues are trying to understand the process of this loss by exploring the progressive disconnection of nerve and muscle in a mouse model of ALS, both before and after the onset of disease symptoms.

In the mouse model, muscle weakness is apparent at approximately 90 days of age. Hegedus has found that the nerve-muscle connections are lost before the onset of weakness. Further, she has found that the motor neurons are affected in a specific order, with the largest motor neurons, those that normally contact the greatest number of muscle fibers, losing their contacts first, as early as 50 days before symptom onset. She has found that the smaller motor neurons, which have fewer nerve-muscle connections, maintain their connections until much later.

In examining the muscles of pre-symptomatic ALS mice, Hegedus found little evidence of new compensatory nerve-muscle connections being made. The ALS mouse model's remaining motor neurons do not branch out to supply more muscle fibers than usual. This important initial finding has led to additional discoveries concerning axonal sprouting and the role of Schwann cells in ALS.

Hegedus completed her B.Sc. at the University of Alberta in 2001 and began her PhD with Gordon that same year. Other ALS researchers at the

continued on page 10...

Hegedus continued

University of Alberta include Drs. Sanjay Kalra and Ming Chan.

Funding for this project has been provided by the NRP. Hegedus has also received funding from the Natural Sciences and Engineering Research Council and the Alberta Heritage Foundation for Medical Research.

Deciphering Programmed Cell Death in Motor Neurons

Dr. Jeff Henderson

Since ALS was first identified by French neurologist Jean-Martin Charcot in 1869, researchers have identified a number of features of motor neuron degeneration. These include abnormal glutamate metabolism, elevated intracellular free radicals, cytoskeletal proteins dysfunction, and the accumulation of abnormal protein deposits within affected motor neurons. It is not clear, however, if any of these features cause motor neuron death. Indeed, there is evidence that these different forms of cellular injury are the result of motor neuron destruction in the brain and spinal cord through a common process called PCD, or programmed cell death. The identification of the ALS genes SOD1 and ALS2 (Alsin), which encode proteins with different cellular properties, suggests that the neural injury seen in ALS is different, and that it can be initiated by different types of cellular stimuli.

Dr. Jeff Henderson, Assistant Professor at the Leslie Dan Faculty of Pharmacy, and Director of the Murine Imaging and Histology Core at the University of Toronto, studies the process of PCD in motor neurons of living mammals. In his laboratory he investigates how neurons of the brain are damaged during acute injury or in degenerative diseases such as ALS. "The overall goal is to manipulate the processes of PCD so that the cellular death which accompanies these forms of injury no longer occurs," says Henderson.

Henderson's model to study motor neuron injury and repair within the central nervous system is mice. Why mice? The benefits of using mice are twofold. First, the biology of the motor neurons which are responsible for movement is virtually identical in mouse and man. Second, the genetics of mice allow Henderson and his team to make specific modifications in individual genes, enabling them to assess the effect of a given gene on motor neuron survival following injury. Henderson uses gene modification procedures (the creation of transgenic, "knockout", and Cre recombinant mice) to identify the role which individual cell death proteins play in a given genetic pathway as it really occurs in living motor neurons in mammals such as man.

To augment their studies in living animals, Henderson's laboratory also examines the process of PCD in primary neural explants – slices of nervous system tissue which are kept alive outside the body. His laboratory is developing innovative high-throughput culture and transfection methods for use with these neural explants to dissect the process of PCD in greater detail.

To help look inside the brain and spinal cord of living ALS-like mice to see the process of neural degeneration as it occurs, Henderson has, together with U of T's Dr. Mark Henkelman, developed new procedures to non-invasively scan the mouse brain at high resolution and perform detailed 3D morphologic analyses which lets him compare the neuroanatomical changes of mouse brain and spinal cord cells with those of people with living with ALS. The objective of this work is to both better characterize the structural changes which occur in PCD mutants, and to support the laboratory's ongoing neuroanatomic analysis of new ALS-like mutants, generated at the Centre for Modeling Human Disease at Mount Sinai Hospital.

The unique combination of Henderson's innovative methods is being utilized by few other neuroscience centres in North America. His work is supported by the CIHR and the NRP.

The ultimate aim of these studies is to develop therapeutic compounds which will protect motor neurons from programmed cell death. As a number of different stimuli (such as glutamate, and reactive oxygen species) can trigger the processor PCD, it is expected that the development of such compounds would be capable of inhibiting neural cell death resulting from a wide range of stimuli, including ALS.

Henderson's graduate studies in biochemistry and molecular biology were completed at California State University, (Fullerton). He obtained his PhD in biochemistry at the University of Illinois at Chicago.

Over the past decade, Henderson and others have made progress in identifying the protein families and pathways involved in regulating each phase of PCD. The current challenge is to identify which of the identified PCD pathways is dominant within a given cell type – i.e., motor neurons – following a particular form of injury. "We've developed methods to delete the key player(s) in each of the candidate PCD pathways. The race is on to determine which components of these pathways are critical for controlling the forms of PCD which occur in motor neurons during ALS," explains Henderson.

Unusual Suspects

Canadian ALS researcher Dr. Jean-Pierre Julien investigates the role of rogue proteins in ALS

Dr. Jean-Pierre Julien

Dr. Jean-Pierre Julien seeks to discover and demonstrate the molecular and cellular mechanisms of ALS that contribute to the loss of motor neurons. His research will have important implications for the discovery of new therapeutic targets and for the development of more effective treatments for neurodegenerative diseases.

"Provocative" is the word being used to describe Dr. Jean-Pierre Julien's newest research project, supported co-operatively by The ALS Association (U.S.) and the ALS Society of Canada. Julien, a leader in ALS research at Laval University, Quebec City, will use the grant toward a new mouse model to study a formerly unknown ALS pathway. "Role of Chromogranin-mediated Secretion of Superoxide Dismutase Mutants in ALS Pathogenesis," is the title of the research grant.

It was 1993 when researchers discovered the gene responsible for the inherited or "familial" form of ALS. The discovery held promise because it allowed researchers to genetically engineer mice that model familial ALS. The mouse models allow scientists to study the pathway of the mutant SOD1 protein produced by the faulty gene. But after more than a decade of research into how SOD1 causes damage within the nerve cells, the link between SOD1 and ALS remains unexplained. Now, Julien and his team have discovered a tiny hint that suggests researchers may have been looking in the wrong place all along.

"Everyone assumes that the defect is within the nerve cell," says Julien. "We are proposing something very different – that it is not within, but that it is extracellular. The SOD1 mutant would be secreted *outside* the cell and this would cause the damage."

The hint came from protein molecules called chromogranins. Chromogranins are made by nerve cells. They usually help to make and package other proteins. Julien's team detected the chromogranins binding to the mutant SOD1 protein in the spinal cord of ALS mouse models and in cultured nerve cells in the laboratory. The location of the bound proteins was a surprise: instead of appearing in the guts of the cell, the proteins were in vesicles that secrete materials from the cell to the outside.

"We wondered what to do with this, because it presents in the secretory pathway," says Julien. "So we did further research and found that we can indeed detect the mutant with this protein in the cells. We are now asking what the consequence is of the secretion of this protein together with the mutant SOD1."

The next step is to observe the behavior of the chromogranins in SOD1 mice. "In culture, if we produce more chromogranins, more SOD1 becomes secreted," says Julien. "But in vivo [live animals], we don't know. We cannot go further in culture."

Mouse models are one of Julien's particular areas of expertise. Over the past decade, he has studied mouse models of the familial form of ALS, and recently developed a novel mouse model to study the juvenile form of the disease.

To investigate the role of chromogranins in the disease process, the team will use specially engineered viruses to increase the production of chromogranins in the spinal motor neurons of the mice to see if their condition worsens. In other mice, they will under-produce the chromogranins to address the alternative: Does the condition lessen?

Julien is optimistic about where the research might lead, even if the new pathway raises a few eyebrows. "It is a very different concept but we have good data to support it," he says. "It is a new avenue."

Holder of a doctorate in biochemistry and possessing special expertise in neurobiology Julien is a board member of the ALS Society of Canada. His research is supported by the CIHR, the NRP, The ALS Association, The Robert Packard Center for ALS Research at Johns Hopkins and Réseau de Recherche en Transgénèse du Québec.

Canada Research Chair in the Mechanisms of Neurodegeneration at Laval University (Tier 1)

As Canada Research Chair in the Mechanisms of Neurodegeneration, Julien will devote most of his time to studying the mechanisms implicated in the selective loss of motor neurons. One aspect of his research seeks to better understand the deleterious effects that occur when neurofilaments accumulate on intracellular transports. Julien will also study the role of inflammation in the pathogenesis of ALS.

Tier 1 Chairs, tenable for seven years and renewable, are for outstanding researchers acknowledged by their peers as world leaders in their fields. For each Tier 1 Chair, the university receives $200,000 annually for seven years.

Biomarkers and ALS

Dr. Sanjay Kalra

Magnetic resonance spectroscopy (MRS) is being used to examine the chemical composition of the brain in ALS. The data collected will improve our understanding of ALS. Eventually, it may allow the diagnosis of ALS and the evaluation of drug therapies to be made more efficiently. MRS is a technique that can measure various chemicals in the brain. Both MRI and MRS use the same scanner.

Techniques based on MRI have the potential to improve our understanding of ALS and the way that clinical trials are done. MRI is a tool that can be used to examine the structure of the central nervous system. And, Dr. Sanjay Kalra, a neurologist at the University of Alberta, is exploring this ground-breaking technology.

MRI systems with high magnetic field strengths are becoming more common. There is a lot of evidence implicating altered neurotransmitter functioning as an important mechanism responsible for the death of neurons. Neurotransmitters are chemical substances that communicate impulses from one nerve cell to another. The ability to measure these neurotransmitters using MRS will be very useful to further our understanding of what is happening inside the body of a person living with ALS.

As the technology improves we will be able to measure more compounds with greater accuracy. Magnetic resonance techniques are non-invasive and have no side effects or risk of injury; doing studies repeatedly is completely safe.

"I use MRI technology to learn more about what could be the cause of ALS and to find biomarkers," explains Kalra. Biomarkers, also known as surrogate markers, are biologically-derived indicators that reflect the extent of disease without having to physically examine tissue. Kalra is studying various chemicals measured with MRS as potential biomarkers to detect and monitor the progression of ALS.

"One of the greatest hurdles clinicians and researchers face is the lack of a definitive test for ALS," says Kalra. This is one of the reasons why it is not uncommon for the diagnosis to be made 12-18 months after symptoms begin, and by that time there is already significant damage to the nervous system. An earlier diagnosis would mean that the person with ALS could start treatment earlier and participate in clinical trials sooner. Kalra makes a comparison with other diseases, such as diabetes, where having an appropriate test makes a huge impact on patient management and the search for new treatments.

The lack of a test to accurately quantify the extent of the disease means that one has to rely on indirect measures such as muscle strength as indicators of effectiveness of new drugs in trials. These trials are lengthy and involve many patients.

"It is very disheartening to tell a patient after 12 months in a study – 'Sorry, the drug didn't work'. That is a long time for a person with ALS who only has a few years to live," says Kalra. By that time the disease has likely progressed too far for the person to participate in another trial. Kalra hopes that his techniques will speed up drug studies, allowing more drugs to be screened faster and with fewer subjects; this could lead to the discovery of an effective drug sooner. MRS could also assist in the fine-tuning of treatment for individuals, just as glucose measurements do for the diabetic.

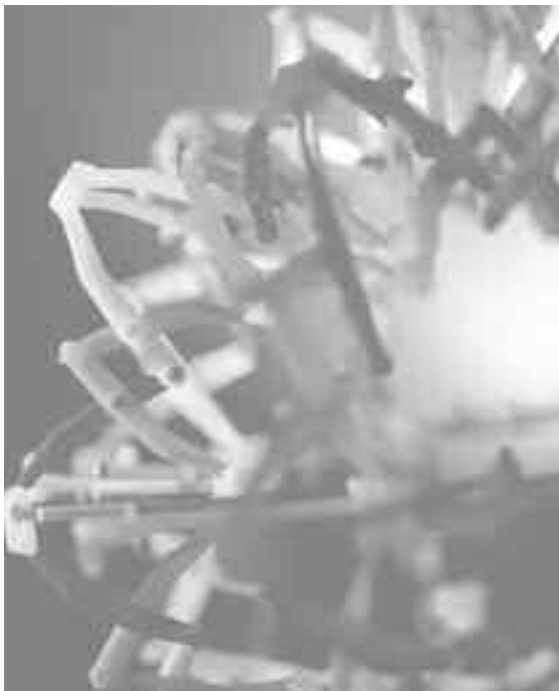
The people affected by the illness were a strong motivation for Kalra to make ALS his focus. "You see them regularly and really get to know them. They've taught me a lot about life," he says.

Kalra continues to focus on ALS, specifically on the development of biomarkers using MRS and other imaging techniques. He is also interested in the study of other clinical areas in ALS, such as cognitive impairment. While he would like to work with animal models of the disease in the future, Kalra expresses the privilege that he feels in having had the opportunity to work directly with people.

Kalra obtained his B.Sc. and MD degrees at the University of Toronto, followed by neurology residency training at McGill University. During his residency, Kalra started a research project using MRS to study drug effects in ALS. He stayed to do a fellowship at the Montreal Neurological Institute in the MRS Unit to continue his study of ALS using MRS and other advanced magnetic resonance techniques.

During his residency Kalra had not expected to be eventually working in the ALS field. "Once I started, I became more and more hooked on ALS as the project developed. At the biological level, the disease itself fascinated me. It is so simple in the sense that motor neurons are the prime targets, but the manner in which motor neurons degenerate is very complex and not well understood," he explains.

Kalra's research is funded by the University of Alberta Hospital Foundation and the MSI Foundation of Alberta.



Inflammatory Cells in ALS

Dr. Charles Krieger (l)
Dr. Fabio Rossi (r)

Since obtaining three years of funding from the NRP in 2004, Drs. Charles Krieger and Fabio Rossi have focused on the role of microglia, inflammatory cells of the nervous system in ALS. These inflammatory cells are being examined in a mouse model of ALS. The mice have mutant human superoxide dismutase (mSOD) that causes the development of a disease like ALS.

"In addressing the issue of how these inflammatory cells get into the nervous system of the mice, we have completed the first series of experiments examining the distribution of green fluorescent protein-labelled bone marrow cells in spinal cords of recipient mSOD mice," explains Krieger, a neurologist at Simon Fraser University and Acting Director of the ALS Centre at the GF Strong Rehabilitation Centre in Vancouver.

Krieger and Rossi found that the transplantation of green fluorescent protein-labelled bone marrow cells did not affect the rate of disease progression in mSOD mice. These mice developed their ALS-like illness at the same rate as mice which were not given a bone marrow transplant.

Mean numbers of microglia and green fluorescent protein (GFP+) cells in the spinal cords of control mice were not significantly different from those in mSOD mice. The number of green fluorescent protein-labelled cells and microglia within the spinal cord of mSOD mice increased as compared to age-matched controls at a time when mSOD mice exhibited disease symptoms, continuing to the end-stage of the disease. Green fluorescent protein-labelled bone marrow-derived cells and microglia were more numerous in motor neuron-associated areas in the spinal cords of the mice, suggesting that these are targeting areas of neuron degeneration.

Although an increase was observed in the number of GFP+ cells in spinal cords of mSOD mice with disease symptoms, mean numbers of GFP+ microglia (microglia derived from the bone marrow) comprised less than 20 per cent of all microglia and did not increase with disease progression.

In this work it was demonstrated that the microglia present in spinal cord tissue of mSOD mice (and presumably in people living with ALS) is primarily explained by an expansion of resident microglia and not the recruitment of cells that ultimately will become microglia from the circulation. "However," states Krieger, "there is evidence for some modest recruitment of cells that will ultimately become microglial cells from the circulation in mSOD mice."

The research was presented as an abstract at the Society for Neuroscience meeting in November 2005. Together with colleagues, Krieger was involved in a presentation to those living with ALS and their families and caregivers on November 13, 2005 in Surrey, BC that was organized by the ALS Society of BC. There is much interest in the possibility of using bone marrow-derived cell therapy in ALS and Krieger described what is possible and what is not at the present time. Based on the current work Krieger says that, "from our data there is no reason to think that bone marrow-derived cells could enter the nervous system and turn into neurons. However, this question is of such importance that we will continue to do further experiments to investigate."

Krieger and Rossi also receive funding for their work through the Natural Sciences and Engineering Research Council of Canada and the CIHR.

Krieger received his M.Sc. at the University of Montreal, his MD from the University of Toronto, and PhD from the University of London. His current research interests also include the evaluation of the roles of protein and lipid kinases in a cell culture model of excitotoxicity and the determination of the role of protein kinases in an animal model of motor neuron disease. Protein kinases are enzymes that modify many proteins in the body and have wide-ranging effects.

In addition, Krieger is the Principal Investigator of a study at the GF Strong Rehabilitation Centre entitled *The Genetics of Amyotrophic Lateral Sclerosis (ALS) and Other Motor Neuron Diseases.* This study will examine SOD1 mutations in individuals and families with ALS/Motor Neuron Disease in British Columbia.

Rossi – Canada Research Chair in Regenerative Medicine (Tier 2), Michael Smith Foundation for Health Research Fellow and stem cell expert – is an Assistant Professor at the University of British Columbia. He received his MD from University of Genoa in Italy and his PhD at the European Molecular Biology Laboratories in Germany. Rossi is renowned for his work with adult stem cells and has shown that circulating hematopoietic stem cells can contribute to the regeneration of peripheral tissues such as skeletal, muscle, heart and liver.

Etymology of ALS

Amyotrophic comes from the Greek language

A- absence of ***myo*** - muscle ***trophic*** - nourishment ***Lateral*** - side (of spine) ***Sclerosis*** - hardening

Nesca: a Novel Intracellular Signaling Adapter

Dr. Susan Meakin

As the Director of the Laboratory of Neural Signalling at the Robarts Research Institute in London, Ontario, Dr. Susan Meakin's research efforts have been geared towards a better comprehension of the complexity of neuron growth and development. Emphasis has been placed on the identification of new cellular mechanisms that make this process possible. The objective of the grant that Meakin has been awarded by the NRP is to determine the mechanism(s) which facilitate a novel intracellular (inside the cell) protein that Meakin and her group have identified – Nesca – to facilitate the process of neuronal growth.

'Nesca' stands for "new molecule containing an SH3 domain at the carboxy-terminus." Named by a Japanese group who recognized that the protein had the characteristics of an intracellular signaling molecule, although its role was not clear at the time. The term carboxy-terminus refers to the region at the end of a protein. Meakin and her group are the first to describe Nesca's role.

"Nesca has been observed to promote neuronal survival and neurite outgrowth, initially in a cell model line termed PC12 and then in primary cortical and sensory neurons. It is hoped that these data will facilitate future efforts to promote neuronal survival, re-growth and/or function following neuronal injury," explains Meakin. "The mechanisms causing motor neuron loss and how this loss may be delayed or repaired are not completely understood," she states, outlining the relevance of her research to ALS. "In fact in many cases, axons degenerate or retract prior to the onset of death in the cell body. Consequently, research directed at understanding the underlying mechanisms regulating neuronal survival and axonal growth are absolutely essential to develop and test new approaches to intervention."

In her initial description of Nesca – published in the *Journal of Cell Biology* in March 2004, Meakin described how the protein moves from the cytosol to the nucleus in response to growth factor stimulation. Cytosol is fluid contained within the cell, in which most biochemical reactions take place. Meakin explains how over-expression of Nesca can greatly increase the length of neuronal growth. "Of the four aims outlined in the application, we have made considerable progress on three of them."

Meakin and her research team have demonstrated that the three different domains or region called RUN, SH3 and leucine zipper (LZ) that are found on Nesca are essential to neuronal growth. The RUN domain is fundamental to the nuclear re-distribution process, while the SH3 and leucine zipper domains are essential to the facilitation of neurite outgrowth once Nesca is in the nucleus.

The term leucine zipper refers to the appearance of a repetitive number of residues in a protein that predicts that the leucine-rich region of the protein will have a particular conformation. Leucine zippers are usually involved in mediating protein to protein interactions; therefore, it is possible to predict that this region of Nesca will be involved in its ability to interact with another protein in the cell. This predicted model can then be tested to determine which molecule(s) the leucine zipper region binds to.

"We have recently addressed the functions of the SH3 and LZ domains by mutation studies. Essentially, when Nesca is missing the SH3 domain, both the process of Nesca's transport to the nucleus and the process of neuronal growth are delayed. By contrast, the replacement of three leucine residues in the zipper region disrupts the structure of the LZ, a conserved region within certain classes of nuclear proteins which mediates interactions between proteins," says Meakin. This mutant's effect is striking as it completely impedes neurite outgrowth. These data reveal that the SH3, RUN and LZ are interacting with intracellular proteins, and that each one is important to neuronal growth.

In addition, Meakin has recently discovered that when Nesca is imported into the nucleus, it is phosphorylated there. Phosphorylation is the process by which a phosphate residue is added onto one of the three usual acceptor amino acids (tyrosine, serine, or threonine). The phosphate residue increases the negative charge on the protein, which is sufficient for the regulation of changes in the activity of a molecule, its conformation, and its ability to interact with other proteins. "This is a hallmark protein modification that is observed in nuclear proteins that regulate nuclear processes, in particular, transcription," she explains. "From these data, we can hypothesize that Nesca acts as either a transcriptional activator or co-activator by binding to and regulating the activity of a transcription factor. Currently we are making progress in identifying proteins that serve as binding partners for Nesca."

Over the last 10 years, Meakin's laboratory has identified five new molecules that are involved in neurotrophin signaling for developing and adult nervous systems. The focus of her ongoing research will be to determine the roles of these molecules in regulating neuronal growth and re-growth in the developing and injured nervous systems.

Meakin's research is supported by: The Cancer Research Society; the CIHR; the NRP; and, The National Cancer Institute of Canada.

Meakin obtained her PhD in the Department of Molecular and Medical Genetics at the University of Toronto. Her postdoctoral research fellow was completed at the Stanford University School of Medicine in California.

Calcineurin's Orchestral Maneuvers in Muscle

Dr. Robin Michel

In the inaugural issue of the *Northern Neuron*, an article highlighting the work of Dr. Robin Michel's Neuromuscular Research Laboratory, at Laurentian University, Sudbury, Ontario, discussed novel features of the calcineurin molecule and its key role at the nexus of signaling pathways leading to muscle growth. Calcineurin is an enzyme that acts as a sensor for calcium levels in cells. When nerves drive their muscle partners and make them work in a way that would prompt them to grow and become more efficient, calcium waves or oscillations are triggered in muscle cells that serve as a sort of molecular code. Calcineurin and its partners are brought into play to decipher this code and enact mechanisms that will activate the genes required for growth.

Michel likens the signals responsible for muscle growth to "a perfect symphony" with calcineurin "acting as first violinist, playing in concert with all other instruments in the orchestra." With long-time colleague Dr. Jasmin at the University of Ottawa, Faculty of Medicine, a membrane protein, utrophin, has recently been identified as an important molecular target of calcineurin. Utrophin is an evolving key player in the therapeutic fight against muscular dystrophy.

Muscular dystrophy occurs in people who have a mutation in the gene for dystrophin, preventing their muscle cells from making a large membrane-associated protein called dystrophin (hence the disease name). Dystrophin is a protein found in membranes surrounding individual muscle fibers. Its deficiency is one of the root causes of muscular dystrophy. The absence of dystrophin renders the muscle membrane fragile and more susceptible to breakdown as a result of the wear and tear related to muscle contraction. The ability of muscle fibers to protect themselves from this wear and tear, and their ability to regenerate, eventually fails and the muscle mass becomes infiltrated with connective and adipose tissues. People afflicted with muscular dystrophy don't have dystrophin, but their muscles do have dystrophin's "twin sister" utrophin. Utrophin performs a different cellular role and is found in a different cellular location. If utrophin could be enticed to be expressed to a large extent and at the correct membrane location, then it could functionally compensate for its twin sister, dystrophin.

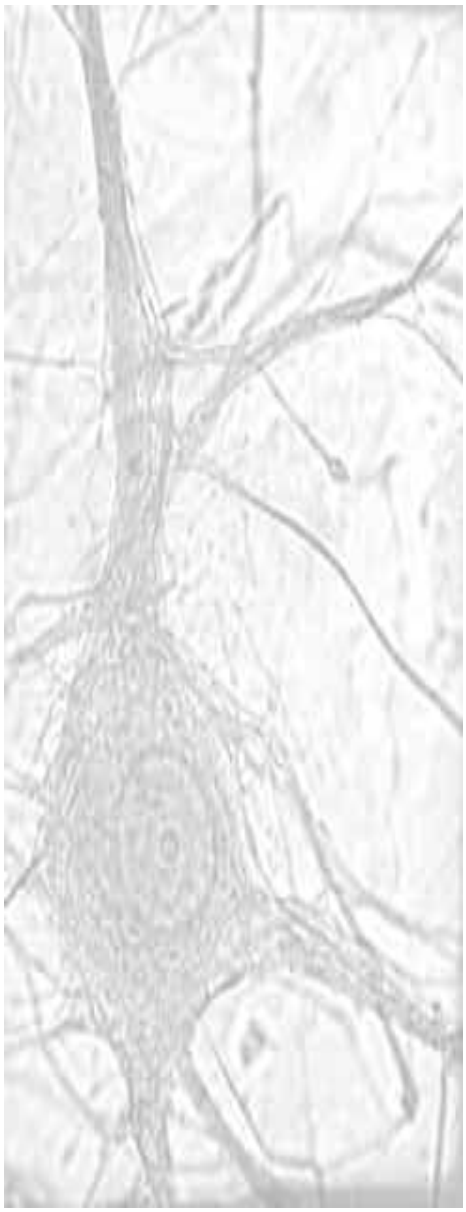
Doctors Michel and Jasmin found that muscle cell expression of utrophin is under the control of calcineurin. More importantly, calcineurin can be made to direct this utrophin-to-dystrophin transfer. These researchers showed that by crossing dystrophic mice (mdx) with transgenic mice over-expressing a constantly active form of calcineurin (CnA*), they were able to rescue muscle fibers from developing classic signs of muscular dystrophy in crossed progeny. Increased understanding of these and other mediators of muscle growth may provide additional strategies for the development of effective therapeutics to counter muscle weakness and wasting related to diseases such as ALS and muscular dystrophy.

Cellular and molecular neuromuscular physiology are Michel's area of expertise. His educational background includes a B.Sc., from McGill, followed by an M.Sc., and PhD from the University of Montreal.

Last fall, Michel moved his laboratory and has taken a post as Full Professor at Concordia University in Montreal.

His funding sources include: the NRP and the Natural Sciences and Engineering Research Council of Canada.

Michel was recently awarded a Tier 1, Canada Research Chair in Cellular and Molecular Neuromuscular Physiology.



Rehabilitation and Quality of Life

Dr. Colleen O'Connell

The purpose of rehabilitation is to improve function and quality of life. The process of neuro-rehabilitation relies on an interdisciplinary team to provide integrated and co-ordinated care.

"Out of all the diseases I've seen, I can honestly say that ALS is the most devastating," says Dr. Colleen O'Connell, a physiatrist at the Stan Cassidy Centre for Rehabilitation (located in Fredericton, N.B.) who specializes in neuro-rehabilitation. A physiatrist is a physician who specializes in physical medicine and rehabilitation.

Just two months after O'Connell started her practice as a specialist, she received a phone call from a neurologist who had a patient in his office who had just been diagnosed with ALS. "She (the patient) was extremely distraught. She was only 42-years-old. They had a daughter who, at the time of diagnosis, was only two-years-old."

O'Connell describes how she had to learn along with the woman. "I had to work with her so that she could still be a mother: still comb her daughter's hair, read her a story, and go on a family outing," she says.

After this experience, O'Connell was determined to do her best to improve the lives of those living with ALS. Fortunately, she works in a field which enables her to do so. She works with engineers and therapists to develop ways to remotely monitor activities such as arm movements and lung function of people who have ALS, which allows them to stay in their milieu as opposed to traveling to the Centre.

For people who have ALS and live in rural areas, a long trip to a medical centre can be exhausting. The type of technology that is being developed and tested at the University of New Brunswick's Institute of Biomedical Engineering could be extended to help people with other diseases where transportation is difficult. One type of technology is a device for monitoring data points such as range of motion and strength, constructed of thin strips of fiber-optic tape. It has been developed by a company called Measurand, and through unique partnerships between the University of New Brunswick and the Stan Cassidy Centre, they are hoping to proceed with small clinical trials to determine its effectiveness.

"I can monitor what is happening to the arm function of someone who is three hours away," explains O'Connell. Presently, single case trials are taking place with the software that has been developed.

"We believe we can potentially have an impact on quality of life. For example, a spouse/patient may not have to miss a day of work, family time, recreational activity, etc. to accompany a patient. Patients and families may feel more secure knowing that we can "check them" easily from home. This is especially important for rural patients for whom even a day trip may be fraught with difficulty. It may open up opportunities for more patients to participate in drug trials for access purposes," explains O'Connell.

The Centre offers both in- and out-patient services, is the only tertiary neuro-rehabilitation center in New Brunswick, and provides widespread outreach programs throughout the province.

"People who have ALS need to be able to normalize their lives," says O'Connell. "Techniques have to be non-invasive, and a level of comfort has to be ensured."

O'Connell graduated from medical school at Memorial University of Newfoundland in 1995 and completed her residency at Dalhousie in 2000, specializing in Physical Medicine and Rehabilitation.

International health is O'Connell's other passion. She has worked in international rehabilitation initiatives in Africa, Asia and the Caribbean. She is the founder of Team Canada Healing Hands, a non-profit volunteer organization that is dedicated to administering rehabilitation care, education and training in areas of need. For the past three years Team Canada has worked with Healing Hands for Haiti Foundation International, sending teams of Canadian volunteers to Haiti.

Assistive Technology and ALS

Glen Hughes

A professional engineer with a background in electrical engineering Glen Hughes has observed that the needs of people with ALS were more complex than any of the other groups with whom he was working.

Glen Hughes, a Project Engineer at the Institute of Biomedical Engineering, at the University of New Brunswick in Fredericton, N.B., works towards developing various assistive technologies to support people living with ALS.

Currently, Hughes is working with O'Connell at the Stan Cassidy Centre for Rehabilitation, to develop a proposal for a set of tools that would allow for more efficient tracking of the progression of ALS, in the hopes that such tools could be used for evaluating the effects of different drugs in clinical trials, and to better monitor the diminishing strength of people who have ALS.

Alternate ways of tracking the illness – methods such as video conferencing and in-home monitoring – are necessary because people with ALS can live far from specialty rehabilitation centres. The small differences in results between individuals during clinical trials are important, but have been difficult to compare. In addition, information needs to be collected quickly, owing to the devastating speed of loss of function that is a characteristic of ALS.

Hughes advocates having alternate ways of doing things for people with disabilities. He was involved with evaluating the potential of using Internet-based video conferencing tools for use by people with ALS.

"It was very interesting to investigate the use of Internet-based video communication technology for people with speech disabilities. During limited trials, ALS patients indicated they were able to communicate as well as if they were face-to-face with the people they wanted to communicate with."

Hughes explains that as technologies evolve, access issues for people with disabilities are not always considered. Confirming that the use of Internet-based interactive video communication equipment will not be another obstacle but is of potential use for people with ALS is significant and may lead to a better quality of life for people living with ALS.

Recently, Hughes was approached about making an adapted "call bell" that would be worn on the wrist of the person with ALS. It would be a small transmitter and the receiver would be worn by the caregiver. If the person with ALS needed anything s/he could notify the caregiver using the call bell.

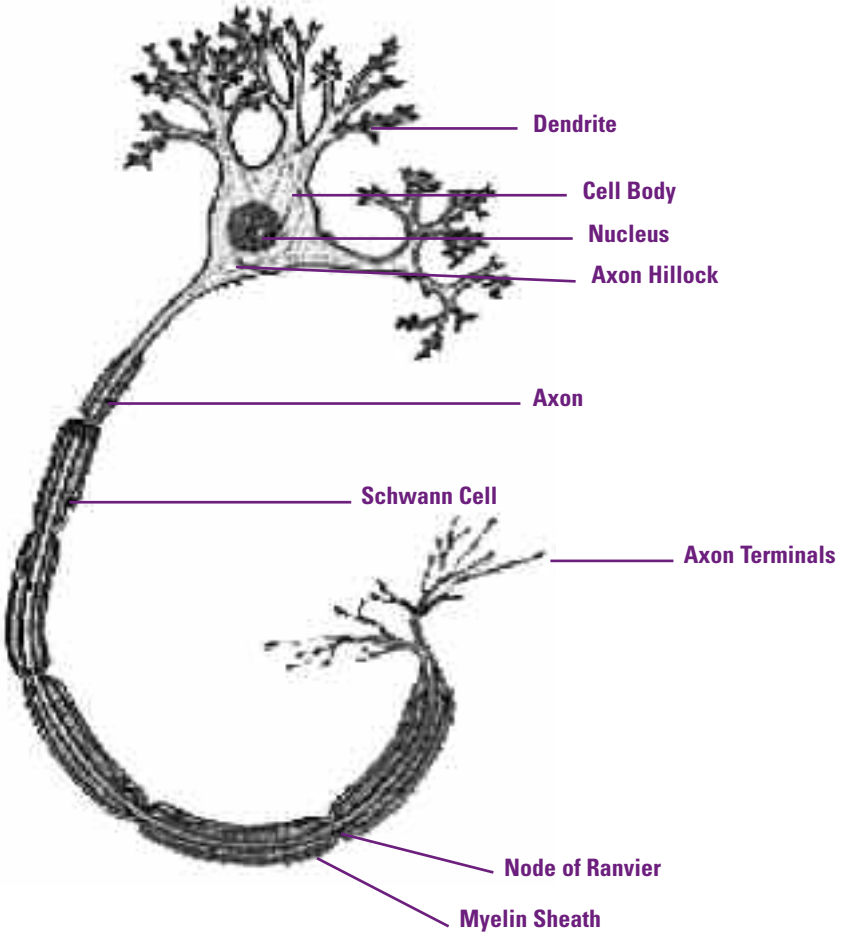
"There would be benefits for everyone. There is increased independence for the person living with ALS, and the caregiver can move around without needing to continuously check if the person needs assistance," Hughes explains.

"At the Institute of Biomedical Engineering we've created adaptations for people who are living with ALS to use, says Hughes. "We have developed a prototype that allows someone with ALS to use a cell phone with ability switches."

For the prototype cell phone access device – there was a single switch to turn the phone on/off – and another switch to access incoming calls or dial using speech recognition. The person living with ALS could press an ability switch and simply say the name of the person that s/he would like to call. Hughes explains that this kind of project is important because it allows for the person with ALS to have more independence.

Funding for the accessible cell phone prototype came from the Stan Cassidy Centre for Rehabilitation.

Diagram of a Motor Neuron



Adenovirus Vectors for Muscle Gene Therapy

Dr. Robin Parks

Dr. Robin Parks and his team at the Ottawa Health Research Institute have been working towards developing efficient and useful gene therapy vectors – elements that help carry genes into cells – to treat neuromuscular disorders.

Gene therapy involves the delivery of "therapeutic" DNA to the cells and tissues of an individual with a hereditary disease. The therapeutic DNA is typically a normal copy of the defective gene causing the disease. Currently, engineered viruses are the most efficient method to deliver DNA to cells.

Dr. Robin Parks, Senior Scientist at the Ottawa Health Research Institute is exploring the use of a new class of adenovirus (a DNA virus that is used in gene therapy) called helper-dependent Ad vectors (hdAd) which has shown promising results in pre-clinical studies. Over the last year, Parks and his team have focused in two areas: characterizing immune responses to Ad vectors, and improving Ad infection of muscle cells for muscle-directed gene therapy.

"Existing vector systems have proven inadequate to achieve high level, long-term expression of foreign genes due either to poor infection efficiency or strong vector-directed immunological responses leading to elimination of infected cells," explains Parks.

The earliest events in the cells' response to Ad infection are initiated by the binding of the virus to the cell, and culminate with the activation of an immune response. Immune responses to viral vectors limit their effectiveness. The delivery of traditional Ad vectors to mice results in a strong inflammatory response that persists for several days after administration. In contrast, although hdAd do induce an early phase of inflammation within a few hours of administration, this response does not persist. These findings suggest that hdAd vectors are better able to "hide" from the host immune system, a characteristic which allows them to persist and express a therapeutic protein for a longer period of time.

In another series of experiments, Parks showed that hdAd could be easily modified to improve infection of muscle cells, simply by altering one of the proteins used by the virus to attach to cells. Infection of mature muscle cells in tissue culture was more than 20 times more effective than with an unmodified virus. However the promising results that were witnessed in cells in culture were not recapitulated in animal studies where only a two-fold enhancement in infection was observed. The results demonstrated that modified hdAd can lead to enhanced infection of mature muscle, but that other barriers may also contribute to poor infection of muscle by Ad – aside from low levels of the viral receptor on muscle cells.

In the above-described study, infection with modified viruses in isolated tissue culture cells was more effective than infection with viruses in intact muscle. Parks explains, "the protective sheath that surrounds each muscle fiber, called the basal lamina, only allows molecules of a certain size to pass through easily. Our virus is larger than this size, so it doesn't easily get through the basal lamina to be able to bind to the muscle cells." The basal lamina acts as a protective sheath that surrounds each muscle fiber.

Parks and his team showed that the Ad vector seems to accumulate in pockets between the muscle fibers that are created as a result of the injection process. This occurrence can be observed shortly after the injection of virus into mouse muscle. Although Ad does disperse from the site of injection along the length of adjacent fibers, in general it does not travel far. Failure to efficiently disperse in the muscle may be one reason why Ad does not infect muscle well. He is currently exploring methods to allow for better dispersion of the virus.

"The methods that we are developing to improve hdAd infection of muscle can also be applied to improve infection of motor neurons, the cell type affected in neuromuscular diseases," explains Parks. However, he cautions that current gene therapy studies are mostly in the early, pre-clinical stages of development, and it will be several years before such approaches might reach the clinic.

Parks completed an M.Sc. and PhD in the department of Molecular Biology and Genetics, at the University of Guelph. In 1996, he began a Postdoctoral Fellowship with Dr. Frank L. Graham at McMaster University. At McMaster, Parks worked on the development of novel adenoviral vectors for use as gene therapy vehicles for gene therapy applications.

Parks' funding sources include the NRP, the CIHR, the Jesse Davidson Foundation for Gene and Cell Therapy, and a Premier's Research Excellence Award.

The Ottawa Health Research Institute is the research arm of The Ottawa Hospital and a major part of the University of Ottawa Faculties of Medicine and Health Sciences.

The three major hurdles to successful gene therapy using Ad are poor entry into muscle, inability to infect every muscle cell, and immune responses. Current research is focusing upon ways to improve effectiveness by modifying the virus or the ways in which the virus is delivered. Gene therapy is any disease treatment-regimen that uses genetic information as a therapeutic.

The Roles of Ribonucleic Acid and Peripherin

Dr. Janice Robertson

Reproduced courtesy Torstar Syndication Service.

Dr. Janice Robertson's research focuses on understanding the disease mechanisms causing ALS. Robertson's research group was recently established at the Centre for Research in Neurodegenerative Disease at the University of Toronto. Her lab focuses exclusively on ALS where she uses a multi-disciplinary approach to investigate the molecular mechanisms of ALS using a combination of protein-biochemical analyses, molecular biological techniques, primary neuronal culture, transgenic mouse technology and analysis of human ALS pathological material.

One aspect that Robertson is investigating is the role of ribonucleic acid (RNA, a single-stranded nucleic acid that contains the sugar ribose) in ALS. "Fewer than five per cent of ALS cases are caused by single gene mutations; the remaining 95 per cent have no known cause. DNA forms RNA that is then processed to form protein. It seems that one piece of DNA can generate several RNAs which can then, in turn, generate many proteins," says Robertson. RNA is one of the two types of nucleic acids found in all cells, the other is DNA. Most genes contain the information needed to make functional molecules called proteins. The journey from gene to protein is complex and tightly controlled within each cell. It consists of two major steps: transcription and translation. Together, transcription and translation are known as gene expression.

The most recent figures estimate that humans only have approximately 25,000 genes yet there are estimates of more than 250,000 proteins (the proteome), which indicates the importance of RNA processing in expanding the proteome. "Since there are not enough gene mutations to account for all cases of ALS, it seems increasingly likely that defects of RNA processing could lead to abnormal protein generation – such as I have found for peripherin – thereby causing disease," Robertson explains. Peripherin is a key protein which is involved in the accumulations that clog motor neurons. One theory is that toxic mutations in peripherin have a negative impact on cells.

"Peripherin is a protein that has been shown to have increased expression after injury," she says. The association between peripherin and cell death was uncovered when Dr. Jean-Pierre Julien discovered that when peripherin is over-expressed in transgenic mice, the mice develop a motor neuron disease mimicking aspects of ALS.

An important discovery that Robertson made was the demonstration that abnormalities of alternative splicing of peripherin occur in ALS. Alternative splicing isoforms are differing forms of mRNA that can be produced from the same gene. Messenger or mRNA is a copy of the information carried by a gene on the DNA. The role of mRNA is to move the information contained in the DNA to the translation machinery. Robertson's research reveals that alternative splicing (a process that allows one gene to generate a variety of proteins) of the mouse peripherin gene creates at least three peripherin isoforms: Per 58, 61, and 56. Isoforms are proteins that are derived from the same gene but have distinct physical, (and sometimes even biological) properties. The research shows that each of the isoforms has different assembly characteristics, and Per 61 is neurotoxic when expressed in motor neurons in primary culture. Primary culture is taking motor neurons directly from a mouse embryo and growing them in a culture dish.

In studies, Per 61 is present in the motor neurons of transgenic mice with a mutated SOD1 gene but not in the control or peripherin transgenic mice, showing that mutant SOD1 ALS includes expression of a neurotoxic spliced variant of peripherin. The results of the research, which were published in the March 17, 2003 issue of *The Journal of Cell Biology*, suggest that the expression of peripherin in alternatively spliced forms may contribute to neurodegeneration in ALS.

This work was arrived at through the collaboration of Drs. Walter Mushynski, Heather Durham, Michael Strong, and Jean-Pierre Julien, among others. However, Robertson has shown that in some cases the presence of peripherin alone is not a sufficient cause for cell death.

"We have shown that when an unhealthy neuron is placed in an inflammatory environment the neuron dies. This demonstrates the important link between inflammation and neuronal death. Neuronal death together with inflammation are two key pathological features in ALS," says Robertson.

Robertson is investigating how and when the inflammatory response is initiated. "I think there are two events occurring in ALS, the initiating event and the propagating factor(s)." Disease in ALS tends to start focally and then propagates. I think the propagating factor is inflammation," she says.

The inflammatory cells that are activated in ALS are called microglia and these are recruited to areas of neuronal degeneration. How the microglia is recruited to these areas is not entirely clear but likely involves signals from motor neurons in distress. This in Robertson's view would be the initiating event. Microglia provides both good and bad functions and work from Robertson's laboratory suggests that motor neurons in ALS have a special vulnerability to chemicals released by activated microglia that leads to their death. Moreover, once microglia become activated they appear

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Robertson continued

to remain activated in ALS, allowing them to exert their detrimental effects on other surrounding motor neurons, thereby, in Robertson's view, propagating the disease.

"A process that spreads like a tidal wave of catastrophic death, all due to one initiating event but propagated by activated microglia," explains Robertson, who would like to explore the mechanisms by which cells trigger the inflammatory response and then determine how the negative impacts of the response can be stopped.

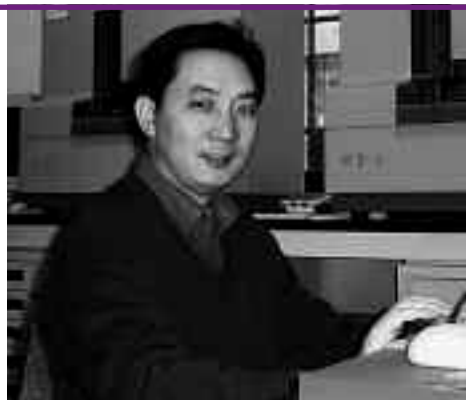
After receiving her PhD from the University of London, Robertson trained with Professor Nigel Leigh at the Institute of Psychiatry in London, U.K., followed by a Wellcome Trust International Prize Travelling Fellowship to train with Drs. Jean-Pierre Julien and Heather Durham at McGill University. She is currently supported by grants from the U.K. Motor Neurone Disease Association, The ALS Association and the NRP.

Robertson Awarded Tier 2 Research Chair

As Canada Research Chair in Molecular Mechanisms of Amyotrophic Lateral Sclerosis, Robertson is focusing her research on the mechanisms that kill the motor neurons in the brain, brain stem and spinal cord. Her cell biology research includes studying ALS in mice and using modern imaging technology to look at living cells without destroying them. She examines human ALS pathological tissue to validate and build on the results. By tracking down the causes of ALS, Robertson hopes to find a way to treat or even cure it.

A Genetic Approach to ALS

Dr. Shangxi Xiao



Dr. Shangxi Xiao is an ALS researcher who works with Robertson. Xiao's ultimate aim is to identify the causes of alternative splicing isoforms in the disease.

Xiao's research interest in ALS began with his study of the major role of genetics in the disease. He was also drawn to examining the illness owing to the devastating course that ALS takes. "ALS is a destructive disease that ultimately paralyzes the body. In addition to the pain felt by the person who has the disease, there is also the pain of the family and caregivers to consider," Xiao says. He is referring to the pain experienced by society as a whole when there is an illness that can't be cured or effectively treated.

Xiao seeks to identify the combination of genetic and environmental factors that initiates ALS. This could lead to the development of better interventions and the design of new screening tests as well as more effective therapies in the future. He also stresses the importance of having more animal models so that the molecular mechanisms of ALS can be explored.

Xiao received his MD in 1990 at Anhui Medical University (China), his PhD in 2001 at the University of Science and Technology in Shanghai.

"My research is focused on human genetic diseases," says Xiao. While in China, Xiao assisted in the discovery of the dentin sialophosphoprotein gene, the gene responsible for causing a tooth-decaying disease called *dentinogenesis imperfecta*. His findings were featured in the February 2001 edition of *Nature Genetics*.

ALS and Immunotherapy

Dr. Shirley Liu

Dr. Shirley Liu is interested in the mechanisms of disease. "ALS captured my attention because it has no cure or effective treatment." She is optimistic that her knowledge of glutamate and oxidative stress will contribute not only to treatment and cure for ALS, but for other motor neuron diseases as well.

Liu is participating in a novel project within Robertson's laboratory that could potentially lead to a treatment for ALS. The project uses transgenic mice to study the misfolded protein aggregates produced by the mutant SOD1 gene responsible for a hereditary form of ALS.

Liu is interested in an active immunization approach to the disease. A transgenic animal is one that carries a foreign gene that has been deliberately inserted into its genome. The foreign gene is constructed using recombinant DNA methodology. Recombinant DNA is DNA that has been created artificially. In addition to a structural gene, the DNA usually includes other sequences to enable it to be incorporated into the DNA of the host and to be expressed correctly by the cells of the host.

Using mice with genetic mutations (transgenic mice) scientists are able to simulate and study conditions that may occur in the course of ALS. Mutant SOD1 inside cells has a propensity to

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Liu continued

misfold and aggregate in a manner generally believed to cause toxicity. Robertson's lab in collaboration with Dr. Avijit Chakrabartty (also at U of T) now has evidence that misfolded SOD1 exists not only inside the cells of mutant SOD1 transgenic mice, but outside the cells as well.

"It's a fascinating discovery," says Liu. "It suggests that mutant SOD1, acting outside the cell, could be the triggering factor of the inflammatory response." Liu and her colleagues plan to test whether immunotherapy (treatments designed to restore or stimulate the immune system's ability to fight disease and infection) would remove SOD1 aggregates and so offer therapeutic benefits. The project, which began in June 2004, involves vaccinating ALS SOD1 transgenic mice with mutant SOD1 aggregates to induce B cell-mediated clearance of mutant SOD1. The purpose of this project is to see if vaccinating the mutant SOD1 transgenic mice can remove the misfolded SOD1 proteins and in doing so provide therapeutic benefit.

Liu is also involved in a second ALS project, examining the role of a proinflammatory cytokine – tumor necrosis factor alpha (TNF-α) – in peripherin-mediated neuron death. Cytokines are chemicals involved in growth and regulation, and can be used by the immune system to send messages between cells.

Liu earned her B.Sc. (Pharmacy) from Taipei Medical College in Taiwan, and completed her PhD at McGill in 2003, studying multiple sclerosis. She has been a member of Robertson's ALS team since June 2004.

Looking at Genetics with Dr. Guy Rouleau

Dr. Guy Rouleau

In 2002, the Rouleau lab identified a candidate region harboring a gene responsible for ALS on chromosome 18. This region has been exhaustively analyzed to determine which gene is in fact involved.

Dr. Guy Rouleau focuses his research efforts on characterizing defective genes that lead to nervous system dysfunction. Rouleau is the Director, Centre for the Study of Brain Disease, Centre hospitalier de l'Université de Montréal. Work in his lab has recently led to the discovery that the disruption of the peripherin gene – through mutation – in humans accounts for a proportion of ALS cases. The disruption leads to the destabilization of intracellular structures that hold the cell together.

This was the first report of a human peripherin mutation, and it corroborates well with evidence that peripherin mutations can cause ALS in mouse models of the disease. Peripherin is found in neurons of the central nervous system and in sensory neurons of the peripheral nervous system and thus is a useful neuronal-specific marker.

A collaborative group has been established between Rouleau and ALS researchers from the U.S. and Europe to identify both this gene and others which may be responsible for ALS. This group includes – as principal investigators – Drs. Robert Brown of Harvard University, Teepu Siddique of Northwestern University, Eric Lander of the Massachusetts Institute of Technology, Jackie de Belleroche of Imperial College in London, U.K., Pieter de Jong of the Children's Hospital in Oakland, California, and Christopher Shaw of the Institute of Psychiatry in London, U.K.

A substantial database of DNA from French-Canadians with ALS is in place at Rouleau's lab. This collection of cases is ongoing from centres in Montreal and Quebec City. By examining the limited genetic diversity of this specific population, it is hoped that additional genes that predispose for this disease can be identified. Through collaborations with Drs. William Camu and Vincent Meininger in France, hundreds of additional familial and sporadic ALS DNA samples have been collected. This enables other genetic studies to progress rapidly, and will help validate results from the French-Canadian population.

As a neurologist, Rouleau sees those living with ALS and understands the clinical aspects of the disease. In 1990, he participated in the El Escorial (Spain) conference, a meeting that first defined the diagnostic criteria for ALS. Rouleau is part of The ALS Association consortium of genetics experts from North America and Europe that contribute resources and strategies to detect subsequent ALS genes.

Rouleau's lab was the first to report that SOD1 toxicity does not result directly from the presence of the mutant protein in neurons, but also requires expression in other cells. Rouleau has been involved in the mapping and cloning of both the SOD1 and aslin genes, responsible for ALS 1 and 2 respectively. ALS2 is also known as juvenile ALS. This disease generally arises when children are 12 years of age and progressively leads to the death of nerves that feed the muscles.

Work in his lab has resulted in the identification of the first neurofilament heavy chain gene mutations, as well as detection of a frameshift mutation in the peripherin gene.

Rouleau graduated from the University of Ottawa with a medical degree in 1980, received a specialty certificate in Neurology five years later and his PhD from Harvard University in 1989. He has been working on the genetics of ALS since he and Dr. Robert Brown started the genetics of ALS project at Harvard in 1986.

Rouleau's work is supported by The ALS Association and the Muscular

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Rouleau continued

Dystrophy Association.

The Centre for the Study of Brain Diseases is an integrated research unit headed by Rouleau that is comprised of four clinical scientists with an interest in the genetics of various neurological diseases. The facilities include state-of-the-art genetic equipment that will enable the processing of a high volume of DNA samples, as well as cell culture and genotype (the particular genetic makeup of an individual) analysis.

Rouleau Awarded Tier 1 Research Chair in Genetics of the Nervous System

His research aims to understand the causes of diseases of the nervous system and lead to the development of new treatments for these diseases. As the first Director of the recently created Institute for Neurosciences and Mental Health (INMH), based at the Centre hospitalier de l'Université de Montréal, Rouleau is working with researchers from different backgrounds and fields on the design of new drugs and treatments for diseases that affect the brain and the nervous system. His research projects include the use of genetics and genomics to identify the genes responsible for simple and complex diseases as well as the use of techniques such as gene expression and nano-imaging to uncover the function proteins affected by disease genes. In addition, Rouleau is carrying out an epidemiological analysis to study how the environment influences disease symptoms and severity.

Comparing Notes with François Gros-Louis

François Gros-Louis

"At first I became interested in ALS research because of the genetics aspect. When one has the opportunity to work for a cause that is this important, it should be taken," explains François Gros-Louis, who is completing a PhD in human genetics at McGill University.

Gros-Louis is screening candidate genes for a study on the genetic causes of ALS in a cohort of mainly French and French-Canadians. The genetics behind ALS interests Gros-Louis as mutations in the SOD1 gene only account for two to three per cent of cases. He is looking for other genes that may be responsible for the disease or that may affect onset and progression.

"I would like to be involved in the development of an animal model," says Gros-Louis. Existing mouse models live too long for the effects of implanted mutated ALS genes to take effect right away. He would like to work on other animals where the disease would show up more quickly, such as zebra fish, and answer questions that could be hard to assess with existing animal models. "Extrapolating data found in smaller organisms to understand human biology is a process known as comparative genomics," Gros-Louis explains.

Comparative genomics is the comparison and analysis of genomes – genetic material in the chromosomes of an organism – from various species. The purpose of the comparison is to better understand how species have evolved, and also to determine the function of the genes. To date, researchers have learned a lot about the function of human genes through investigating their counterparts in organisms such as the mouse. The process of comparing the different features of the genomes involves computer programs that are able to line up multiple genomes and search for regions of similarity between species.

Gros-Louis is also working on a second project. He is researching ALS2, a newly discovered gene that has been suggested to be causal for a juvenile-onset form of familial ALS. He is looking for its pattern of expression in adult mice to better understand its normal function. ALS2 was identified in 2001 by a group of Japanese researchers with the help of Dr. Guy Rouleau and colleagues. Gros-Louis explains that the current research in Canada for this gene is being done in collaboration with a team of researchers in Vancouver.

Gros-Louis graduated in 1998 with an undergraduate degree in medical biology from the University of Quebec in Trois-Rivières, and then completed an M.Sc. in cytogenetics at Laval University. For his PhD in human genetics at McGill, Gros-Louis is working with Rouleau, and has recently completed a certificate in bioinformatics from the University of British Columbia. Gros-Louis is now looking for a post-doctoral fellowship, and wants to continue focusing on ALS.

The cause of ALS is not yet understood. To date, there is no effective treatment or cure. But researchers are striving to develop drugs to slow the progress of the disease, and investigating many possible (and likely combined) causes including:

- **environmental toxins**
- **genetic predispositions**
- **viral or infectious agents**
- **immunological changes**
- **aging-related changes**
- **cellular changes**

Environmental Factors also Part of the ALS Puzzle

Dr. Chris Shaw

Owing to the overlapping symptoms of several progressive neurological disorders, ALS-PDC may hold the key to unlocking important information about degenerative neurological disorders, including ALS.

On the West Coast at the University of British Columbia, Dr. Chris Shaw studies ALS-parkinsonism dementia complex (ALS-PDC), a motor neuron disease found mainly on the island of Guam. He studies mice that have been fed seed flour from the cycad, an ancient plant native to Guam. Consumption of cycad flour is linked to ALS-PDC. Treated mice develop a motor disorder much like ALS; they also have outcomes resembling parkinsonism and Alzheimer's disease.

From the 1950s to the late 1960s, Guam's ALS incidence rate was up to 400 times that of the Canadian/U.S. rate, creating a flurry of research interest. Then the rates declined, and most researchers moved to other challenges. Shaw, however, became intrigued by the ALS-PDC phenomenon. "Clusters of people afflicted with the same disease provide important clues to the factors leading to the disease. ALS-PDC is one of only two major ALS clusters and is thus potentially very important," he explains. He believes his work on ALS-PDC can unlock many questions surrounding ALS, including whether or not the disease does have environmental factors. Shaw also believes his work on ALS-PDC will help us to better understand a number of other motor neuron diseases.

"The first thing that we thought when we heard about cycad causing ALS-like symptoms in humans was – is there anything to this story? There was evidence that cycad was toxic, so we thought we'd do a simple experiment and feed washed cycad to mice as 25 per cent of their diet," says Shaw. The mice were then put through a battery of behavioral tests, tests that rapidly showed emerging motor deficits. The death of cells in the spinal cords of the mice was similar to what happened in humans with the disease. In the Guamanian disease, ALS symptoms usually manifest before parkinsonism and dementia. The same was true in the cycad-fed mice.

In each experiment using the cycad flour, careful measurements at many levels were made from each mouse. The progression of motor outcomes was measured from four different perspectives:

- the overall behavior of the mice was observed (olfactory, motor, and cognitive functions);
- following post-mortem examination, MRI measurements of spinal cord volumes were done;
- through conventional histology we examined individual neural cells;
- biochemical measurements of molecules linked to ALS were performed.

With Shaw's mouse model, a number of crucial questions can be addressed more quickly than in human studies. Work is already underway in the Shaw lab to determine if the SOD1 mutation linked to some familial forms of ALS uses the same cell death pathway as that induced by cycad feeding.

"Now that we have an environmental model, we can probe the interaction with genetic factors – these pieces of information are complimentary to a gene-environment interaction experiment," says Shaw.

One key study in the Shaw lab is the study of the "timeline" of cycad-induced motor neuron death, which is crucial for obtaining a better understanding of ALS in humans. "By the time that people go to a neurologist, the damage has already been done," says Shaw. "One of our goals is to study what happens before behavioral symptoms begin. At these early phases, it might still be possible to prevent cells from dying." With the ALS-PDC mouse models, the disease processes can be observed in sequence.

Examining different stages of disease development has another advantage: tests of various potential ALS medications can be used to see if they prevent or slow down the cell death cascade. As Shaw explains, the advantage of this type of study in cycad-fed mice is that there is more time for observing different stages of the cascade, "whereas the SOD1 transgenic mouse dies much more quickly."

The cycad model of ALS lends itself well to studies of gender differences in ALS. In the human disease, males predominate. This observation is mirrored in cycad-fed mice in which females have fewer behavioral and neuronal losses than their male counterparts.

Shaw's ALS research plans for the future include identifying the molecule in cycad that initiates the disease and understanding its mechanism of action. The greatest challenge that he foresees in the future will be taking what is happening in the mouse model and translating it to people; issues will arise with the question of how to screen people before they have ALS and how to locate those who may be in pre-symptomatic stages of the disease.

"Polio is now prevented by better hygiene and immunization," Shaw explains. "This is an example of dealing with a disease before it takes hold." Another future challenge will be to explore the possibility that something that occurs early in fetal development increases sensitivity to toxin exposure later in life. If this hypothesis turns out to be true, it would help us to understand how a single toxin could be lethal to a variety of neurons in a complicated disease such as ALS-PDC.

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Shaw continued

Having a complete model of the disease and an accurate timeline are the first steps towards approaching these potential questions.

After graduating with a B.Sc. degree (Biology) at the University of California in Irvine, Shaw obtained an M.Sc. (Physiology) at the Hebrew University of Jerusalem, obtaining his PhD (Neurobiology) at the same school in 1979. Shaw completed his post-doctoral work in 1988, the same year that he became part of the medical faculty at the University of British Columbia in the Ophthalmology Department. Shaw also holds cross appointments to the Neuroscience graduate program and the Department of Experimental Medicine at UBC.

"I first became involved with ALS research indirectly," he says. "Dr. Charles Krieger was studying molecular events that occur in ALS in a particular mouse model. We did a few studies together, I became interested in working with ALS, and the interest carried on and on."

Shaw's work is funded primarily by the U.S. Army's Department of Defense, with additional support from the Natural Sciences and Engineering Research Council of Canada and the Scottish Rite Charitable Foundation of Canada.

If environment proves to be a causal feature of ALS, and the molecules that cause the disease can be found outside the body, then there may be other sources besides cycad that can be responsible for triggering ALS. "We've identified a potential toxin and the timeline, now we need to learn how to block and stop the disease," says Shaw.

Washed Cycad and ALS-PDC

Dr. Jason Wilson

Jason Wilson, who recently completed his PhD at the University of British Columbia has dedicated a lot of time to researching ALS-parkinsonism dementia complex. When he was an undergraduate student, Wilson began working with Shaw and became involved in Shaw's laboratory.

ALS-parkinsonism dementia complex (ALS-PDC), originally described on the island of Guam, is a progressive neurological disorder that either shows the characteristics of ALS, the dementia that is associated with Alzheimer's with features of parkinsonism, or a combination of symptoms. Exposure to cycad seed toxins, through diet, has been hypothesized to be the primary factor leading to the neurodegeneration that develops in ALS-PDC. To confirm this, Wilson completed behavioral and histological experiments with mice that have been fed washed cycad flour (a flour made from the extracted starch of cycad seeds), which showed a progressive loss of motor and cognitive abilities as well as central nervous system pathology that mimicked human ALS-PDC.

Recently Wilson and Dr. Krieger, of the School of Kinesiology at Simon Fraser University, have been working to describe some of the similarities and differences between the ALS-PDC and SOD1 models of ALS. They have completed MRI volume analysis of spinal cords in SOD1 and ALS-PDC mice. This information may describe a potential difference between familial and sporadic forms of ALS and may help researchers determine the mechanism of behavioral loss in ALS. Wilson explains that collaboration happens in this type of research by seeing how one research model compares to another model, and then compares to people. Wilson points out that in the future, early detection of the disease will be an important topic.

Wilson's desire has always been to be a medical doctor and he has begun a post-doctoral fellowship with Shaw and plans to begin his medical school studies next year.

Based on the research that he has done, Wilson has identified a huge need to apply newly acquired knowledge to human beings. "Once I am an MD, I will be able to relate my clinical background to people," says Wilson. He credits Shaw, whose idea it first was to feed washed cycad to the mice.

Wilson recipient of Brain Star Award

In recognition of his article entitled *Quantitative measurement of neurodegeneration in an ALS-PDC model using MR microscopy,* University of British Columbia PhD fellow Jason Wilson was recently awarded the Brain Star awarded by the Institute of Neurosciences Mental Health and Addiction of CIHR .

The article, published in 2004 by Elsevier, a multiple-media publisher of scientific information, describes a study which demonstrates how Magnetic Resonance Microscopy (MRM) can be applied to mouse models of progressive neurodegenerative diseases. MRM works under the same principle of traditional MRI; however, it's modified for smaller biological systems. Higher magnetic field strengths – combined with larger gradients – permits imaging of mice and rats with optimal spatial resolution.

Another important feature of the study is a description of the effects that cycad toxins induce in mice – information which can lead to future studies in neurodegenerative research. MRM volume analysis indicated early stage brain and spinal cord morphology changes that corresponded with behavioral changes.

Wilson explains that refinement of the techniques examined in the study may be applied to "repeated in vivo scanning in animal models of progressive neurological disease, and potential MR screening for pre-clinical stages or features of human neurological disorders based on central nervous system (CNS) structures shown to be affected in the paper."

The objective of the Brain Star award is to acknowledge the outstanding research of Canadian graduate students, residents, and post-doctoral fellows in the different fields that the Institute of Neurosciences Mental Health and Addiction of CIHR covers.

ALS-Related Frontotemporal Dysfunction

Dr. Michael Strong's laboratory and clinical research group has been involved in researching the frontal lobe dysfunction of ALS since the early 1990s. Strong is the Chief of Neurology and Co-chair of the Department of Clinical Neurological Sciences at the University of Western Ontario, and a research scientist at the Robarts Research Institute

There is an increasing awareness that frontotemporal dysfunction can occur in ALS and that this may present a wide variety of manifestations that include behavioral changes, alterations in cognition, or a more robust frontotemporal dementia. Strong has used magnetic resonance spectroscopy imaging (MRSI) to investigate the early changes in neuronal loss in the anterior cingulate gyrus (a section of the brain that is involved in emotional and attentional functions) in those living with ALS, and published these results in a 1999 study. The study demonstrated that an early marker of cognitive impairment in ALS was a loss of neurons within this region of the brain, a region distinct from that normally involved in motor function. MRSI reveals brain metabolism. MRI can pinpoint a physical lesion in the brain, whereas MRSI is a more sensitive marker of disturbed neuronal function. In current studies, Strong and colleagues are utilizing a more widely accessible imaging tool, CT scanning with cerebral blood flow studies, to determine if changes in cerebral blood flow can be used as a surrogate marker for the early manifestations of cognitive dysfunction in ALS and in a related disorder, primary lateral sclerosis (PLS).

Following his studies using MRSI, Strong and his team then undertook a neuropathological study investigating the pathology of the anterior cingulate region of the brain and determined that the syndrome of cognitive impairment in ALS was neuropathologically similar to that which is seen in related frontotemporal dementias (for instance, in Pick's and Parkinson's diseases). While this study suggested that those patients with ALS who also had cognitive impairment suffered from a frontotemporal lobar degeneration (the neuropathological correlate of the frontotemporal dementia), it did not provide the cause for this process. To address this issue, Strong and his laboratory undertook additional neuropathological studies and, in a paper published in the journal *Neurology* in late 2003, stated that alterations in tau protein metabolism were evident in patients with cognitive impairment and ALS. This was marked predominantly by changes in immunohistochemical studies in which he saw deposition of tau immunoreactive protein aggregates (abnormal assemblies of proteins) within neurons, astrocytes (supporting cells of the nervous system), and in the surrounding background matrix. Strong and his colleagues have recently examined nine decades of life and quantitated the amount of tau protein (a protein that binds to and regulates the stability and assembly of neuronal microtubules, or protein structures, within cells) deposited within the region of the brain that is involved in ALS. This study, published in *Developmental Brain Research* (2005) demonstrated that the deposition of tau protein in this region of the brain is not a normal aging phenomenon; thus, observing such a protein aggregation in ALS is abnormal. In the most recent studies, abnormalities in the tau protein itself have been characterized, and there is evidence that a unique change in the phosphorylation (a process in which a phosphate group is added to another molecule or protein) of the tau protein underlies cognitive impairment in ALS.

Although these observations have met with controversy, the finding of altered tau metabolism in ALS defines a new biological substrate for the pathology of ALS. Previously, Strong and his research group investigated the deposition of neurofilament protein (microscopic threads that are found in the cytoplasm of neurons) as being important in ALS. By finding an association between cognitive impairment in ALS and alterations in tau protein metabolism, the biology of ALS is now better understood. The critical issue still remains to determine the extent to which alterations in tau metabolism are unique to ALS patients with cognitive impairment, and whether such changes are also seen in other forms of frontotemporal dysfunction in ALS. It will also be key to understand whether such changes in the phosphorylation state as defined by Strong and colleagues are the primary cause of this process. These remain areas of key activity within the Strong laboratory.

Incredible gift: Dr. Michael Strong and Chris Halls watch as PhD student Teresa Sanelli works in the lab conducting research looking for a cure for ALS. Halls was on hand to mark the donation of $3.5 million from his brother Michael's estate to the London Health Sciences Centre for ALS research. Photo courtesy of the London Free Press.

Strong's research facilities are located at the Robarts Research Institute in London, Ontario. Eight individuals work in the lab, including research associates, graduate students and technicians. Within Strong's laboratory, his team is fully equipped for cell culturing, protein analysis, histological studies and molecular studies.

Regarding Strong's future plans, his research will continue to focus not only on the clinical and biological characteristics of cognitive impairment in ALS, but also on the mechanisms by which the stability of neurofilament RNA is regulated within degenerating motor neurons in ALS. This has turned out to be a critical area in

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understanding the biology of motor neuron degeneration in ALS. Strong is intensively following this avenue, having discovered novel binding proteins that regulate the stability of neurofilament RNA, and that appear to be differentially expressed in ALS. He is also interested in the inflammatory response in ALS and believes that this response, which is mediated by microglial cells is critical to the disease progression. Experimental evidence suggests that there is a connection between the extent of inflammatory response and the presence of alterations in neurofilament RNA stability.

A graduate of Queen's University and The University of Western Ontario, Strong spent three years as a researcher at the National Institutes of Health in Bethesda, Maryland focusing on the biology of ALS. In 1990, he returned to London to take over the clinic and continue his research. Having the opportunity to participate in caring for people with ALS, and to ultimately understand the pathology underlying the disease process, were important to Strong.

"The Canadian ALS research community is small and well-acquainted with each other," Strong says. "An advantage to the Canadian research milieu is the willingness to collaborate – results can be shared and future directions discussed. There is collaborative work in place with Doctors Jean-Pierre Julien and Serge Rivest, looking towards modifying the immunological response in ALS. We have collaborated with Dr. Heather Durham, specifically looking at the nature of aggregate formation in degenerating motor neurons in ALS and the role of oxidative stress, the process in which the build up of free radicals occurs, resulting in cell damage. We also collaborate with Dr. Janice Robertson looking at the peripherin and the factors regulating its expression." In addition, he has collaborated with Dr. Guy Rouleau looking at the genetic aspects of cognitive impairment in ALS.

On the clinical front, Strong's research has been collaborative across a number of centres looking at the role of feeding tubes in ALS (six Canadian centres in total, led by Strong's) and in the most recent study he collaborated in the phase IV study of Riluzole.

Strong's research is supported by: The ALS Association, the ALS Society of Canada, Muscular Dystrophy Association, The Scottish Rite Charitable Foundation, and the Canadian Institutes of Health Research (NET grant, Serge Rivard lead investigator).

"In London, we are amongst the largest clinical and research clinics for ALS patients in Canada and what we learn from our patients drives our research lab. We conduct a fair number of drug trials, but we also see patients and can observe subtle differences in how the disease progresses. Some ALS patients have a form of dementia that first shows up as change in verbal fluency. We're trying to develop better tools for detecting these early symptoms of ALS and possibly diagnose it before there is motor neuron loss," says Strong.

Examining the Effects of Aggregates

Currently working on a PhD in pathology under the supervision of Dr. Michael Strong, at the University of Western Ontario, Teresa Sanelli has the potential to become a major researcher of ALS.

Sanelli first became interested in ALS through her studies in neurotoxicolgy where she was introduced to various neurodegenerative disorders in which environment was hypothesized to play a role.

"What really drew me into ALS was meeting Dr. Strong and his team, and seeing the multidisciplinary approach being used to diagnose and provide care for people with the disease. Strong and his team are involved in basic science and clinical research, both leading towards the same goal: understanding of the mechanisms to lead to a cure," says Sanelli. She was also interested in researching ALS because it is a terminal illness with the characteristic of not usually having an effect on the cognitive capacities of those who suffer from it. "I wanted to see what I could do to help these people who were able to witness the failing of their own bodies," she explains.

Sanelli works with primary motor neuron cells from transgenic mouse models of ALS that display ALS-like pathology (aggregates) in culture. She examines aggregates, composed of neuronal cytoskeletal and structural proteins called neurofilaments that become clumped together when disease and/or genetics change their normal conformation in the motor neuron and their role in disease progression. There is a debate underway as to whether or not aggregates are a consequence of ALS or if, in fact, they are involved in disease progression, actively causing further detriment. Sanelli believes that the aggregates are harmful.

Sanelli would like to decipher some of the complex, multifaceted processes that lead to cell death in ALS. Examining these processes "could give indications as to how to more successfully treat the disease – a combination of drugs and timing of administration will be key," she says.

After completing her PhD, Sanelli aspires to post-doctoral work on neuronal degeneration and development. "I want to see how problems in development can lead to degeneration," she explains. She would like to explore this because, although there is a familial variant, the majority of ALS cases are sporadic – therefore, there is a need to uncover the cause of the disease. "I am also interested in the clinical aspect and would like to one day become a medical doctor," she says.

In 2000, Sanelli graduated from the University of Toronto with a B.Sc., specializing in toxicology.

Strong has recently been honored by the American Academy of Neurology for his outstanding contribution to worldwide ALS research. He received the $25,000 Sheila Essey Award for ALS Research at an awards ceremony last April in Miami, Florida.

***"This is a tremendous honor not only for me but for the members of my lab who have worked toward understanding this devastating illness. It is also a tribute to the ALS community of Southwestern Ontario, which has long supported our research,"* says Strong, who holds the Arthur J. Hudson Chair in ALS Research, an endowed chair made possible by Michael Halls, who recently died from ALS.**

An ALS Virus?

New research suggests a retrovirus may be behind ALS

Dr. John Turnbull

Dr. John Turnbull's chief research interests are in motor neuron diseases and education. He has an active laboratory investigating models of ALS using transgenic animals and molecular biological approaches, and is involved in clinical research trials in ALS.

Ever since the discovery of the polio virus, researchers have wondered if ALS could also be a viral disease. In recent years, a class of viruses called "retroviruses" has become suspect. Retroviruses are viruses which contain RNA as their genetic material. The virus's RNA translates into DNA, which inserts itself into an infected cell's DNA. Retroviruses can cause a number of diseases, including AIDS and some cancers.

Sometimes, ALS coincides with a known retroviral disease. For example, one retrovirus causes motor neuron disease in mice. Some people with retroviruses, including HIV, have developed ALS-like symptoms which have completely reversed with retroviral therapy.

In 2003, ALS clinician and researcher Dr. John Turnbull of Hamilton's McMaster University Hospital began treating Elizabeth Grandbois (who has ALS) with the common HIV therapy, Highly Active Anti-Retroviral Therapy (HAART). Grandbois did not have HIV, but Turnbull reasoned that HAART might have an impact on the progress of her ALS, especially if a retrovirus was behind the ALS.

When Grandbois's ALS symptoms decreased, Turnbull began a small clinical trial to investigate the effect of this retroviral treatment on others with ALS. The results were not what he had hoped, and he stopped the trial.

"I didn't see any major improvements, as I did with Elizabeth," says Turnbull. "I suspect there is a real, beneficial effect, but that it is not major enough to warrant the empirical use of HAART at the present time."

The idea of retroviral involvement in ALS is gaining ground. In a study, *Detection of serum reverse transcriptase activity in patients with ALS and unaffected blood relatives*, funded by Project A.L.S., U.K. researchers found that many people with ALS have a marker of retrovirus activity in their blood. The findings were reported in the February 8, 2005 issue of the medical journal *Neurology*.

The study detected "reverse transcriptase" activity in the blood of 47 per cent of ALS patients, confirming the results of an earlier study. Reverse transcriptase is an enzyme associated with retroviruses. Its activity implies the presence of a retrovirus. Interestingly, the activity was found in equal proportion in direct relatives of the ALS patients in the study, but not in their spouses. This finding suggests the possibility that an inherited retrovirus plays a role in ALS.

Turnbull cautions that the results are difficult to interpret. "It may be that the increase reflects new viral activity, or a re-activation of retrovirus fragments that we all carry around with us in our cells, and that we inherit from generation to generation. If that's the case, it may be that the reactivation has occurred because of the ALS – not the other way around."

For now, Turnbull does not advise retroviral therapy for people with ALS. "Until further work is done to sort out some of the above difficulties, I think further clinical trials into retroviral therapies in humans might be premature."

Follow-up studies have already begun. A statement from Project A.L.S. reads, "The next phase of this exciting

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Detection of serum reverse transcriptase activity in patients with ALS and unaffected blood relatives

A.J. Steele, PhD, A. Al-Chalabi, PhD, FRCP, K. Ferrante, BA, M.E. Cudkowicz, MD, MSc, R.H. Brown, Jr., MD, DPhil and J.A. Garson, MD, PhD

Methods:

A highly sensitive product–enhanced RT assay was employed to test coded sera obtained from 30 American patients with sporadic ALS and from 14 of their blood relatives, 16 of their spouses, and 28 nonrelated, nonspousal control subjects.

Results:

Serum RT activity was detected in a higher proportion of ALS patients (47%) than in non-blood-related controls (18 %; p = 0.008). The prevalence of RT activity in the serum of spousal controls (13 %) was similar to that in other non-blood-related controls. Unexpectedly, the prevalence of serum RT activity in blood relatives of ALS patients (43 %) approached that in the ALS patients themselves.

Conclusions:

These results confirm that patients with ALS have a significantly higher prevalence of serum reverse transcriptase (RT) activity than that seen in unrelated control subjects. The finding of a similarly increased prevalence in blood relatives of ALS patients raises the possibility that the observed RT activity might be due to an inherited endogenous retrovirus.

work, funded by Project A.L.S., has recently commenced. It is hoped that further progress in this area will lead to the development of improved diagnostic tests and novel therapeutic strategies, and to a greater understanding of the fundamental causes of this progressive and ultimately fatal neurological disease."

Viral Delivery vehicles for RNAi

Currently, Turnbull and his team are researching ways to implement viral delivery vehicles for RNAi (ribo nucleic acid interference). Their techniques are being tested in transgenic mice, and also in cell cultures.

Turnbull is hopeful that RNAi will have a role in battling familial ALS and, later on, sporadic ALS. As Turnbull points out, there are more than 100 possible mutations of the SOD1 gene responsible for familial ALS.

The project started approximately one year ago and Turnbull is working in collaboration with Drs. Kenneth Rosenthal from the Viral Vaccine Division at McMaster's Centre for Gene Therapeutics, and Jack Gauldie – who is the Director of the Centre.

Although the techniques that are being examined are still new, "they do look promising," says Turnbull.

There are still some difficulties that need to be worked out before RNAi can be used. Delivery systems are one of the primary concerns. For example, how will the RNAi get into motor neurons? Also, while it may be easy to show positive effects of RNAi therapy in a Petri dish, it is more difficult to display the same results in transgenic mice. "This leads to the big question" which Turnbull points out, "Can the results be extrapolated into humans?"

For future use of RNAi, carriers of the SOD1 gene may need life-long therapy. Vectors may also be used. With retroviral vectors, however, there is a significant risk of dangerous side effects such as leukemia.

"There will be many ethical concerns surrounding this type of therapy. It's going to be difficult for people who are pre-symptomatic. They will have to weigh the risks and benefits very carefully," explains Turnbull."

Turnbull received his Honors B.Sc. (Theoretical Physics and Chemistry) from York University, a Masters Degree (Mining and Metallurgical Engineering) from Laval University and a PhD (Pharmacology and Toxicology) from the University of Montreal. He received his MD degree from the University of Western Ontario, obtained his fellowship in Internal Medicine from McMaster University, and his fellowship in Neurology from the University of Western Ontario. He undertook two years of post-doctoral training at the University of California (Irvine), at the Centre for the Neurobiology of Learning and Memory. Turnbull is also the Douglas Professor and Head of Neurology at McMaster University.

ALS Events Support Research

The following events make significant contributions to funding ALS research:

Betty's Run for ALS
(also supporting the ALS Society of Alberta)

Jim Hughes Golf Classic

Supporters associated with one of the 10 Provincial ALS Societies in Canada, organize events, large and small, throughout the year, and especially during June ALS Awareness Month.

In addition, there are planned gifts (wills, estates, life insurance policies and securities) In Memoriam and In Recognition donations, made in support of ALS research.

ELIZABETH'S CONCERT OF HOPE
(also supporting the ALS Society of Ontario)

HIKE 4 ALS

WALK for ALS
MARCHE pour la SLA

ALS PROVINCIAL SOCIETIES

ALS Society of Alberta (and NWT)
320 - 23rd Avenue SW, Suite 400
Calgary, AB T2S 0J2
Tel: (403) 228-3857
Toll free: 1-888-309-1111 (Alberta and NWT)
Fax: (403) 228-7752
alsab@alsab.ca
www.alsab.ca

ALS Society of British Columbia (and Yukon)
#119-1600 West 6th Avenue
Vancouver, BC V6J 1R3
Tel: (604) 685-0737
Toll free: 1-800-708-3228
Fax: (604) 685-0725
info@alsbc.ca
www.alsbc.ca

ALS Society of Manitoba
2109 Portage Avenue, Room N108
Winnipeg, MB R3J 0L3
Tel: (204) 831-1510 or (204) 254-5337
Toll free: 1-866-718-1642
Fax: (204) 837-9023 or (204) 255-1743
als@deerlodge.mb.ca
www.alsmb.ca

ALS Society of New Brunswick
P.O. Box 295
Moncton, NB E1C 8K9
Tel: (506) 855-1239
Toll free: 1-866-722-7700 (in New Brunswick)
Toll free: 1-866-625-7257 (in the Maritimes)
Fax: (506) 388-7466

ALS Society of Newfoundland and Labrador
P.O. Box 844
Corner Brook, NF A2H 6H6
Tel: (709) 634-9499
Toll free: 1-888-364-9499
alssocietyofnfld@nf.aibn.com

ALS Society of Nova Scotia
3433 Dutch Village Road, Unit #3
Halifax, NS B3N 2S7
Tel: (902) 454-3636
Toll free: 1-866-625-7257 (in the Maritimes)
Fax: (902) 453-3646
info@alsns.ca

ALS Society of Ontario (and Nunavut)
265 Yorkland Blvd., Suite 300
Toronto, ON M2J 1S5
Tel: (416) 497-2267
Toll free: 1-800-267-4257
Fax: (416) 497-1256
info@alsont.ca
www.alsont.ca

ALS Society of Prince Edward Island
c/o 103 Maple Avenue
Charlottetown, PEI C1A 6G2
Tel: (902) 892-7102
Toll free: 1-866-625-7257 (in the Maritimes)

Société de la SLA du Québec
ALS Society of Québec
6300 Park Avenue, Suite 502
Montreal, Québec H2V 4H8
Tel: (514) 725-2653
Toll free: 1-877-725-7725 (in Québec)
Fax: (514) 725-6184
info@als-quebec.ca
info@sla-quebec.ca
www.sla-quebec.ca

ALS Society of Saskatchewan
Box 31028, Normanview Mall
Regina, SK S4R 8R6
Tel: (306) 949-4100
Fax: (306) 949-4020
alssocietyofsask@sasktel.net

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ALS Society of Canada
265 Yorkland Boulevard, Suite 300
Toronto, Ontario M2J 1S5
Tel: (416) 497-2267
Toll Free: 1-800-267-4257
Fax: (416) 497-1256
www.als.ca