



## **RESEARCH IN THE WORKS 2007:**

Neuromuscular research was a driving reason for the formation of Muscular Dystrophy Canada in 1954. At that time, little research was being done in Canada or elsewhere in the world. Since its inception, Muscular Dystrophy Canada has provided over \$60 million dollars in funding to Canadian scientists who are determined to discover the causes, treatments, and eventual cures for muscular dystrophy and other neuromuscular disorders. Great progress has been made, but much more work needs to be done.

The Neuromuscular Research Partnership is the cornerstone of Muscular Dystrophy Canada's research program. Muscular Dystrophy Canada collaborates with ALS Society of Canada and the Canadian Institutes of Health Research (Institute of Musculoskeletal Health and Arthritis, Institute of Genetics, and the Institute of Neurosciences, Mental Health and Addiction) to fund the most promising neuromuscular research projects in Canada. Applications are subject to a rigorous peer-review and relevancy grading process, and the top rated applications are awarded grants.

This partnership arrangement allows Muscular Dystrophy Canada to leverage our donor dollars; for every dollar that Muscular Dystrophy Canada contributes, more than 3 times that amount is spent on research.

The following broad categories are eligible for funding:

- Basic research involving muscle or nerve biology relevant to neuromuscular disease.
- Focused research directed toward an understanding of neuromuscular disease.
- Applied research encompassing research designed specifically to translate promising research advances from basic research and focused research into pre-clinical and clinical investigations relevant to treatment of neuromuscular disease, but not including drug trials.

In 2007, the Neuromuscular Research Partnership jointly invested a total of \$4 million dollars in 12 operating grants.

## **2007 Neuromuscular Research Partnership grants:**

1). Interplay of oxidative stress and protein misfolding in amyotrophic lateral sclerosis.

Period: 3 years

Principal Investigator(s): CHAKRABARTTY, Avijit

Co-Investigators:

Institution: University Health Network (Toronto)

**A genetic mutation causes an important enzyme to become toxic causing a type of amyotrophic lateral sclerosis (ALS). This research will explore the mechanisms leading to this toxicity.**

The formation of chemical byproducts (including free radicals) derived from oxygen is a normal part of cellular mechanisms but these free radicals are chemically unstable and present a major threat to the cell. Normally, the cell utilizes complex defenses to help cope with the damage they cause, for example, the enzyme superoxide dismutase (SOD). More than 100 mutations in the gene that makes this enzyme have been linked to causing a type of ALS, most of these mutations appeared to make the enzyme toxic. It was hypothesized that cellular oxidation induces misfolding and abnormal aggregation of SOD1. This research team confirmed that misfolded SOD1 is found within degenerating motor neurons – an important finding that will soon be published in the prestigious journal *Nature Medicine*.

This research project will further explore the interaction of oxidative stress and SOD1 misfolding as a causative factor in ALS. A mouse model will be utilized to measure the distribution of misfolded SOD1. Manipulation of oxidative components will be used to see if increased oxidative stress induces SOD1 misfolding. The relationship between SOD1 misfolding and mitochondrial function will also be examined. In addition, the ability of an antioxidant to reduce or reverse misfolding will be examined.

2. Investigating the role of diacylglycerol kinase- zeta in the assembly and maintenance of the myofibrillar apparatus in skeletal muscle

Period: 3 years

Principal Investigator(s): GEE, Stephen H

Co-Investigators:

Institution: University of Ottawa

**This research will investigate myofibrils, a key component of the muscle and examine the mechanisms involved in their formation and normal disassembly, shedding light upon neuromuscular disorders related to the abnormal deterioration of these muscle structures.**

Myofibrillar myopathies (MFMs) are a group of skeletal muscle diseases that are characterized by an abnormal pattern of muscle deterioration associated with the excess accumulation of multiple proteins including desmin, causing progressive weakness and often heart problems. This research will examine the components and mechanisms

involved in the normal disassembly of myofibrils. In addition, currently, little is known about the formation of myofibrils and this research is also designed to examine the mechanisms involved in the creation of the proteins that form myofibrils. This knowledge will be important in eventually developing treatment approaches to MFM disorders.

3). Molecular basis of ryanodine receptor regulation and function in skeletal muscle

Period: 5 years

Principal Investigator(s): GRAMOLINI, Anthony O

Co-Investigators:

Institution: University of Toronto

**This research is designed to increase our understanding of a cluster of interacting proteins in skeletal muscle that are involved in normal muscle contraction and further, to learn about the mechanisms involved in genetic disorders involving mutations in one or more of these proteins.**

The operation of skeletal muscle involves excitation and contraction which in turn depends upon a large protein cluster called the “calcium release unit.” The major component in this protein complex is the ryanodine receptor (RyR), a component involved in the movement of calcium ions – a key aspect of regulating muscle contraction and relaxation. Genetic defects in any of these proteins result in neuromuscular disorders including malignant hyperthermia and central core disease. This research will utilize a number of experimental techniques to better understand the regulation and function of these proteins and receptors in skeletal muscles. This knowledge will help identify targets for therapeutic intervention in disorders related to the ryanodine receptor.

4). Combinatorial use of viral vectors for the gene therapy of muscle

Period: 2 years

Principal Investigator(s): HOLLAND, Paul C NALBANTOGLU, Joséphine

Co-Investigators:

Institution: Montreal Neurological Institute

**This research will attempt to improve the efficiency of current techniques used to transfer genes into muscle cells – a critical step in the eventual treatment of several types of muscular dystrophies.**

Much research on Duchenne muscular dystrophy has focused upon methods to restore the function of dystrophin protein within the muscle. The introduction of a full-length dystrophin gene is the ideal but is difficult due to the extremely large size of this gene. Introducing a therapeutic gene is normally done by associating it with a virus and introducing the virus into the cell. This method, called a viral vector, is under intense investigation. One problem has been that mature muscle is difficult to infiltrate with the viral factor containing the full gene product for dystrophin.

This research details several experiments in a mouse model designed to increase the success of utilizing an adenovirus vector in mature muscle to would facilitate the

introduction of full-length dystrophin DNA. First, mature muscle fibers will be treated with a receptor designed to enhance the efficiency of gene transfer using injected adenovirus. Research will monitor the time needed to achieve maximum expression of this receptor in mature muscle and to determine the optimal time of administration for the adenovirus. Finally, measurements of dystrophin expression will be made. This research will determine if the overexpression of the adenovirus receptor is a feasible and safe strategy to increase efficiency of the delivery of the dystrophin gene into mature muscle cells. If this is the case, it supports further research in larger animal models and ultimately in humans.

5). Live imaging and analysis of disease onset and progression in amyotrophic lateral sclerosis

Period: three years

Principal Investigator(s): KRIZ, Jasna

Co-Investigators:

Institution: Université Laval

**This research will use live imaging to examine some of the early cellular changes associated with amyotrophic lateral sclerosis (ALS) and refine this technique as a new research tool.**

Recent research has implicated the involvement of several non-neuronal cells in the pathway leading to amyotrophic lateral sclerosis (ALS). These cells include astrocytes and microglia. The main objective of this research is to clarify the events associated with disease onset and progression in a mouse model using live imaging of astrocytosis, microgliosis and neuronal damage. This research will examine the role of astrocyte activation to determine if this is involved in the early stages of disease onset. If so, the modulation of astrocytosis may represent a potential therapeutic target. Developments of light imaging technologies will help understand and characterize the role of astrocytes and microglia in ALS as well as neuronal damage and will also present a new tool for target drug screening.

6). Characterization of PABPN1 for the development of an Oculopharyngeal Muscular Dystrophy (OPMD) treatment

Period: 3 years

Principal Investigator(s): ROULEAU, Guy A

Co-Investigators:

Institution: Centre Hosp. de l'Université de Montréal (CHUM)

**This research will focus on very early protein abnormalities produced by a genetic mutation that eventually leads to oculopharyngeal muscular dystrophy (OPMD) and examine several drugs that may reduce the toxicity of these proteins.**

Oculopharyngeal muscular dystrophy (OPMD) primarily causes weakness in the eyelids and muscles that control the pharynx. In most cases symptoms come on between the age of 40 and 60 and eventually also involve proximal limb muscles, half of cases eventually

become wheelchair-bound. A high rate of OPMB is found in Québec making this an especially important type of muscular dystrophy for Canadians. In the 1990s this research group found a gene (poly A binding protein nuclear 1 gene – PABPN 1) that causes OPMD and has been active in studying laboratory and mouse models for research. The development of a mouse model of this disorder was critical to research into the testing of potential treatments. Another new model involving *C. elegans*, a type of worm, will also be important in studying drugs that may lead to a treatment of this disorder. This research will focus on strategies designed to reduce the toxic effects of the proteins produced as a result of the mutated PABPN 1 gene before the formation of protein inclusions, a feature associated with the later progression of the disorder. Several nontoxic drugs have been chosen for examination.

7). Development of therapeutic strategies for dysferlin deficiency

Period: 3 years

Principal Investigator(s): SINNREICH, Michael

Co-Investigators:

Institution: Montreal Neurological Institute

**This research will help create the foundation for the eventual gene therapy of dysferlin related disorders (the limb girdle muscular dystrophies).**

Limb girdle muscular dystrophy represents a group of diverse disorders; many are associated with specific genetic mutations and many remain of unknown origin. All cause muscle weakness and wasting in the pelvic and shoulder girdle. Mutations in dysferlin are a frequent cause of the recessively inherited limb girdle muscular dystrophies, comprising about 15% of cases in clinical practice. Currently no treatment exists for patients suffering from dysferlin abnormalities – now often called the dysferlinopathies.

Strategies utilizing gene therapy and adeno-associated virus (AAV) vectors would be suitable for use with an abbreviated dysferlin (micro-dysferlin) gene. This research will guide the strategy to produce functional micro-dysferlin molecules that can be tested in the laboratory to determine their effect on abnormal (dysferlin deficient) human myotubes. These experiments will lay the groundwork for eventual future genetic transfer experiments in mouse models using micro-dysferlin as a treatment for the dysferlinopathy related muscle disorders.

8). Motor neuron differentiation, connectivity, and regeneration

Period: 4 years

Principal Investigator(s): STIFANI, Stefano.

Co-Investigators:

Institution: Montreal Neurological Institute

**This research examines the role played by a key protein involved in the generation of motor neuron cells and the possibility that this protein could be taken advantage of in a stem cell approach to regenerating motor neurons destroyed as a result of amyotrophic lateral sclerosis.**

Amyotrophic lateral sclerosis (ALS) causes degeneration of the motor nerve cells in the brain and spinal cord. Understanding the mechanisms involved in this degeneration is important to develop therapeutic approaches using stem cells. This research group believes that the genetic mechanisms involved in developing motor neurons may be harnessed to stimulate their replacement using stem cells. A key protein (Runx1) is expressed during the development of motor neurons in the mouse and it is hypothesized that this protein may be involved in the generation of motor neurons from embryonic stem cells. This research will develop a number of experiments to test the hypothesis. Three aspects will be considered, first, whether Runx1 promotes the differentiation of motor neuron cells, whether it is involved in establishing the correct pattern of connections of the motor neuron cells and third, to determine if Runx1 can be used as part of a therapy designed to create new motor neuron cells using a stem cell approach.

9). Exercise-induced upregulation of mitochondrial gene expression: Therapeutic strategies for mitochondrial disease

Period: three years

Principal Investigator(s): TAIVASSALO, Tanja

Co-Investigators:

Institution: McGill University

**This research will consider the safety and possible benefit of different types of exercise in patients with mitochondrial myopathies.**

Mitochondrial DNA defects are associated with neuromuscular diseases – the mitochondrial myopathies. Two different types of exercise training have been proposed as treatment strategies, one, resistance training, based on the rationale that activation of muscle satellite cells through exercise will help increase the number of normal (“wild”) mitochondrial DNA present within the skeletal muscle and second, endurance training. The effect of endurance training is less clear and may have long-term deleterious effects. The current research is based on the idea that increases in the overall number of normal mitochondrial DNA within skeletal muscle will improve cellular and full body oxidative function. The proposal is that resistance training followed by endurance training is a safe and effective technique for these patients. The analysis of the response of the normal mitochondrial DNA within individual muscle fibers will bring important insight into the cellular effects of exercise and will determine if exercise training can help contribute to the management of this disorder and/or improve the quality of life of patients with mitochondrial DNA defects. This research will also help illuminate the critical question – “should exercise be encouraged or discouraged or avoided altogether in these cases?”

10). Improving MPC transplantation by increasing IGF-1 or MGF stimulation

Period: 3 years

Principal Investigator(s): TREMBLAY, Jacques P.

Co-Investigators:

Institution: Université Laval

**This research will examine ways to increase the efficiency of the transplantation of muscle stem cells as a treatment in muscular dystrophy.**

This project builds upon previous research intended to use genetically treated muscle precursor cells (MPCs) to improve function in patients with recessive muscular dystrophies. A phase 1 clinical trial was recently successfully completed showing that dystrophin expression was improved in eight out of nine patients treated. The long-term objective of the current project is to increase the proliferation and fusion of these cells into muscle and increase muscle strength. This research will use insulin growth factor 1 and a synthetic growth factor. If these treatments increase muscle mass, the effects of this treatment on muscle strength will be investigated further. These experiments will indicate whether increases in growth factor will increase muscle mass and improve the success of myoblast transplantation.

11). Identification of the mechanisms of motor neuron degeneration in amyotrophic lateral sclerosis (ALS)

Period: 3 years

Principal Investigator(s): VANDE VELDE, Christine Diane C

Co-Investigators:

Institution: Centre Hosp. de l'Université de Montréal (CHUM)

**This research will help clarify the mechanisms leading to one type of amyotrophic lateral sclerosis (ALS).**

One form of familial amyotrophic lateral sclerosis (ALS) is caused by mutations in copper/zinc superoxide dismutase 1 (SOD1). This mutation somehow creates a toxic property in the SOD1 but the biological basis for this toxicity remains unknown. A portion of these mutant proteins are found in association with mitochondria derived from affected spinal cords. This association may shed light on the toxic mechanism involved. This research project will examine if any temporal correlation exists between any mitochondrial impairments and the association of the mutated SOD1s. A number of experimental techniques will be used to address this question. Specifically, mitochondrial positioning and movement will be carefully examined. The effects of mutant SOD1 on mitochondrial transport in motor axons will be determined in relation to the disease stage. The research will also examine whether mutant SOD1 mitochondrial localization is unique to motor neurons. It is expected that these experiments will reveal a connection between mitochondrial damage and the clinical manifestations of ALS.

12). Amyotrophic lateral sclerosis: Rescue of mutant sod1-induced motor neuron death by targeting the BNIP3 death gene family

Period: 1 year

Principal Investigator(s): KONG, Jiming

Co-Investigators:

Institution: University of Manitoba

**This research will examine the a key gene involved in the regulation of cell death in amyotrophic lateral sclerosis and attempt to protect nerve cells by modulating the activity of in this gene.**

The BNIP3 gene is involved in the regulation of cell death. This research group has previously demonstrated that levels of BNIP3 gene family expression increase in motor neurons before the onset of amyotrophic lateral sclerosis (ALS) in mice genetically bred with mutant SOD1 and that reducing the effect of this gene reduced mutant SOD1-induced motor neuron death. The BNIP3 pathway may show commonalities linking oxidative stress, mitochondrial dysfunction and motor neuron death in ALS. This research will examine this pathway and test strategies to protect neurons by reducing the activity of this gene. This has the potential to lead to new approaches to treatments.