

**Duchenne's Muscular Dystrophy:****Gene Therapy and the Current Effort to Overcome Treatment Challenges**

Duchenne's Muscular Dystrophy (DMD), a rapidly progressive form of Muscular Dystrophy, is an inherited disorder characterized by the enlargement, and the ultimate deterioration, of muscles. DMD affects approximately 2 out of 10,000 people, making it one of the most common forms of muscular dystrophy (other forms of muscular dystrophy include Becker, Emery-Dreifuss, distal, facioscapulohumeral, oculopharyngeal, and limb-girdle). Until recently, Duchenne's Muscular Dystrophy has been virtually untreatable, with the only remedies being temporary treatments such as physical therapy and walking braces for muscle support. However, a very challenging Genomics project of this decade has been the effort to employ gene therapy as a means of altering the course of this tragic genetic disease.

Like all other forms of muscular dystrophy, Duchenne's muscular dystrophy is X-linked, and thus it primarily affects males. Although symptoms generally appear in males before age 6, they can appear as early as infancy. Symptoms are manifested in the form of progressive muscle weakness of the legs and pelvis. This muscle weakness is associated with a loss of muscle mass ("wasting"), and tends to also occur in a less severe form in the arms, neck, and other areas of the body. Calf muscles enlarge initially, and the enlarged muscle tissue is ultimately replaced by fat and connective tissue (pseudohypertrophy). The subsequent shortening of muscle fibers and fibrosis of connective tissue, resulting from muscle contractures in the legs and heels, causes an inability to use the muscles. As a result, affected children often need braces for walking by age 10 and may even be confined to a

wheelchair by the age of 12. Skeletal deformities of the chest and other areas are caused by abnormal bone development, and the combination of these skeletal deformities and muscle weakness often contributes to breathing disorders in the affected individuals. Intellectual impairment occurs in some cases, but it does not worsen as the disorder progresses. Affected individuals usually die by the age of 20 years. Diagnosis of Duchenne's is given when (a) a serum CPK is highly elevated; (b) an EMG (electromyography) indicates that weakness is caused by destruction of muscle rather than by nerve damage; (c) a muscle biopsy confirms the diagnosis.

Duchenne's Muscular Dystrophy is caused by an absence of the protein dystrophin, which is necessary for the structural support of muscle. Without this protein, a 427-kd protein that is an essential component of the inner surface of the sarcolemmal membrane, muscles weaken to the point at which the victim can no longer survive. The full-length dystrophin gene is massive, 2.4 megabases, and most mutations of the gene are frame-shift deletions (as occur in Duchenne's Muscular Dystrophy), or internal in-frame deletions (as occur in other forms of MD such as Becker's Muscular Dystrophy).

As of yet, there is no way of significantly altering the long-term course of this disease. However, the identification of the genes and protein products responsible for the disease has been an essential development for establishing an accurate diagnosis and for reliable genetic counseling and prenatal diagnosis. In addition, advances in gene manipulation and stem-cell therapy indicate that there is hope for the discovery of an effective treatment at some point in the near future. Gene transfer research (using DMD animal models), progress in vector design (such as high-capacity adenoviral vectors, targeted adenoviral vectors, and heterodimerization of DNA delivered by adeno-associated virus vectors), have marked significant advances in the study of Duchenne's. Also, the introduction

of point mutation correction involving the use of chimeric oligonucleotides has proven to be a considerable advance in research on DMD.

The cloning of the dystrophin gene is one of the most recent discoveries that has fostered a better understanding of the molecular genetic basis of DMD. Information about the nature of the dystrophin gene and the glycoprotein complex has led many researchers to believe that somatic gene replacement is the most promising approach to treatment of the disease. Researchers in gene therapy have been searching for years for effective ways to introduce the dystrophin gene into the bodies of DMD patients to replace the missing gene. The effort in this arena has led to the development of a strain of mice who lack the dystrophin gene (the “mdx mouse model”). The mdx mouse model has been used to demonstrate the potential use of the somatic gene replacement strategy. This mouse model has also shown that an increase in the production of utrophin (closely related to dystrophin) to compensate for the low levels of dystrophin in the mouse may provide the basis for an effective treatment for DMD in the future.

Although researchers have been able to insert a “copy” of the missing dystrophin gene into newborn mice via adenoviral vectors, those vectors have had many viral properties, and thus results have been limited because adult mice and mice that have begun to develop symptoms of the disease have developed a sharp host immune response that eliminated the therapeutic gene. Furthermore, results of gene replacement at this stage remained limited by the large size of the dystrophin gene; until now, there has been no effective way to deliver the entire gene and to have it remain in the muscles of the mice.

In 1999, Dr. Xiao Xiao and colleagues from the University of Pittsburgh created miniature versions of dystrophin genes that were one-third the size of the gene itself. These adeno-associated “viral packages” carrying truncated dystrophin genes were injected into the calf muscle of the mice that were unable to produce the dystrophin protein. Functional

dystrophin protein was consequently expressed in approximately 90% of the muscle tissue treated. This “functional protein” expression lasted for a minimum of one year. By delivering the dystrophin protein into mouse-muscle tissue using the adeno-associated virus, Xiao’s team succeeded in preserving muscle function. However, as Xiao himself was quoted as saying, “Our treatment provides a new avenue for treating this disease, but this has to be tested in human trials to see whether it’s effective. Results from animal trials cannot directly translate to humans”.

Current research with gene therapy as an approach to the treatment of DMD has been focused on the resolution of these limitations caused by the size of the dystrophin gene and by certain viral properties of the adeno-viral vectors. The development of gene delivery technology, specifically improvements in vector safety, analysis of biodistribution, and GMP manufacturing of viral and non-viral gene transfer systems, has been a major advancement in the quest for more effective treatments for DMD. Five years ago, investigators tested every vector for every potential disease indication; now, however, an accumulated database enables investigators to select a single vector based upon its performance in a wide number of animal models and human clinical studies (Finer, M. and Meyer, F., 2001).

In addition, researchers have expanded their goals to include the attempted restoration of normal muscle function in patients suffering from the disease. Researchers have recently succeeded in proving that gene therapy can reverse the pathological features of muscular dystrophy in a larger animal model, as opposed to simply being able to prevent further muscle-wasting in mice. “We expect to build on these results in the continuing search for a way to treat a horrible disease,” says Jeffrey S. Chamberlain, professor of neurology at the University of Washington School of Medicine in Seattle, “Our results indicate that gene therapy could be used not only to halt or prevent this disease, but also to restore normal muscle function in older patients” (Neary, Walter, 2002). Chamberlain and his colleagues,

like many other current researchers in gene therapy, have been focusing on ways to overcome the limitations posed by the size of the dystrophin gene. Until this point, there has been no effective method for injecting the entire gene into the muscles of the mice. Last spring, Chamberlain and his colleagues reported that they had succeeded in delivering a “micro” version of the dystrophin gene, similar to Xiao’s miniature version, into the mice. Although even the micro version appeared to effectively reverse the muscle-wasting process, it was not as effective as the full dystrophin gene itself.

In his September 16 paper, however, Chamberlain and his colleagues describe their development of a “stripped-down” vector that did not raise a host immune response and which delivered the full-length, muscle-specific dystrophin gene. Chamberlain demonstrated that the complete gene could be injected into the muscles of both young and old mice, even long after severe muscle damage had occurred. In addition, he showed that normal muscle function could be restored to a level that directly corresponded to the amount of the gene that had been delivered. “These results are extremely encouraging,” Chamberlain says. “We have shown that replacing the dystrophin gene will correct this disease, even in older animals. In future research, we hope to develop better methods to deliver the gene to all the muscles of the body, as currently we are limited to treating relatively small muscles. We believe these results also support the need to move forward with human clinical trials to assess the safety of these methods in patients.”

There has been a major effort to translate these results of gene therapy in mouse models to human trials in the laboratories of Helen Blau and Thomas Rando of Stanford University. The research of Helen Blau and her colleagues has made fundamental contributions to gene therapy by proving for the first time that the differentiated state of a gene is not fixed and irreversible. To the contrary, Blau’s research has shown that previously silent genes can be activated in highly specialized cell types. In heterokaryons, Blau has

induced human muscle gene expression from the non-dividing cell fusion products formed between mouse myoblasts and human primary cells derived from tissues of all three lineages (endoderm, ectoderm and mesoderm). Blau reports that these discoveries have indicated that myogenic activators exist in cells which have already initiated differentiation, are recognized by nuclei of another species, and do not diffuse between unfused cells.

Blau's research has in turn demonstrated that undifferentiated muscle precursors, or myoblasts, can gain access to and enter mature multinucleate myofibers surrounded by basal lamina (Blau 2002). In a phase I clinical trial of myoblast mediated gene therapy for DMD patients, Blau's team showed that human myoblasts, like mouse myoblasts, are efficiently incorporated into the muscle fibers of the recipients, but that the patient's disease state determines whether the dystrophin gene introduced by this approach is actually expressed. Blau and her colleagues concluded that normal myoblasts might thus be particularly well-suited as a delivery system for non-myogenic and their therapeutic products to the circulation (Blau 2002). Blau's research provided the first evidence of sustained delivery over an extended period at physiologic levels of the therapeutic protein, human growth hormone. The results of these experiments using myoblasts as gene therapy delivery mechanisms have been replicated by numerous laboratories and, because this approach yields the physiological expression of therapeutic proteins without an associated immune response, it offers further hope for the effective treatment of DMD.

Researchers today still have a long way to go before they discover a completely effective treatment for the tragic genetic disease Duchenne's Muscular Dystrophy. Until recently, treatments for DMD have been limited to temporary treatments that, although slightly improving the everyday lives of patients suffering from DMD, could do nothing to alter the long-term course of the disease. However, researchers in gene therapy have made discoveries that could revolutionize the treatment of muscle diseases like DMD. These recent

advances in gene therapy, such as the cloning of the dystrophin gene and the introduction of myoblast mediated gene therapy, provide new avenues of hope and possibility for a cure for DMD in the future. In the medical and scientific world today, there is a serious debate surrounding the ethics of research in cloning and in gene therapy. However, all aspects considered, one must remember that diseases like Duchenne's Muscular Dystrophy continue to exist without an effective treatment, and that scientific advances like gene therapy could provide the sole hope for a cure in the near future.

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