



May 2006

Research updates reflect a sampling of what is important and new in muscle research.

General Developments

► Human gene therapy typically attempts to re-introduce a normal functioning gene into a genetically mutated cell/organism or repress the activity of a defective gene. Therapy for recessive disorders may only require the introduction of a healthy replacement gene to restore gene function (because recessive conditions generally have both copies of the affected gene being defective) and there are now many examples of gene therapy techniques for recessive disorders. On the other hand, dominant conditions, which can be due to haploinsufficiency or to a gain-of-novel function, are more difficult to address and few therapies have been developed for them. Therapies for dominant conditions require silencing the dominant mutated gene copy without interfering with the expression of the other normal gene copy. This article describes several techniques available for gene therapy of dominantly inherited conditions. The overview includes considerations and comparisons of antisense DNA and RNA, ribozymes and RNA interference. The authors conclude that with further understanding and refinements, RNA interference will become the likely choice for therapy. Examples of candidate conditions for this treatment include myotonic dystrophy and familial amyotrophic lateral sclerosis (ALS).

Source: RNA based gene therapy for dominantly inherited diseases. By: Pelletier R, Caron SO, Puymirat J. *Curr Gene Ther.* Feb;6(1), Pages: 131-46, 2006.

Dr. Puymirat is an MDC funded researcher. He and his team are at the Human Genetics Research Unit, Laval University, CHUQ, Pavillon CHUL, Ste-Foy, Quebec, Canada, G1V 4G2.

► Gene treatments for muscle disorders require a means to deliver the modified gene into the muscles. Normally, the gene is packaged into a “vector” that delivers it to the target. Many types of vectors have been created, both viral and non-viral. A virus vector known as adeno-associated virus (AAV) is currently the choice for gene transfer into muscle. The last couple of years have seen major breakthroughs in the field of vector delivery systems, particularly those using the vascular route -- intravenous injection, such that gene therapy of muscular dystrophies has become a clinical possibility. The author notes that successful gene transfer of dystrophin may slow or stop the progression of Duchenne muscular dystrophy but that the treatment is unlikely going to result in increased muscle strength; he feels that other treatments will be required to deal with the consequences of existing damage, for example, using growth factors to correct the damage. One complication that must be managed successfully is the body's natural immune response to both the vectors and the modified genes.

Source: Viral and non-viral methods for gene transfer into skeletal muscle. By: Wells D.J. *Curr Opin Drug Discov Devel.* Mar;9(2), Pages:163-8, 2006.

Note: MDC is also funding research on muscle gene vectors:

-Dr. Robin Parks & Jonathan Bramson, Ottawa Health Research Institute: Adenovirus vectors for gene therapy of muscle.

► A chapter titled “Diseases of Muscle and the Neuromuscular Junction”, in a recent publication of The American College of Physicians describes the common disorders of muscle and the neuromuscular junction with an emphasis on the clinical description, causes, diagnosis and therapy. The chapter was written by Marinos C. Dalakas, MD, Senior Investigator, Neuromuscular Diseases Section, National Institute of Neurological Disorders and Stroke. In the past, these disorders were classified clinically, today they are better described by the gene defect and corresponding protein abnormality involved. Each disorder is given a brief but comprehensive review. An excellent illustration of the dystrophin–glycoprotein complex (DGC) accompanies the chapter.

Source: *ACPMEDICINE* A Publication of the American College of Physicians, Vol. 29, No. 4 April 2006. The chapter by Dalakas appeared in *ACP Medicine Neurology: III Diseases of Muscle and the Neuromuscular Junction*, pages: 1-20, April 2006 update.

► Physical therapy often plays an important role in muscular dystrophies. This article suggests that physical therapy offers the most promise in caring for the majority of patients with muscular dystrophy conditions, because it is unlikely that advances in gene therapy will significantly alter the clinical treatment of patients in the near future. This review presents a basic biological summary together with the clinical manifestations of the muscular dystrophies and the latest approaches to their physical management. The article discusses the role of physical therapy in dealing with some of the complications of these conditions including pain management, the use of night splints to help prevent contractures (often a more debilitating side effect than the muscle weakness itself), and the use of hydrotherapy to develop mild exercise programs.

Source: The Muscular Dystrophies: From Genes to Therapies, By: Richard M Lovering, Neil C Porter, Robert J Bloch, *Physical Therapy*, 85, Number 12, December 2005.

► The dystrophin–glycoprotein complex (DGC) is a large multi-protein structure in the wall of cells. The DGC acts as a bridge connecting the inside "skeleton" of the cell with the outer environment that surrounds cells, the extra-cellular matrix (ECM). In addition to a mechanical function, this complex of proteins also appears to play a role in sending chemical signals between the cell's interior and its nearby environment. The DGC is very prominent in muscle cells and mutations in any one of its proteins leads to a disruption of function and a form of muscular dystrophy, the type depending upon which protein is involved. Loss of the DGC leads to wasting of muscle and loss of muscle function. Perhaps the best known DGC protein is dystrophin (defects in which cause Duchenne muscular dystrophy). This article considers several of the complex roles played by the DGC in the muscular dystrophies. A more integrated picture of the DGC is slowly emerging but a comprehensive understanding remains to be achieved. Excellent illustrations accompany the article.

Source: Sparks, signals and shock absorbers: how dystrophin loss causes muscular dystrophy

By: Clare L. Batchelor and Steve J. Winder *TRENDS in Cell Biology*, 16 No.4, Pages: 198-205, April 2006.

Note: MDC is also funding research on the dystrophin glycoprotein complex:

-Dr. Hakima Moukhles, University of British Columbia, Dystroglycan function in glial cells.

Duchenne Muscular Dystrophy

► Duchenne muscular dystrophy is caused by mutations in the gene that produces the protein dystrophin, a gene that is very large and difficult for researchers to work with. A very similar protein called utrophin is found in the muscles in very early development but very soon, its levels fade and dystrophin assumes its role. For more than 10 years, researchers have been experimenting on ways to stimulate the muscle cells to create extra utrophin in the hopes that it could compensate for defective dystrophin. An article by two researchers from the University of Ottawa, Pedro Miura and Bernard J. Jasmin, reviews utrophin and several different strategies that have been used to increase utrophin in the muscles of research animals. Three main approaches are reviewed that have been successful in at least partly compensating for dystrophin defects in animals. The authors conclude that significant progress has been made but that further research on mice and dogs is required. They believe that in the future, utrophin therapy will likely be one part of a combined approach to ameliorating the effects of dystrophin mutations.

Source: Utrophin upregulation for treating Duchenne or Becker muscular dystrophy: how close are we? By: Pedro Miura and Bernard J. Jasmin, [University of Ottawa] *TRENDS in Molecular Medicine*, 12, No. 3, Pages: 122-129, March 2006.

Note: MDC is also funding research on boosting utrophin levels:

-Dr. George Karpati & Joséphine Nalbantoglu, Montreal Neurological Institute & McGill University: Molecular therapies for dystrophin deficiency.

-Dr. George Karpati, Montreal Neurological Institute & McGill University: Extrasynaptic Endogenous Utrophin Upregulation in Dystrophin Deficient Muscle: A Therapeutic Approach For Duchenne Muscular Dystrophy.

► Deflazacort is a man-made corticosteroid that is derived from prednisone. These medications are similar to natural steroid hormones produced in the body by the adrenal glands. They have complex roles in controlling the various systems in the body especially in response to stress. Man-made corticosteroids, primarily prednisone, have been used to decrease inflammation in muscle disorders and remain the most effective treatment for the inflammation related to musculoskeletal disease. The use of these medications has been complicated by a number of serious side effects, including obesity, bone abnormalities, diabetes and hypertension. Deflazacort appears to have fewer side effects than other corticosteroids. Duchenne muscular dystrophy (DMD) has often been treated with either prednisone or deflazacort. A recent paper written by a team from the University of Toronto reviews the long-term effectiveness of deflazacort in the treatment of boys with DMD. This research, done on 74 boys, demonstrates that deflazacort has a very significant positive impact on the quality of life and health of boys with DMD and is associated with few side effects. Deflazacort extends mobility for three to five years and as well, has significant impacts on a number of health related issues including; improved cardiac and pulmonary function, and reduced scoliosis (spinal curvature usually requiring surgery). The use of these drugs needs to be adjusted and carefully considered as maturation continues.

Source: Long-term benefits of deflazacort treatment for boys with Duchenne muscular dystrophy in their second decade. By: W. D. Biggar, V.A. Harris, L. Eliasoph, B. Alman. [University of Toronto] *Neuromuscular Disorders*, 16, 249–255, 2006.

Other recent major reviews of DMD can be found here:

Splicing intervention for Duchenne muscular dystrophy. By: Graham McClorey, Susan Fletcher and Stephen Wilton. *Current Opinion in Pharmacology*, 5, 529–534, 2005.

Molecular, cellular, and pharmacological therapies for Duchenne/Becker muscular dystrophies. By: Joe V. Chakkalakal, Jennifer Thompson, Robin J. Parks, and Bernard J. Jasmin. [University of Ottawa] *FASEB Journal*, 19, 880–891, 2005.

Experience and Strategy for the Molecular Testing of Duchenne Muscular Dystrophy. By: Thomas W. Prior and Scott J. Bridgeman, *Journal of Molecular Diagnostics*, Vol. 7, No. 3, August 2005.

► Duchenne muscular dystrophy (DMD) is caused by a mutation in the gene that manufactures the dystrophin protein. Some of the specific mutations in dystrophin prevent the whole message (mRNA) of the gene from being read, thus impairing dystrophin production. For the majority of DMD mutations, if this defective section can

be bypassed (skipped), then the rest of the message of the gene will be correctly read and amounts of dystrophin protein manufactured – enough to significantly ameliorate DMD symptoms. A short man-made piece of genetic material (an oligonucleotide - a short stretch of nucleic acid) is introduced into the cell to block the mistake and allow it to be skipped over. The actual sequence of the man-made material is designed to correspond to the natural genetic code containing the mistake: it attaches to the faulty code and “covers it up”. Now, when the cell reads the message, the mutated code is skipped over and the rest of the message gets read and put to work making dystrophin - it may not be perfect but it is a major improvement. The approach is called antisense oligonucleotide (AON) mediated exon skipping.

This research presents a modified approach to AON therapy that produces encouraging results in mice, with a resulting improvement in muscle function. Although problems remain to be overcome (notably the level of resulting dystrophin expression varies considerably between different muscles) the authors feel that this approach may lead to an eventual treatment for a majority of individuals with DMD.

In this approach, the short man-made sequence of genetic material consists of a small number of genetic letters (bases) that are constructed on a backbone of morpholine molecules -- this differs from natural DNA (which is formed by a backbone of deoxyribose sugar molecules). One of the key challenges is how to deliver the man-made genetic code into the body. The researchers note that using their approach has a number of advantages over the other leading method which uses a virus to deliver a modified genetic sequence.

Source: Systemic delivery of morpholino oligonucleotide restores dystrophin expression bodywide and improves dystrophic pathology. By: Alter J, Lou F, Rabinowitz A, Yin H, Rosenfeld J, Wilton SD, Partridge TA, Lu QL. *Nature Medicine*, Feb, 12(2):175-7 2006.

Note: MDC is also funding research on a similar approach:

-Dr. J. Puymirat, Centre hospitalier de l'Université de Québec: Ribozyme and antisense RNA as a tool to study myotonic dystrophy.

In a related story, dated May 11, 2006, CureDuchenne have revealed that a Dutch biotech company (Prosensa) is working with researchers from the Leiden University Medical Centre (LUMC), South Holland, The Netherlands, to perform the first in-human trial using antisense oligonucleotides (AON) to treat Duchenne Muscular Dystrophy (DMD). The exploratory study focuses on the efficacy, safety and tolerability of a single dose of antisense oligonucleotides injected into a muscle to restore production of dystrophin, the protein defective in DMD patients. This study is the first step in demonstrating the general safety and effectiveness of the method (proof-of-principle). If the study is successful, future studies will move to systemic, full-body, delivery. Approval for the study was granted from the Netherlands Central Committee on Research involving Human Subjects (CCMO) and the Medical Ethics Committee of LUMC.

Source: <http://www.cureduchenne.org/>

► A press release from the University of North Carolina at Chapel Hill dated March 29, 2006 (number 175) reports that the first gene therapy human trial in the United States for a form of muscular dystrophy (Duchenne muscular dystrophy (DMD)) is now under way. The release states that: “The trial was launched March 28, at Columbus Children's Hospital in Ohio, an affiliate of Ohio State University's School of Medicine. In the trial, six boys with DMD will receive replacement genes for an essential muscle protein. Each of the boys will receive replacement genes via injection into a bicep of one arm and a placebo in the other arm. Neither the investigators nor the participants will know which muscle got the genes. After several weeks, an analysis of the injected muscle tissue's microscopic appearance, as well as extensive testing of the health and strength of the trial participants, will reveal whether gene therapy for DMD is likely to be safe and whether it's likely to result in persistent production of the essential protein in muscle cells. . . . The new Biostrophin therapy uses a novel combination of advanced technologies, including a miniaturized replacement dystrophin gene and nano delivery technology called biological nanoparticles. Developed from a virus known as adeno-associated virus (AAV), the nanoparticles are engineered specifically to target and carry the "minidystrophin" ["mini-D"] gene to muscle cells.”

Source: Stephanie Crayton (919) 966-2860

<http://www.unc.edu/news/archives/mar06/mdtrial032906.htm>

► This article reviews gene therapy approaches to treating muscular dystrophy with an emphasis on Duchenne muscular dystrophy. One challenge is that a safe and reliable means of delivering a modified gene into the cells needs to be developed. One of the most widely researched delivery mechanism uses a virus, usually based on adeno-associated virus (AAV). These delivery mechanisms are called vectors and are critical in gene therapy as their safety must be guaranteed at the doses required to deliver sufficient gene product. Efforts to develop safe and effective gene vectors go hand-in-hand with trying to create small but efficient gene treatments or replacements for genes, for example the development of micro-dystrophin to treat mutations in the large dystrophin gene. The authors conclude that “gene therapy as a potential treatment tool for muscular dystrophy is poised for clinical trials. There is an overwhelming consensus that AAV is the vector of choice for muscular dystrophy gene therapy” (page 54). Several questions need to be addressed, including requirements for immune suppression, the specific subtype of virus to use, and the possible creation of hybrid vectors. The authors end by saying: “The future looks bright with a steady, consistent assault by this means of treatment. Successes will most likely be achieved in a stepwise manner, with regional delivery clearly in sight and systemic [body wide] vascular delivery [through the blood] is now in view on the horizon.”

Source: Challenges for Gene Therapy for Muscular Dystrophy. By: Jerry R. Mendell, and K. Reed Clark, *Current Neurology and Neuroscience Reports*, 6, Pages: 47–56, 2006.

► Myoblast transfer therapy (MTT) has long been viewed as a potential therapy for Duchenne muscular dystrophy. This technique involves the transplantation of muscle precursor cells (called myoblasts) directly into the muscle fibres of the patient. Fusion of

donor myoblasts with the muscle cells of the patient creates a mosaic of defective and healthy genetic material and ideally, enough healthy dystrophin is produced to improve function. The article notes that the early clinical trials where donor myoblasts from the father were used were controversial and unsuccessful and thus interest in MTT waned. This approach has had little success in clinical trials because the majority of donor myoblasts cells die very quickly after transplantation. The authors suggest that chemical conditions before the transfer adversely affect the cells.

The recent discovery of a population of cells in skeletal muscle tissue, which have the ability to differentiate into both myogenic (muscle creating) and non-myogenic offspring has interesting implications for the future of traditional myoblast cell transplantation therapies. These muscle-derived stem cells (MDSC) found within adult muscle have stem cell-like characteristics. This review focuses on the characterization and application of these muscle-derived stem cells (MDSC) to myoblast transfer therapy. The exact location of these MDSC cells within the muscle has not yet been fully identified. Researchers need to discover whether they are located in the satellite cell position (and therefore represent a satellite stem cell) or if they lie outside the muscle cell (and represent an interstitial stem cell).

Source: Critical Review: Muscle-Derived Stem Cells: Implications for Effective Myoblast Transfer Therapy, By: Tracey F. Lee-Pullen and Miranda D. Grounds, *IUBMB Life*, 57(11), Pages: 731 – 736, November 2005.

Note: MDC is also funding research on transferring myoblasts:
-Dr. J. Tremblay, Laval University: Treatment of Duchenne muscular dystrophy: Correction of mutated dystrophin mRNA with ribozymes.

► A growing appreciation of cardiac care in muscular dystrophy patients is occurring. An editorial by John Bourke mentions that the heart is almost always affected by a progressive cardiomyopathy in Duchenne muscular dystrophy (DMD). Patients with DMD are now living longer thanks to better ventilatory support, steroid therapy to bolster muscle strength, and orthopaedic procedures to address scoliosis. Dr. Bourke notes that “with increased quality of life and prolonged survival, however, heart failure and arrhythmias now contribute more directly to premature death. Yet, most cardiologists are unfamiliar with the nature of cardiac involvement in DMD and its management” (page 164). He concludes that “managing cardiac involvement in neuromuscular disorders is a new frontier in the overlap between cardiology, neuromuscular genetics, and neurology. Cardiologists need to become familiar with these conditions and the implications for their practice” (page 164).

The accompanying article by English and Gibbs explores cardiac issues related to several types of muscular dystrophy. The article examines the use of routine screening and medications and recommends a pragmatic approach focused on conditions which will benefit from early intervention.

Source: Cardiac monitoring and treatment for children and adolescents with neuromuscular disorders. By: English KM, Gibbs JL. *Dev Med Child Neurol*. Mar;48(3), Pages: 231-5, 2006.

Source: Cardiac monitoring and treatment for children and adolescents with neuromuscular disorders. Editorial comment by John Bourke, *Dev Med Child Neurol*. Mar;48(3), Page: 164, 2006.

Other Disorders

► Spinal muscular atrophy (SMA) is caused by mutations in a gene that produces SMN protein. SMN protein has many functions and it has not been clear which functions are implied in causing SMA. Recently, a research team from Germany found that one of the SMN defects in SMA patients is an inhibition in the production of what are called ribonucleoprotein (RNP) subunits. Researchers developed a model to explain SMA and identified the chemical pathway that appears to be affected in SMA thus providing the first real insight into the chemical effects of SMN deficiency. Knowledge of this pathway will lead to new ideas about therapy for this very complex disorder. The article also provides an excellent overview of SMA as well.

Source: Spinal muscular atrophy: the RNP connection. By: Christian Eggert, Ashwin Chari, Bernhard Lagerbauer and Utz Fischer. *TRENDS in Molecular Medicine*, 12, No. 3, 114-121, March 2006.

Another recent major review of SMA can be found here:

Spinal Muscular Atrophy: A Deficiency in a Ubiquitous Protein; a Motor Neuron-Specific Disease. By: Umrao R. Monani. *Neuron*, Vol. 48, 885-896, December 22, 2005.

Note: MDC is also funding research on SMA:

-Dr. Louise Simard, Hôpital Sainte-Justine: Characterization of "survival of motor neurons" (SMN) gene regulation.

-Dr. A. Mackenzie, Children's Hospital of Eastern Ontario: Modulation of apoptosis in mouse models of spinal muscular atrophy.

► Facioscapulohumeral muscular dystrophy (FSHD) is considered a common muscular disorder. Unfortunately, it has a reputation for being difficult to diagnose. Efforts to develop a genetic test for FSHD have also been difficult. FSHD is caused by extra DNA in a region of chromosome 4 known as D4Z4. Current testing methods are labor-intensive, time-consuming and can only estimate the size of the defect present. In this article, Japanese researchers report the development of a simpler, cheaper and faster technique to examine the D4Z4 section. This technique also gives a precise number of extra DNA pieces -- this is important information as the number of extra pieces is related to the severity of symptoms seen. The development of this new screening technique for FSHD appears to be a major step ahead.

Source: Rapid and accurate diagnosis of facioscapulohumeral muscular dystrophy. By: Kanako Goto, Ichizo Nishino, Yukiko K. Hayashi, *Neuromuscular Disorders*, 16 256–261, 2006.

Another recent major review of FSHD can be found here:
The D4Z4 Repeat – Mediated Pathogenesis of Facioscapulohumeral muscular dystrophy. By: Silvere M. van der Maarel and Rune R. Frants, *American Journal of Human Genetics*, 76, 375-386, 2005.

► Myotonic dystrophy type 2 (DM2) affects, and is affected by, pregnancy. This study reviewed 96 pregnancies involving DM2. Nine women presented the first symptoms during pregnancy and worsening of symptoms in subsequent pregnancies. Some 13% of these pregnancies ended early and preterm labor occurred in 50%, resulting in 27% preterm deliveries. This research suggests that pregnancy can have an adverse affect on DM2 and vice versa. The authors believe that gestation causes a shift toward an earlier onset of the illness in women who give birth. If the disorder shows symptoms before pregnancy, or in pregnancy, there is often a marked improvement after delivery followed by a reoccurrence of symptoms in subsequent pregnancies.

Source: Outcome and effect of pregnancy in myotonic dystrophy type 2. By: S. Rudnik-Schoneborn, MD; C. Schneider-Gold, MD; U. Raabe, MD; W. Kress, MD; K. Zerres, MD; and B.G.H. Schoser, MD, *Neurology*, 66, 579–580, 2006.

► Motor neuron diseases (MND), involve degeneration of upper and/or lower motor neurons. Examples are amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA). Unfortunately, clinical trials have made no significant impact on survival for either illness. This article reviews gene based therapies for MNDs and focuses on neuroprotection in ALS and SMA. The authors conclude that further understanding into the causes of these disorders is urgently required. Improved detection needs to be developed to allow the targeting of the early stages of disease which may help prevent neuron damage (rather than trying to correct existing damage). Combining several therapeutic agents may help increase treatment effects. The authors suggest that clinical trials over the next decade will likely be needed before success is realized.

Source: Gene-Based Treatment of Motor Neuron Diseases, By: Thais Federici, and Nicholas M. Boulis, *Muscle Nerve* 33, Pages: 302–323, 2006.

Note: MDC is also funding research on motor neuron diseases, including:
-Dr. H. Durham, McGill University & Montreal Neurological Institute: The role of protein chaperones and proteasome-mediated proteolysis in the pathogenesis of motor neuron diseases.
-Dr. Jean-Pierre Julien, Université Laval: Pathogenic mechanisms associated with neurofilament disorganization.
-Dr. Louise Simard, Hôpital Sainte-Justine: Characterization of “survival of motor neurons” (SMN) gene regulation.

-Dr. Fabio Rossi, Simon Fraser University: Functional role of hematogenous inflammatory cells in amyotrophic lateral sclerosis.

-Dr. E. Meiering, University of Waterloo: Folding and aggregation of ALS-associated mutant superoxide dismutases.

End.