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Cover Page Footnote
We would like to thank Dr. DebBurman for guiding us through this project. We also acknowledge Krista Kusinski and Josh Haas for their continued support of our research. Finally, we thank Lital Silverman for reviewing and editing our final paper.

Stepping Forward With MD: *Current Research on Five Common Types*

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Summary

The muscular dystrophies (MDs) encompass a range of genetic diseases characterized primarily by skeletal muscle wasting, with possible weakening of the heart. Clinical manifestations of these diseases have been documented for more than a century; however, the molecular defects that cause MD were only discovered in the last twenty years. Some forms of the disease, including Duchenne muscular dystrophy (DMD) and congenital muscular dystrophy (CMD), result from mutations in genes coding for proteins crucial to muscle cell structure. In these forms of MD, symptoms result from a deficiency of dystrophin or an associated protein in the dystrophin-glycoprotein complex (DGC), a structure that preserves function of normal muscle fibers. Another form of MD, limb-girdle muscular dystrophy (LGMD), is caused by a mutation encoding a protein component of the plasma membrane. Still other forms of the disease exist; fascioscapulohumeral muscular dystrophy (FSHD) and myotonic dystrophy (DM) result from nucleotide repeats and silencer deletions on skeletal muscle genes, respectively. In these forms of MD, symptoms are due to abnormal pre-mRNA splicing. This paper reviews current literature on the molecular basis and therapeutic strategies of five types of MD.

Introduction

The muscular dystrophies (MDs) represent a range of inherited disorders in which progressive muscle wasting is manifest primarily in skeletal muscle, with possible weakening of the heart (Worton et al., 2001). Symptom severity varies between disease forms and may even fluctuate within one type of disorder (Worton et al., 2001).

The MDs were initially classified by clinical criteria; however, the 1980's discovery of dystrophin deficiency as the cause of Duchenne muscular dystrophy (DMD) represented the beginning of MD classification at the molecular level (Worton et al., 2001). In the last twenty years, genetic research has explored the molecular defects responsible for the disease. Some forms of MD are caused by protein deficiencies in the dystrophin-glycoprotein complex or muscle cell membrane; other forms are the result of abnormal splicing of pre-mRNAs necessary for muscle cell regulation (Figure 1; Worton et al., 2001; Gabellini, et al). Although the molecular defects of the former MDs are well known, the defects of the latter have undergone significant research in the last five years and continue to be studied today (Worton et al., 2001).

Despite progress in determining the molecular defects that cause these diseases, treatments and therapies for all forms of MD require additional research. Studies in the last five years have explored the possibilities of drug therapy and gene therapy for MD treatment (Worton et al., 2001). Treating MD is a complex process: several types of the disease exist, and the scientific understanding of each form has progressed differently throughout history.

DMD was first characterized by Guillaume Duchenne in the 1860's. DMD is an X-linked recessive disorder caused by a mutation in the dystrophin-coding DMD gene (Hoffman et al., 1987). The disease affects approximately one in 3500 males; however, boys born with DMD appear phenotypically normal until the age of three to five years (Hoffman et al., 1987). DMD symptoms include skeletal muscle atrophy and cardiomyopathy, and these typically progress until death, which usually occurs around age 17 (Worton et al., 2001). Scientists uncovered the molecular basis of DMD in the late 1980's. Modern DMD research focuses on the development of new strategies to treat this disease.

Congenital muscular dystrophy (CMD) is an autosomal recessive disease that exists in many forms. The classic form is due to merosin deficiency caused by a mutation in the laminin α2 gene (LAMA2), an extracellular protein of the dystrophin-glyoprotien complex (DGC); (Helbling- LeClerc et al., 1995). Fukuyama MD (FCMD) and muscle-eye-brain disease (MEB) are congenital muscular dystrophies also associated with variable central nervous system involvement (Michele et al., 2002). These secondary forms are unique due to the observed brain defects, but the mechanism of abnormality is poorly understood and is the focus of current research. CMD often results in early childhood death, thus the search for an effective treatment is also a current research obstacle.

Limb-girdle muscular dystrophy (LGMD) exists in more than ten forms. Limb-girdle may be inherited as either an autosomal dominant or an autosomal recessive disease (Worton et al., 2001). Four of the eight autosomal recessive forms of LGMD have mutations in genes that encode for different forms of sarcoglycan. These mutations often lead to a loss of sarcoglycan in smooth muscle. Thirty-nine distinct mutations were identified by 1997: most were found in the gene's large extracellular domain, and they were determined to cause LGMD (Worton et al., 2001). Shoulder and pelvic-girdle muscle degeneration occur in all LGMD forms. Many LGMD symptoms are milder forms of DMD and BMD symptoms. Sarcoglycan mutations can also lead to cardiomyopathy. Although LGMD itself does not cause death, muscle weakening can lead to the development of fatal secondary disorders.

Facioscapulohumeral muscular dystrophy is an autosomal dominant disease caused by the deletion of D4Z4 repeats containing a gene silencer. This deletion leads to over-expression of genes upstream (Gabellini et al., 2006). *Facioscapulohumeral* refers to the muscle degeneration that primarily affects the face, upper arms, and shoulder blades. Life expectancy is normal, but some patients become disabled and are restricted to a wheelchair.

Myotonic dystrophy (DM), first characterized in 1909, is the most common inherited skeletal muscle disease in adults (Brook et al., 1992). An autosomal dominant disease, DM is caused by an increased number of CTG repeats on the DMPK gene on chromosome 19 (Brook et al., 1992). The most common form is characterized by myotonia (muscle hyperexcitability), muscle wasting, and varied effects on the nervous system (Jiang et al., 2004). DM typically onsets during adolescence or early adult life; however, a large age range is adversely affected, and neonatal mortality is possible (Brook et al., 1992).

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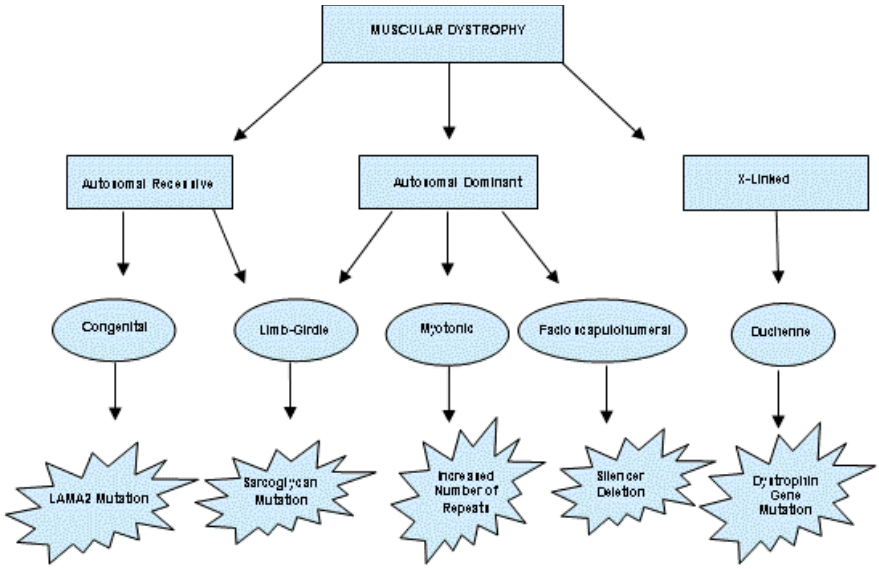

Figure 1. Five Common Muscular Dystrophies. While all muscular dystrophies are characterized by extensive muscle pathology, the numerous forms of the disease show a wide variety in their molecular basis. Of the five MD's discussed in this paper, several share common inheritance patterns, but all are caused by a distinct molecular disorder within muscle cells. Due to the varied causes, muscular dystrophy is largely untreatable.

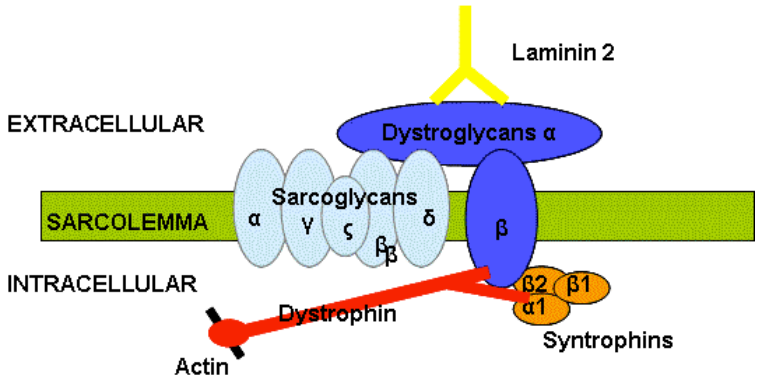

Figure 2. Dystrophin Glycoprotein Complex. Mutations in genes that code for proteins in the dystrophin glycoprotein (DGC) complex result in various forms of MD. This complex mediates stabilization and communication among the extracellular matrix, membrane, and cytosol; thus, when DGC integrity is reduced diverse myopathies can occur.

Duchenne muscular dystrophy

The DMD gene and its protein product, dystrophin

DMD is caused by one of several possible mutations on the 2300-kb DMD gene, which contains 79 exons and is located on the X chromosome (Worton et al., 2001). Mutations result in deletions in the gene's Xp21 region (Worton et al., 2001). These defects alter pre-mRNA reading frames by inserting premature stop codons that terminate translation prior to the synthesis of the gene's protein product, dystrophin (Mann et al., 2000). Point mutations, including stop codons and insertions, have also been found to be responsible for the DMD condition (Worton et al., 2001).

Dystrophin is one of several members of the spectrin family of cytoskeletal membrane proteins (Campbell, 1995). Preliminary research indicating a correlation between dystrophin absence and the DMD condition prompted scientists to investigate dystrophin's role as a membrane protein (Campbell, 1995). They determined that the dystrophin of normal muscle cells localizes on the cytoplasmic face of the muscle cell membrane, connecting the sarcolemma to cytoskeletal sarcomeres (Ervasti, 2000). The sarcolemma-cytoskeleton connection was thus determined as vital to normal muscle cell function; moreover, dystrophin was recognized as integral to maintaining this link (Ervasti, 2000).

Dystrophin function: its role in the DGC

Scientists were interested in the mechanism by which dystrophin preserved the sarcolemma-cytoskeletal connection (Campbell, 1995). Research indicated that the presence of dystrophin correlated with that of several sarcolemmal glycoproteins (Campbell and Kahl, 1989). Additional experiments purified the dystrophin-glycoprotein complex (DGC), and functional analysis confirmed that the dystrophin component of this complex was necessary to maintain the sarcolemma-cytoskeleton attachment (Tinsley et al., 1994).

Protein components of the DGC

Three types of DGC proteins were evaluated according to their location on the cytoskeleton, the sarcolemma, or in the extracellular matrix (Tinsley et al., 1994). From this analysis, scientists developed a DGC complex model (Tinsley et al.,

1994). The cytoskeletal dystrophin protein links transmembrane sarcolemmal glycoproteins to an actin component of the cytoskeleton (Figure 2; Tinsley et al., 1994). The sarcolemmal proteins include three subcategories, and they function as follows: first, intracellular syntrophin proteins bind dystrophin; second, the transmembrane β-dystroglycan protein attaches dystrophin to the extracellular α-dystroglycan protein; finally, α-dystroglycan links transmembrane β-dystroglycan and sarcoglycan proteins to the laminin a2 (also known as merosin) protein in the extracellular matrix (Figure 2; Tinsley et al., 1994). Laminin a2 connects α-dystroglycan to the extracellular matrix (Figure 2; Tinsley et al., 1994).

Dystrophin absence disrupts the DGC

According to the DGC model, dystrophin absence causes a disruption in the link between β-dystroglycan and the cytoskeleton (Figure 2; Tinsley et al., 1994). Scientists were interested in determining whether this disruption correlated with increased sarcolemmal damage; moreover, they explored that sarcolemmal damage could result in cell necrosis and loss of muscle function (Campbell, 1995). This pathogenesis was confirmed by evidence of increased sarcolemmal damage and loss of muscle function after muscle cell contraction in the *mdx* mouse, a dystrophin-deficient animal model for human DMD (Petrof, 1993). This led to the modern understanding that the dystrophin component of the DGC mechanically reinforces the sarcolemma, thus preventing muscle cell death and preserving muscle function (Petrof, 1993).

DMD treatment and strategies for the future

Several therapies have been proposed for DMD treatment. Pharmacologic approaches focus on the use of corticosteroids, especially prednisone, to decrease muscle cell degeneration and enhance muscle cell strength (Worton et al., 2001). Such treatment successfully improves muscle function; however, the negative side effects of long-term corticosteroid therapy have decreased the feasibility of this treatment (Worton et al., 2001).

As a result, recent research has explored gene replacement therapy as a potential DMD treatment option. Both cell-mediated and vector-mediated gene transfer have been studied. Antisense oligoribonucleotide (AO) therapy and adenoviral (Ad) vector therapy are two novel treatment strategies that have shown promise (Mann et al., 2000; DelloRusso et al., 2002).

AO therapy uses antisense oligoribonucleotides (AOs) to block specific pre-mRNA splice sites on mutated DMD genes (Mann et al., 2000). This blockage induces excision of the mutation-containing exon and has enabled previously dystrophin-deficient *mdx* hind limb muscle cells to translate short dystrophin proteins (Mann et al., 2000). The therapy has been successful both *in vivo* and *in vitro* (Mann et al., 2000). Clinical significance of AO therapy depends upon the success of future research in delivering the therapy systemically, rather than through intramuscular injection (Mann et al., 2000).

Adenoviral (Ad) vector therapy is another new DMD treatment strategy (DelloRusso et al., 2002). This therapy uses Ad vectors carrying full-length cDNA dystrophin cassettes to transduce dystrophin-deficient muscle cells, thereby facilitating the production of short dystrophin proteins (DelloRusso et al., 2002). Research on *mdx* mice has shown that a single injection of muscle-specific Ad can restore dystrophin levels in 25-30% of cells one month after treatment (DelloRusso et al., 2002). In addition, transduced muscle cells can exhibit up to a 40% decrease in sarcolemmal damage caused by contraction (DelloRusso et al., 2002). Future research employing uniform, rather than muscle-specific, Ad injection will likely facilitate even greater dystrophin restoration and sarcolemmal stability (DelloRusso et al., 2002).

These DMD therapies have developed from a thorough scientific understanding of this disease at the molecular level. Analysis of the DMD gene and its dystrophin protein product led to an evaluation of protein's role in the DGC (Campbell and Kahl, 1989; Tinsley et al., 1994; Worton et al., 2001). Subsequent research demonstrated dystrophin's vital role in the maintenance of sarcolemmal integrity and the preservation of muscle function (Petrof, 1993; Tinsley et al., 1994).

Congenital muscular dystrophy

Characteristics of CMD

The classic form of CMD results from a merosin deficiency. The absence of merosin is due to a mutation in the laminin α2 gene (Helbling-LeClerc et al., 1995). This protein is an extracellular element of the dystrophin-glycoprotein complex (Figure 2). Absence of this protein results in muscle weakness because the muscle fibers are not properly anchored to the extracellular matrix (Helbling-LeClerc et al., 1995). The CMDs vary in phenotype and are characterized by the presence of the disease at birth, thus most patients rarely become ambulatory (Worton et al., 2001). Despite normal mental development, severe muscle wasting often leads to early childhood death (Qiao et al., 2005). The other forms of CMD, Fukuyama Muscular Dystrophy and muscle-eye brain disease affect the central nervous system. In FCMD and MEB, merosin deficiency is secondary to mutations in other genes. However, the mechanism by which these mutated genes are involved in brain malformation is currently unknown and is the primary focus of modern CMD research.

Alternative Forms of CMD

Secondary deficiency usually occurs in the form of Fukuyama Muscular dystrophy (Worton et al., 2001). This form of the disease is caused by a defect in the gene encoding for *fukutin*, a glycosyltransferase (Worton et al., 2001). This secondary deficiency can also be observed through muscle-eye-brain disease (MEB); (Michele et al., 2002). In MEB, the gene POMGnT1 also encodes for a similar glycosyltransferase. Thus, both proteins are enzymes with the similar function of glycosylation. When hypoglycosylation of dytroglucan occurs, binding to dystroglycan, another main component of dystrophin-glycoprotein complex, is completely eliminated (Figure 2, Michele et al., 2002). Therefore, disruption of α-dystroglycan prevents binding to: laminin, neurexin, and agrin. These specific findings form a unique pathogenic mechanism of congenital muscular dystrophies; abnormal dystroglycan-ligand interactions result in brain malformations (Moore et al., 2002). Additionally, CMD is characterized by lack of a successful treatment. Thus, extensive CMD research focuses around the goal of developing a new and effective therapy.

Gene Therapy for CMD

Fortunately, current research focuses on methods of therapy for CMD patients. Gene therapy using the minigene agrin, known for its part in the formation of the neuromuscular junction, has been extensively studied using transgenic mice (Moll et al., 2001). The mechanism of amelioration suggests that agrin acts by binding the membrane and α-dystroglycan to possibly restore muscle function in CMD patients (Moll et al., 2001). The absence of laminin 2 (merosin) is followed by the upregulation of laminin-4, which leads to upregulation of laminin-8. However, laminin-8 does not bind α-dystroglycan

strongly enough to maintain the connection. Congenital muscular dystrophy pathology could potentially be prevented and possibly reversed by introducing agrin, another extracellular matrix component (Qiao et al., 2005). This occurs because agrin more effectively links the up-regulated laminin-8 to the muscle surface at α-dystroglycan. Thus, agrin amends muscle function in CMD mice by stabilizing the α-dystroglycan to restore strength of the basement membrane (Moll et al., 2001). However, mild signs of dystrophy still exist in the tested mice, suggesting that agrin does not completely rescue all deterioration (Moll et al., 2001).

Modern research allowed the development of a mechanism for secondary forms of CMD such as FCMD and MEB. Understanding the role of α-dystroglycan-ligand interactions in brain malformation and general muscle wasting has guided researchers to new mechanisms of CMD therapy. Gene therapy using the miniagrin gene is the main focus of CMD symptom treatment due the proteins assistance in neuromuscular junctions (Qiao et al., 2005). However, agrin is not the complete answer since all muscle weakening could not be salvaged. Gene therapy via a transgene is also not clinically viable (Qiao et al., 2005), thus new methods by somatic gene transfer are also being explored in mouse models and can hopefully be expanded to studies within human patient muscle tissues.

Limb-Girdle Muscular Dystrophy

Disruption of SG-SSPN

LGMD results from mutations in the genes coding for the sarcoglycan proteins of the DGC. Disruption of the sarcoglycan-sarcospan (SG-SSPN) complex in vascular smooth muscle disturbs vascular function, initiates cardiomyopathy, and worsens muscular dystrophy (Vazquez et al., 1999).

Vazquez et al. (1999) analyzed genetically engineered mice deficient in either α-sarcoglycan (Sgca) or δ-sarcoglycan (Sgcd). They found that while the SG-SSPN complex was absent in both models, only $Sgcd^{-/-}$ mice developed cardiomyopathy and loss of vascular smooth muscle SG-SSPN complex (Vazquez et al., 1999). In contrast, Sa null ($Sgca^{-/-}$) mice showed no morphological signs of cardiomyopathy although the SG-SSPN complex was absent from the cardiac muscle membrane.

The $Sgcd^{-/-}$ and $Sgca^{-/-}$ mice expressed the SG-SSPN complex differently in coronary artery smooth muscle: β-, δ-, and ε-sarcoglycan and sarcospan were expressed in the coronary arteries of $Sgca^{-/-}$ mice. These proteins, however, were absent in $Sgcd^{-/-}$ mice.

The Microfil perfusion technique was used in vivo to demonstrate whether disruption of the SG-SSPN complex in smooth muscle of coronary arteries leads to vascular perfusion abnormalities. $Sgcd^{-/-}$ mice displayed numerous areas of pronounced constrictions (Vazquez et al., 1999). In contrast, $Sgca^{-/-}$ mice showed coronary microvessels normally distributed and smoothly tapered, similar to that of the WT (Vazquez et al., 1999).

Gene Transfer Therapy

Limb-girdle muscular dystrophy types 2E and 2F are caused by mutations in the genes encoding β- and δ-sarcoglycan (Durbeej et al., 2003). Loss of sarcogylcans in smooth muscle was previously shown to lead to constrictions of the microvasculature which can then lead to cardiomyopathy (Durbeej et al., 2003). Studies showed that gene transfer of the deleted sarcoglycan gene can help in preserving the sarcolemmal integrity as well as preventing pathological dystrophy and hypertrophy.

Genes were transferred via an adenovirus vector. Recombinant β- and δ-sarcoglycan adenoviruses were injected into skeletal muscles of corresponding null mice, $Sgcb^{-/-}$, mice that lacked β-sarcoglycan and $Sgcd^{-/-}$, and mice that lacked δ-sarcoglycan. The adenovirus targeted only skeletal muscle, as it was unable to cross the perimysium. Both the SG-SSPN and DGC complexes were restored in skeletal muscle post-treatment (Durbeej et al., 2003).

Treatment did not restore these complexes in vascular muscle. In β- and δ-sarcoglycan gene transfer, sarcoglycans were only reconstituted at the sarcolemma but not in the vascular smooth muscle (Durbeej et al., 2003). Adenovirus treatment produced some muscle cell changes: there were, however, fewer centrally nucleated fibers and a decrease in muscle mass (Durbeej et al., 2003). The latter change was found favorable as $Sgcb^{-/-}$ and $Sgcd^{-/-}$ mice displayed severe pathological hypertrophy (Durbeej et al., 2003).

Disruption of Calcineurin as Treatment

Calcineurin (Cn) is a thereonine phosphatase involved in the regulation of differentiation-specific gene expression in diverse tissues (Parsons et al., 2007). Altered Cn treatment has prevented muscle regeneration in *mdx* mice; however, it has a different affect on LGMD mice. The effect of altered Cn activation treatment was studied in $Sgcd^{-/-}$ LGMD mice: deletion of a loxP-targeted *calcineurin B1 gene* significantly reduced skeletal muscle degeneration in this mouse model (Parsons et al., 2007).

NFAT

Calcineurin expression occurs in cells involved in various processes, including muscle development and cardiac hypertrophy (Parsons et al., 2007). Calcineurin activation can directly dephospohorlyate specific transcription factors; this is referred to as nuclear factor of activated T cells (NFAT) (Parsons et al., 2007).

The δ-*sarcoglycan* gene, which encodes SG-SSPN complex proteins that are essential to the DGC complex, is vital for membrane stability (Parsons et al., 2007). In *Scgd* $^{-/-}$ mice disruption of Cn signaling improves both cardiac and skeletal muscle disease: fibrosis was reduced; muscle degeneration and inflammation were improved. Mice exhibiting increased Cn expression, however, displayed worsened skeletal muscle histopathology and heart disease (Parsons et al., 2007).

LGMD Treatment

Different treatments exist for the various LGMD forms. For LGMD types 2E and 2F there is some hope in gene therapy via adenoviruses. Recombinant β- and δ-sarcoglycan adenoviruses injections have restored SG-SSP complexes in LGMD mice (Durbeej et al., 2003). Calcineurin signaling disruption has successfully improved cardiac and skeletal muscle disease in LGMD mice (Parsons et al., 2007). In the future, these treatments may be applied to human patients.

Fascioscapulohumeral muscular dystrophy

Symptoms of FSHD

Facioscapulohumeral muscular dystrophy is an autosomal dominant disease not due to a gene mutation, but rather to a deletion of a silencer which is located within D4Z4 repeats on chromosome 4q35. Deletion of this silencer results in over-expression of FRG1. A critical determinant of the severity of the disease is related to the number of D4Z4 repeats. Some common symptoms of FSHD are muscle weakness in the face, arms, and shoulders. Another

common symptom of FSHD is kyphosis, the abnormal curvature of the spine.

FSHD and mRNA populations
A study (Tupler et al., 1999) compared FSHD mRNA populations to those of normal muscle. Results showed that muscles affected by FSHD showed intense gene expression alteration observed by severe under-expression or over-expression of specific mRNAs (Tupler et al., 1999); thus, suggesting a misregulation of gene expression which serves as the basis for FSHD, providing more evidence that FSHD is different from other forms of MD.

Repression of transcription
Because FSHD affects transcriptional expression of the 4q35 genes (Gabellini et al. 2002), Petrov et al. (2006) proposed a model for the existence of a repressive element in the D4Z4 repeats. This element binds to a complex leading to transcriptional repression and distribution to adjacent sequences. This model further suggests that deletion of a specific number of D4Z4 repeats will decrease the number of bound repressor complexes. Therefore, the transcriptional repression of 4q35 genes would also decrease eventually leading to FSHD.

Since FSHD studies remain in early stages, future research is dedicated to developing a therapeutic treatment for FSHD patients.

Myotonic Dystrophy

Myotonic dystrophy (DM) is caused by an increased number of non-coding repeats in specific nucleotide sequences. The disease was first characterized in 1909, and its molecular basis was determined in 1992 (Brooks et al.). Recent research focuses on the more severe disease subtype, DM1. In DM1, the dystrohphia myotonica-protein kinase (DMPK) gene of chromosome 19 contains expanded trinucleotide CTG repeats (Brook et al., 1992).

The number of repeats is both proportional to disease severity and inversely proportional to the onset of the disease (Miller et al., 2000). Normal individuals have 5 to 37 CTG repeats; mild or classic cases have 50 to 1000 repeats; severe cases have more than 1000 repeats. Classic cases are characterized by late onset, myotonia and muscle weakness. More severe congenital DM cases can cause neonatal mortality or mental retardation (Brook et al., 1992).

Pre-mRNA gain-of-function
CTG repeat expansion has been linked to DM for many years, and its effect on RNA has been debated for almost as long.

Repeat expansions form hairpin loops in the pre-mRNA structure (Miller et al., 2000). The loops accumulate and form nuclear aggregates, or foci. Foci do not directly cause DM (Ho et al, 2005): instead, repeat expansions create a toxic gain-of-function in RNA, resulting in DM (Phillips et al., 1998). Many models for DM pathogenesis have been proposed (Jiang et al., 2004), but one has surfaced as the focus of recent research. Phillips et al. (1998) proposed that pre-mRNA gain-of-function results from the inappropriate binding of gene regulatory proteins to CUG repeats, which causes aberrant pre-mRNA alternative splicing (Philips et al., 1998; Ho et al., 2004; Charlet-B., 2002).

The effect of nuclear foci on gene regulatory proteins
When first described by Philips et al. (1998), the gene regulatory proteins were known only as CUG- binding proteins (CUG-BP). Current research has identified these proteins.

Two RNA-binding protein families have also been identified as CUG-BP: muscleblind-like proteins (MBNL) are involved in muscle differentiation; CELF proteins affect alternative splicing. Both protein families bind the CUG repeats. MBNL proteins colocalize with foci in the nucleus, which in turn decreases their activity (Miller et al., 2000). In knockout mice missing the mbnl1 gene, characteristic symptoms, such as myotonia, were seen (Kanadia et al., 2003). This suggests that loss of MBNL protein function is a source of pathogenesis. While DM cells have a reduced amount of active MBNL, CELF proteins are found at increased levels and activity rates.

Two gene regulatory protein families determine which pre-mRNA splicing pattern is enacted (Ho et al., 2004). Depending on protein quantity, exon splicing is activated or repressed. In normal splicing patterns, MBNL proteins bind to introns and CELF proteins are rarely bound to CUG repeats (Ho et al., 2004). Danisthong et al. (2004) found that decreased MBNL protein levels determine aberrant splicing occurrence. Without binding sites for CELF proteins, however, cells will not respond to CTG repeat expansion (Ho et al., 2004). DM is caused by splicing patterns formed by either low levels of MBNL proteins or high levels of CELF proteins binding to CUG repeats.

Misspliced pre-mRNA in DM
Once abnormal pre-mRNA splicing was found to occur in DM, the specific mechanism of pathogenesis could be determined.

In DM, normal muscle development is disrupted. Normally, fetal protein isoforms are modified into adult isoforms by a change in specific pre-mRNA splicing patterns. MBNL proteins splice fetal isoform exons and incorporate adult exons. However, nuclear foci sequester MBNL proteins disrupt splicing patterns and include fetal exons. CELF proteins also control fetal exon inclusion: rising CELF levels correspond to increased inclusion and splicing of fetal and adult exons, respectively.

MBNL and CELF proteins regulate the splicing patterns of mRNAs affected in DM: misregulation of the Clcn1 gene, which encodes proteins for skeletal muscle chloride channels, accounts for myotonia in DM (Charlet-B. et al., 2002), and missplicing of the insulin receptor (IR) gene creates over-expression of insulin insensitive tissue, causing diabetes in some DM patients (Savkur et al., 2001).

Recent discoveries linking MBNL proteins to aberrant splicing and DM onset suggest possible therapies. Recent research by Kanadia et al. (2006) suggests that over-expression of MBNL proteins may restore normal pre-mRNA splicing to DM cells. DM mice over-expressing MBNL exhibited neither RNA-splicing defects nor myotonia. Gene therapy may be a very promising future treatment for this type of muscular dystrophy.

Cures for MD are still being discovered

Due to the large variety in forms, treatments for muscular dystrophy are very complex.

Recent DMD research indicates that dystrophin synthesis can be restored via antisense and adenoviral vector therapy. CMD treatment strategies focus on gene therapy using agrin. Modern LGMD treatments involve recombinant β- and δ -sarcoglycan adenovirus therapy, as well as Calcineurin disruption signaling. Current FSHD research is determining the pathogenic mechanism of the disease and developing potential therapeutic strategies. Current DM research suggests that gene regulatory protein over-expression will correct the aberrant splicing characteristic of the disease. Research is ongoing, and treatment strategies continue to be investigated.

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