CHAPTER 11

Childhood Muscular Dystrophies Sharing a Common Pathogenesis of Membrane Instability

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In the premolecular era, there were very few clues that different clinical and genetic forms of muscular dystrophy shared a common pathogenesis of membrane instability. Since the cloning of the gene for Duchenne muscular dystrophy (DMD) in late 1987 (1), our concepts have dramatically changed. Dystrophin is the product of the DMD locus, and we know that it is part of a complex of membrane-associated proteins, the dystrophin-glycoprotein complex (DGC), that span the muscle sarcolemma, providing linkage between the intracellular cytoskeleton and the extracellular matrix (2–4). In relation to human muscular dystrophies, it is useful to consider the constituents of the DGC: dystrophin, dystroglycans, and sargoclycans. Laminin-2, a trilaminar molecular complex in the basal lamina, serves as the extracellular anchor for the DGC (5–7). The syntrophins represent another group of proteins bound to the DGC. They are composed of three isoforms, α1, β1, and β2, each the product of individual genes (8). Syntrophins are thought to function as modular adapters for recruitment of signaling proteins to the membrane. Their role in the pathogenesis of muscular dystrophy remains to be defined (9,10). This chapter reviews essential aspects of childhood muscular dystrophies that share a common pathogenesis of membrane instability.

DYSTROPHIN-GLYCOPROTEIN COMPLEX

Dystrophin is a large protein molecule of molecular weight 427 kDa, localized to the cytoplasmic face of the skeletal muscle membrane in a subsarcolemmal location. The dystrophin domains include the amino terminus, the rod domain composed of 24-helical repeats, and a carboxy terminus (1). At the amino terminus, dystrophin is bound to the cytoskeletal protein, f-actin. The carboxy terminus includes a binding-site for the syntrophins, but more importantly its cysteine-rich region serves as a ligand to the dystroglycans. α/β-Dystroglycan is encoded by a single gene on chromosome 3p21 (1–4). Two proteins are proteolytically cleaved and undergo posttranslational modification. β-Dystroglycan serves as the ligand for the cysteine-rich region of dystrophin. α-Dystroglycan serves as the ligand for laminin-2 in the basal lamina. Thus, the dystroglycans establish a bridge across the membrane from the cytoskeleton and dystrophin to laminin-2 in the extracellular matrix.

The sargoclycan subcomplex is an integral part of the DGC, composed of at least four transmembrane constituents: α-sargoclycan (50 kDa, formerly called adhalin), β-sargoclycan, λ-sargoclycan, and δ-sargoclycan (1–4). The precise relationship between the sargoclycans and dystroglycans is not well established, but transgenic mice deleted for certain domains of dystrophin demonstrate that the sargoclycan complex binds dystrophin via dystroglycan (8).

Laminins are ubiquitous integral constituents of the basal lamina of all tissues; laminins in skeletal muscle are designated laminin-2 (5–7). Laminin-2, as already noted, bears an integral relationship to the DGC but is considered to be a separate component (2–4,8). Its heterotrimeric molecular structure is arranged in the shape of a cross with one heavy α chain and two light chains, β and λ. Originally called merosin, the laminin heavy chain of skeletal muscle and Schwann cells is designated laminin α2. The gene for laminin α2 chain maps to chromosome 6q22-23 (11).
UNIFYING HYPOTHESIS OF MEMBRANE INSTABILITY

There is general agreement that the DGC confers stability to the muscle membrane (12). In DMD and the other muscular dystrophies, the serum CK elevation is usually seen at the time of birth and persists throughout life. Studies in DMD indicate that dystrophin deficiency disrupts the membrane localization for the dystroglycans and sarcoglycans. The same is true when the sarcoglycans are deficient, particularly α-sarcoglycan, where deficiency disrupts the integrity of localization for many components of the DGC. A strong argument favoring an integrated function of these proteins is the clinical picture of overlapping phenotypes as seen in the dystrophinopathy DMD and Becker muscular dystrophy (BMD), the sarcoglycanopathy limb girdle muscular dystrophy (LGMD), and the lamininopathy congenital muscular dystrophy, as presented. These findings support a unifying pathogenic concept for these muscular dystrophies as follows: Membrane tears from weakening of the sarcolemma cause excess calcium leak into the muscle fiber, initiating a cascade of events leading to muscle fiber necrosis. A combination of observations justifies the hypothesis that the disorders affecting structural proteins of the DGC and related proteins all have associated membrane instability leading to muscle fiber breakdown. The muscular dystrophy phenotype is the final outcome.

DYSTROPHINOPATHIES

Clinical Features

The features of DMD and BMD have been well described (13,14). Clinical symptoms of DMD are unusual in the neonatal period. Occasional patients, especially those with mental retardation, may exhibit delayed motor milestones. In most cases the disease becomes clinically apparent between ages 2 and 3 years. The condition shows relentless progression, with weakness and wasting more profoundly affecting the proximal lower extremity muscles. As the disease progresses, contractures develop that limit function, especially at the ankles and hips. Scoliosis is common after wheelchair confinement, which typically occurs about age 12. Most patients die of complications of respiratory insufficiency at about age 20 or slightly thereafter.

Cardiac involvement is a consistent part of DMD (15). The heart demonstrates fibrosis in the posterobasal portion of the left ventricular wall. The right ventricular septum and the right ventricular and atrial myocardium have much less involvement. Degenerative changes affecting the conduction system are infrequent. Despite known cardiac disease, most Duchenne patients remain surprisingly free of cardiovascular symptoms. Congestive heart failure and cardiac arrhythmias usually occur only in the late stages and especially during times of stress from intercurrent infections. Rarely, however, DMD patients have overt signs of congestive heart failure and, in fact, may die of cardiac failure with relative sparing of respiratory muscle function.

Clinical and pathologic involvement of smooth muscle of the gastrointestinal tract, although frequently overlooked, can be an important manifestation (16). A syndrome of acute gastric dilatation, also referred to as intestinal pseudo-obstruction, leads to sudden vomiting, abdominal pain and distention, and possibly death if untreated (16). Patients dying of this syndrome show degeneration of the outer longitudinal smooth muscle layer of the stomach; other regions of the gastrointestinal tract can be affected, causing symptoms such as severe constipation.

The average intelligence quotient falls approximately 1 standard deviation below the mean (17). The impairment of intellectual function appears to be nonprogressive and affects the verbal ability more than performance. The neuropathologic correlate for mental retardation in DMD has not been established.

In BMD, the pattern of muscle wasting closely resembles that seen in DMD. The natural history of the illness permits distinction between the two disorders. Most Becker patients experience difficulties between ages 5 and 15 years, although onset in the third to fourth decade or even later can occur. By definition, Becker patients ambulate beyond age 15, allowing clinical distinction from Duchenne dystrophy. Becker patients have a reduced life expectancy, but most patients survive at least into the fourth or fifth decade.

The preceding discussion implies a clear distinction between Duchenne and Becker patients, but a great heterogeneity of clinical presentation and course of illness can be recognized, emphasizing a continuous spectrum ranging from very severe to very mild (14). A well-recognized subgroup of patients with an intermediate course are referred to as outliers or the intermediate-form of dystrophinopathy. These patients can be recognized usually by age 3 years because of relative preservation of antigravity neck flexor muscle strength, whereas Duchenne patients lack this ability throughout their entire life. Patients with an intermediate phenotype retain the ability to climb stairs and walk after age 12 years but not beyond age 15 (18).

Other phenotypes of dystrophin deficiency have been recognized, adding to the clinical heterogeneity of the dystrophinopathies. A disorder of myalgia and myoglobinuria without persistent weakness has been described (19), and even a cardiomyopathy with few or absent skeletal muscle signs can occur (20). Perhaps the future will hold observations, indicating a selective deficiency of the brain dystrophin isoforms accounting for certain types of mental retardation.
Genetics

DMD is the most common X-linked recessive lethal disease with an incidence of approximately 1 in 3,500 newborns. It has been estimated that one third of the cases are the result of new mutations (18). The dystrophin gene spans more than 2,000 kilobases (kb) of genomic DNA and is composed of 79 exons that encode a 14-kb transcript (1); its enormity probably accounts for the high frequency of spontaneous mutations (21–29). Approximately 65% of DMD and BMD cases demonstrate large-scale deletions of several kilobases to greater than one million base pairs in the dystrophin gene. Duplications are found in approximately 5% of cases (23). The large gene size, particularly the introns, which average 35 kb, partly accounts for the high deletion rate. The deletions are nonrandomly distributed, occur primarily in the center, and less frequently near the 5' end of the gene; larger deletions usually begin at the 5' end of the gene. Deletions disrupting the open reading frame result in the more severe Duchenne phenotype in most cases. In the milder Becker dystrophy, the deletion maintains the translational reading frame, and a semidunctional truncated protein is produced. The reading frame rule explains the phenotypic differences observed in about 92% of the Duchenne/Becker cases. A major exception to the reading frame rule has been the identification of Becker patients with out-of-frame deletions of exons 3 to 7. It has been proposed that an alternate splicing mechanism or new cryptic translational start sites account for the milder phenotype observed with exons 3–7 deletions and the production of dystrophin (29).

There are several reports of point mutations, small deletions, and duplications in the dystrophin gene in DMD patients (24,26,27). Most of these point mutations have resulted in dystrophin truncation, consistent with the reading frame hypothesis. However, unlike the deletion hot spots, these small mutations are private and randomly distributed throughout the gene.

With the ability to perform direct DNA diagnostics on the deletion/duplication cases, the accuracy of carrier detection has significantly improved. Nevertheless, the carrier state of the mother of an isolated case should be interpreted cautiously from DNA testing. For example, when the mother has no detectable mutation of the dystrophin gene, the risk of carrier status still cannot be excluded because of the possibility of germline mosaicism (30). Mothers not harboring mutations of the dystrophin gene in peripheral blood leukocytes can still manifest mutations in a percentage of the oocytes. Such examples of germline mosaicism have important counseling implications. The sisters of Duchenne patients should be investigated independently of the outcome of DNA testing of the mother. Furthermore, a negative mutation result in the mother does not rule out a recurrence risk for future pregnancies, but the exact risk is unknown because there is no method to estimate the size of the mutant clone. The recurrence risk for the mother of a sporadic DMD case has been estimated to be as high as 14% (30).

Molecular Pathogenesis of the Dystrophinopathies

In DMD, the absence of dystrophin leads to a drastic reduction in all components of the DGC that are normally synthesized but not properly assembled or integrated into the sarcolemma (2–4,12). Based on these observations, it is proposed that disruption of the DGC plays a key role in the cascade of events, leading to muscle cell necrosis. The absence of dystrophin causes a disruption of the linkage between the subsarcolemmal cytoskeleton and the extracellular matrix, leading to sarcolemmal instability, membrane tears, and muscle cell necrosis.

Support for the delicate relationship within the components of the DGC is provided by experimental and clinical studies. For example, in transgenic mdx mice (the genetic mouse model with dystrophin deficiency), systematic studies replacing certain domains of dystrophin demonstrate the critical role of the cysteine-rich region (49–51). A dystrophin gene construct devoid of exons 64 to 70, responsible for transduction of the cysteine-rich domain, prevents localization of all components of the DGC. In clinical situations, even missense mutations can result in conformational changes of the molecular structure of dystrophin, preventing proper assembly of the DGC (27). These results support a model whereby dystrophin deficiency, or in some cases even small changes that alter the structural conformation of dystrophin, can disrupt the DGC, leading to membrane instability and predisposing the muscle to repeated insults, leading to muscle breakdown and a muscular dystrophy phenotype.

Treatment

Appropriate stretching of the heel cords, iliotibial bands, and hip flexors should be done to prevent contractions. Night splints may delay heel cord tightness. Scoliosis often occurs when patients lose ambulation. The segmental spinal stabilization described by Luque (31) is the procedure of choice to correct scoliosis. Patients with progressive curvatures measuring 35 to 45 degrees should be considered surgical candidates. A forced vital capacity of greater than 35% of predicted normal mean is recommended to prevent postoperative pneumonia. Anesthesia for patients with DMD should not include halogenated inhalational anesthetics or neuromuscular depolarizing agents such as succinylcholine because adverse reactions may occur that are similar, but not identical, to malignant hyperthermia (32).

Prednisone and deflazacort have been shown to benefit Duchenne dystrophy patients. In randomized, double-
blind, controlled trials, both significantly increase muscle strength, pulmonary function, and functional ability (33,34). Improvement occurs as early as 10 days, with prednisone treatment reaching maximal improvement by 3 months. The recommended prednisone dosage is 0.75 mg/kg/day. Long-term studies indicate that a dose of 0.65 mg/kg/day can maintain improvement, but similar results cannot be achieved with alternate-day treatment (35,36). The major long-term benefits of prednisone are prolonged independent and braced ambulation. Both ambulatory and wheelchair-dependent patients maintain improved vital capacity measures for longer periods of time compared with control subjects. Significant side effects include growth retardation, weight gain, cushingoid facial appearance, excessive body hair, cataracts, and behavioral changes (33–36). Unacceptable weight gain is the most common reason for reducing or discontinuing prednisone (37).

Prednisone increases strength by increasing muscle mass (33,34) and decreasing muscle degradation (38). The decrease in muscle degradation may be due to an anti-inflammatory effect related to a reduction in total T cells and cytotoxic-suppressor T cells (39). Prednisone has no effect on dystrophin levels in muscle (40). Deflazacort, an oxazolone derivative of prednisone, has fewer side effects than prednisone, especially in bone osteoporosis and weight gain (41–44). In a randomized trial comparing deflazacort and prednisone, both drugs had equal efficacy after 1-year follow-up, but deflazacort-treated patients gained significantly less weight (45). Gene replacement therapy is on the horizon for dystrophin-deficient patients, but the results of clinical trials have not been reported. Alternative methods of dystrophin replacement by myoblast transfer have not been efficacious in controlled trials in DMD patients (46–48), as well as in mdx mice (49–51).

**LIMB GIRDLE MUSCULAR DYSTROPHY**

The term “limb girdle muscular dystrophy” initially referred to a heterogeneous group of disorders with both autosomal dominant (AD) and recessive (AR) inheritance patterns (52); that concept has been incorporated into current nosology (53,54). There are now at least eight diseases that have been classified as LGMDs (Table 1). This number is constantly expanding and will continue to grow. Fortunately, the new classification schema will accommodate change as explained in the discussion to follow.

LGMD1 refers to the dominantly inherited variants, whereas LGMD2 consists of disorders with AR transmission. Presently, linkage has been established for two dominantly inherited disorders, LGMD1A and LGMD1B (55–58). The recessively inherited forms of LGMD now number six. In five, the specific deficiency has been identified: LGMD2A [calpain 3 (CAPN3)] (59–62), 2B (unknown protein) (63), 2C (λ-sarcoglycan) (64–66), 2D (α-sarcoglycan) (67–72), 2E (β-sarcoglycan) (73,74), and 2F (δ-sarcoglycan) (75). One case of β-dystroglycan deficiency has been found but will probably not be officially assigned a number until the mutation is identified (76).

**Clinical Features**

There are features common to all LGMD disorders. Weakness is predominant in a limb girdle distribution with sparing of facial, extraocular, and pharyngeal muscles (53,54). It would be difficult to make a gene-specific diagnosis purely on clinical evaluation. In any of these conditions, the degree of weakness varies from an early onset, severe, Duchenne-like disorder to a mild Becker-like disorder. The heterogeneity of the Becker-like cases is every bit as diverse as that seen in the dystrophinopathies. Calf hypertrophy is common in the recessively inherited LGMDs but is not an invariable feature. The CAPN3-deficient patients are reported to have rather consistent calf muscle contractures resulting in “tiptoe” walking that often serves as the stimulus for the initial consultation (59–61).

Onset of weakness in distal muscles was considered to be tantamount to another diagnosis when criteria for LGMD were established (53,54). However, even this adage is weakening because evidence now indicates that

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<th>Disease</th>
<th>Protein</th>
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<th>Chromosomal localization</th>
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