The Dystrophinopathies

By Mathula Thangarajh, MD, PhD, FAAN

REVIEW ARTICLE

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ABSTRACT

PURPOSE OF REVIEW: **The dystrophinopathies are among the most common neuromuscular conditions, and they include Duchenne and Becker muscular dystrophies. This article reviews the epidemiology, clinical manifestations, genetic cause, management, and new and emerging therapies for this condition.**

RECENT FINDINGS: **New studies have highlighted how oral corticosteroids have changed the natural history of the disease, prolonging ambulation in boys with Duchenne muscular dystrophy and reducing the risk of developing scoliosis and subsequent surgical correction, improving cardiac health, and increasing long-term survival. Additionally, recent publications have provided insights into how newer and emerging treatment options are becoming more common for this condition. With gene therapy being approved in the United States for the severe form, the dystrophinopathies represent model diseases to understand the personalization of genetic treatment.**

SUMMARY: **Improvement in the standardization of care and the use of oral corticosteroids have increased the life expectancy of patients with dystrophinopathy and changed the natural history of the disease. This article presents a summary of clinical features, diagnostic testing, and new and emerging treatment strategies for the dystrophinopathies.**

INTRODUCTION

The dystrophinopathies are a spectrum of progressive muscular dystrophies that are caused by the absence of or decrease in the function of dystrophin protein.[1] Dystrophin protein is the gene product of the dystrophin *DMD* gene, located on the X chromosome.[2,3] Because of its location on the X chromosome, males are affected by this disease, whereas females are carriers of the disease. The clinical spectrum of dystrophinopathies includes the prototypical progressive skeletal muscle weakness, X-linked cardiomyopathy, muscle cramps, and myalgia. In this article, the term dystrophinopathy is used to discuss Duchenne and Becker muscular dystrophies. The landscape of dystrophinopathy has changed considerably in the past 2 decades, with life expectancy extending to the late thirties and early forties, especially in the severe form of the disease, Duchenne muscular dystrophy.[4,5] The understanding of the natural history of the disease has allowed multidisciplinary teams to provide anticipatory guidance and advocate for the standardization of clinical care.[6] This improved clinical outcome is paralleled by scientific advancements in the personalization of genetic therapy, allowing

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Address correspondence to Dr Mathula Thangarajh, Department of Neurology, Virginia Commonwealth University, 1101 E Marshall St, 12th Fl, Room 006-A, Box 980599, Richmond, VA 23298-0599, *mathula.thangarajh@vcuhealth.org*.

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dystrophinopathy to become a model disorder to study the treatment approach for a monogenic disease.[7]

EPIDEMIOLOGY

The dystrophinopathies are among the most common neuromuscular conditions neurologists encounter, and they affect all races and ethnicities equally. The prevalence at birth is approximately 2.9 per 10,000 live male births (1 per 3500) for Duchenne muscular dystrophy and approximately 0.5 per 10,000 (1 per 18,518) for Becker muscular dystrophy worldwide.[8] The population-based estimated prevalence of the number of boys affected by a dystrophinopathy in the United States is approximately 1 per 7250, with Duchenne muscular dystrophy being 3 times more common than the milder but variable phenotype known as Becker muscular dystrophy.[9–11] Knowledge regarding transmission of *DMD* mutation from the mother to the proband comes from the Universal Mutation Database (UMD) for the *DMD* gene[12] and a postnatal cohort of 216 boys studied in the Netherlands. In the 1102 probands in the Universal Mutation Database cohort, 75% of boys inherited the mutation from their mothers, compared with 52% in the Dutch cohort.[13]

Patients with the Duchenne phenotype are now surviving into adulthood.[4,5] This increase in life expectancy has impacted care delivery; adult neurologists and neuromuscular subspecialists are increasingly providing care for those with the Duchenne phenotype, which often presents during early childhood. The improved life expectancy for dystrophinopathy also underscores the importance of a regular, multidisciplinary approach to improve patient-related outcomes.

Clinical Phenotype

The spectrum of the clinical features seen in dystrophinopathy is directly related to the amount of dystrophin present in the skeletal muscle, and dystrophin is integral to the stability of the subsarcolemmal muscle membrane.[14,15] To illustrate, even the presence of some functional dystrophin protein decreases the severity of underlying muscle membrane damage and clinically results in a milder phenotype. Therefore, the clinical spectrum of progressive skeletal muscle weakness in dystrophinopathy is variable. In addition to Duchenne and Becker muscular dystrophies, an intermediate clinical phenotype is increasingly recognized and discussed later in the section on the intermediate phenotype. The complete absence of dystrophin results in Duchenne muscular dystrophy, whereas even the presence of a relatively small amount of dystrophin in the muscle results in Becker muscular dystrophy or the intermediate phenotype. Exceptions to the prediction of clinical phenotype based on *DMD* mutation do occur; therefore, the importance of a thorough history and clinical examination to accurately characterize the clinical phenotype cannot be overstated.

Dystrophin Gene

The dystrophin gene, *DMD*, is the largest gene in humans, approximately 90 times larger than the average gene, and it constitutes up to 0.1% of the entire human genome. It is 2.3 megabase pairs (Mb) in size and consists of 79 exons. Interestingly, the 11-kilobase pair (kb) coding sequence of *DMD* (exons) contributes to only 0.6% of the *DMD* gene. Thus, the bulk of *DMD* consists of

introns or noncoding sequences. *DMD* encodes the dystrophin protein, which belongs to the spectrin superfamily of proteins. Dystrophin is a 427-kilodalton (kDa) protein and is expressed in skeletal and cardiac muscle and by cortical neurons and Purkinje cerebellar neurons. Shorter dystrophin isoforms are expressed by the retina and peripheral nerves. Dystrophin consists of four distinct regions, each of which has a specific functional role. The critical N terminus binds to actin. The central rod region consists of 21 spectrin repeats, followed by a cysteine-rich segment that binds to β-dystroglycan. The spectrin sites R16 and R17 are binding sites for skeletal muscle nitrous oxide. The carboxy terminus consists of many phosphorylation sites. Thus, dystrophin connects the intracellular contractile apparatus (actin) to the extracellular matrix. Together with other sarcolemmal proteins, dystrophin forms the dystrophin-associated protein complex. An analogy is to think of dystrophin as an anchor of the sarcolemmal membrane and that its deficiency is associated with loss of muscle membrane stability (FIGURE 5-1).[16]

KEY POINT

- **Clinical outcomes in dystrophinopathy have improved, and many individuals with the severe phenotype (Duchenne muscular dystrophy) are surviving into adulthood. Thus, adult neurologists are increasingly providing care for these individuals.**

DMD Mutations

The rate of spontaneous mutations is high in *DMD* because of its size, approximately 1×10^{-4} compared with 1×10^{-5} to 1×10^{-6} in other genes. Mutations that disrupt the open reading frame rule result in Duchenne muscular dystrophy, whereas mutations that respect the open reading frame result in Becker muscular dystrophy. The loss of the open reading frame results in the expression of unstable, truncated dystrophin protein that lacks the cysteine-rich domain and carboxy terminus. Intragenic deletions are the most common type of mutation seen in dystrophinopathies, followed by duplications and point mutations.[12,17,18] One of the hot-spot regions of *DMD* mutations involves the *DMD* exon 45 to 55 region, contributing to up to 63% of mutations.[19]

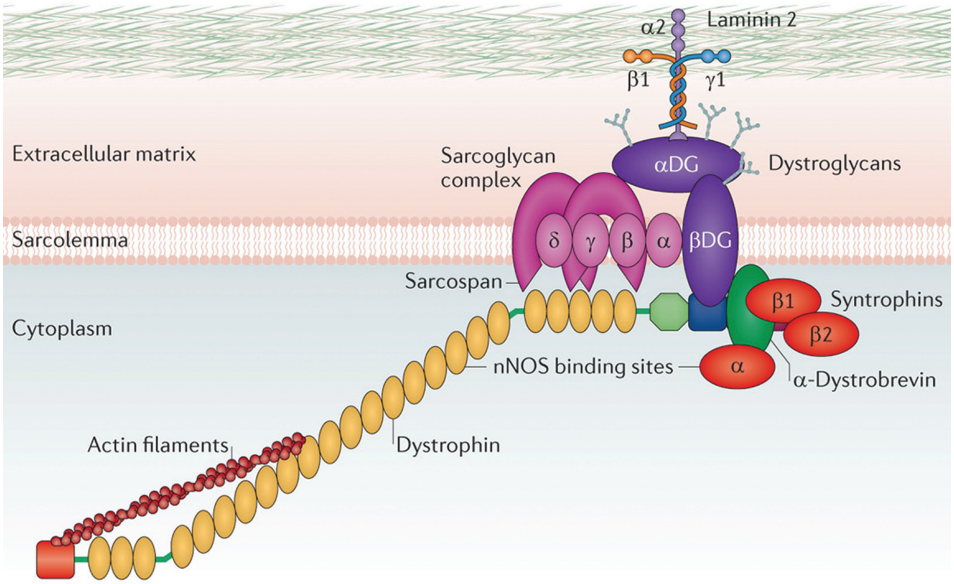

FIGURE 5-1
Structural representation of dystrophin.
αDG = α-dystroglycan; βDG = β-dystroglycan; nNOS = neuronal nitric oxide synthase.
Reprinted with permission from Faircloth RJ, et al, Nat Rev Genet.[16] © 2013 Springer Nature.

DUCHENNE MUSCULAR DYSTROPHY

The clinical presentation of Duchenne muscular dystrophy often occurs within the first year of life. Gross motor delay is one of the most common presenting features in Duchenne muscular dystrophy.[20] Caregivers often report that motor development is delayed in boys with dystrophinopathy compared with typically developing peers. For example, caregivers report that the child has trouble attaining gross motor skills, which may include a delay in walking and difficulty in rising from the floor, jumping, and running. Difficulty in walking is due to weakness of the proximal pelvic muscles. In particular, hip and knee extensors are weak in dystrophinopathy.[21] This weakness presents as exaggerated lumbar lordosis due to weakness of the hip extensors (gluteus maximus) and frequent falls due to weakness of the knee extensors (quadriceps). Gowers sign, seen as a boy "climbing" on himself by supporting his knees, is often seen in Duchenne muscular dystrophy and is due to weakness of hip and knee extensors.[22] Additional history that raises concern for a dystrophinopathy may include frequent falls, especially on irregular surfaces, or difficulty navigating small curbs or steps. Some caregivers note that affected children may not be able to maintain their balance and can be easily toppled or pushed over. Another early symptom in a boy that should alert clinicians regarding the diagnosis of dystrophinopathy is toe walking. In boys 3 to 4 years of age, calf hypertrophy can often be seen. In older boys, hypertrophy of the deltoid and infraspinatus muscles can also be detected (FIGURE 5-2) (CASE 5-1).[23]

Skeletal muscle weakness follows a prototypical pattern, and most boys with Duchenne muscular dystrophy lose ambulation by the age of 12 or 13 years. Most caregivers are accurately able to recall motor milestones, and caregivers of children with Duchenne muscular dystrophy often recall their children beginning to walk much later than the typical 14 to 15 months of age. The author's clinical experience has been that caregivers also recall the age at which their children lose the ability to walk independently, which is defined broadly as a child spending most of his time in a wheelchair. Because skeletal muscle weakness follows a typical pattern, clinicians and care teams can help families set expectations and plan for change in the level of functioning. Loss of ambulation is an indicator of the overall disease severity and predicts other sentinel clinical milestones, such as the need for ventilator support and survival.[24,25]

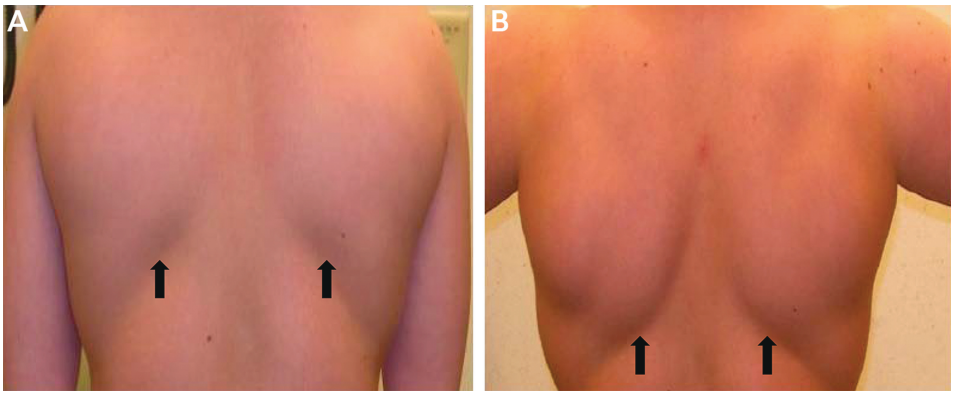

FIGURE 5-2
Prominant infraspinatus muscle (*A, B, arrows*) is shown in a young boy when he holds his arms down (*A*) and when he lifts them (*B*).

CASE 5-1

A 4-year-old boy was evaluated at the neuromuscular clinic for being below peer level for gross motor activities. He could not run well, was unable to climb on alternating feet, had difficulty climbing down, and preferred to scoot downstairs in a seated position. He had difficulty getting up from the floor and was unable to pedal a tricycle. He toe walked frequently. He had never had an episode of rhabdomyolysis and did not report having cramps, fatigue, or muscle pain.

He walked independently at 18 months of age. At age 24 months, his daycare provider noted that his motor skills lagged behind his peers. His family history was unremarkable. His mother also reported that she had severe long-standing muscle cramps and difficulty climbing up stairs.

On evaluation with the Denver Developmental Screening Test (used by most pediatricians to screen for developmental problems), the patient was able to run but was unable to clear his feet off the ground. He was unable to jump up, perform a broad jump, or hop. His motor assessment showed development of approximately 18 months. With regard to fine motor skills, he had difficulty copying simple sequences of colored blocks. His language skills were normal, and he was able to count to 10 and name colors.

His initial laboratory testing was notable for an elevated serum creatine kinase level of 15,842 U/L and aldolase at 110 IU/L. His aspartate transaminase level was elevated at 253 IU/L, and his alanine transaminase level was elevated at 325 IU/L.

His examination showed his weight to be between the 50th and 75th percentiles and his height to be at approximately the 50th percentile. The musculoskeletal portion of his examination showed that he had calf hypertrophy and notable neck flexion weakness. He walked on his tiptoes. He was able to run without clearing his feet off the ground with exaggerated lumbar lordosis. On timed testing, his four-step climb was 5 seconds, and his rise time from the floor was 5 seconds. His deep tendon reflexes were trace at the brachioradialis and biceps, absent at the triceps, and 1+ at the patella and ankle. He had bilaterally tight Achilles tendons.

Genetic testing showed that he had deletion of *DMD* exon 45. His mother also had the same mutation, thus making her a carrier. He was prescribed oral corticosteroids, which led to remarkable stabilization of his motor milestones.

COMMENT

The presentation of delayed motor milestones in a young boy with elevated creatine kinase should alert the medical team to evaluate for dystrophinopathy. Genetic testing not only helps in diagnosis but also in counseling and family planning. Carrier testing of the mother is recommended. With the commencement of oral corticosteroids, it is possible to prolong ambulation by 1.5 to 2 years.

Proximal upper extremity weakness usually begins in boys with Duchenne muscular dystrophy during their early teenage years. This manifests as difficulty in lifting the arms above the shoulder, difficulty in feeding, and increasingly using both the armrest in the wheelchair and elbow support at the table. Distal finger strength is often preserved until late in the course of the disease. As trunk weakness sets in during the teenage years, patients with dystrophinopathy

CASE 5-2

A 10-year-old boy was evaluated in the neuromuscular clinic for long-standing muscle weakness. At age 5, he was first noted to have difficulty with fine motor skills while coloring or drawing. He had a long-standing history of reduced endurance and had leg pain and muscle aches, which frequently occurred while playing. A year before his evaluation, he began to experience difficulty keeping up with his peers while participating in organized sports, such as flag football. His family also had noted some wasting and loss of muscle definition in his thighs, buttocks, and upper arms over the past year. He had no history of fever, joint pain, or muscle swelling. He had no history of recurrent rhabdomyolysis, chest pain, or bulbar symptoms.

His birth history was unremarkable. His early developmental history was normal, and he attained motor milestones at developmentally appropriate times. He had no history of learning difficulties or attentional challenges and no family history of muscle disease.

On initial evaluation, his general, cardiac, pulmonary, and abdominal examinations were unremarkable. His neuromuscular examination showed decreased muscle bulk over the chest and thigh without calf hypertrophy. On manual muscle testing, he had weakness of the proximal muscles of the upper and lower extremities as evidenced by 4/5 strength in his shoulder abductors and 4/5 in his hip flexors. He did not exhibit the Gowers sign, and his rise time from the floor was 3 seconds. He had a Trendelenburg gait. His deep tendon reflexes and sensory and cerebellar examination were normal, and he had no scoliosis.

On laboratory testing, his creatine kinase level was elevated at 1621 U/L. The differential diagnosis included dystrophinopathy. A muscle biopsy of the right vastus lateralis showed a near-normal histologic appearance of the muscle. Immunostaining for exon 50 of dystrophin was absent, accompanied by reduced expression of neuronal nitric oxide synthase, suggesting Becker muscular dystrophy. Genetic testing confirmed that he had a mutation in *DMD* exons 49 to 51. Testing of his mother revealed that she was not a carrier of dystrophinopathy.

COMMENT

This case demonstrates the challenge when a young boy presents with nonspecific symptoms of poor endurance and inability to participate in sports. A thorough medical history combined with ancillary laboratory testing can help in ruling out dystrophinopathy. Muscle biopsy and immunostaining for dystrophin can help to confirm the diagnosis.

experience difficulty turning in bed. As skeletal muscle weakness becomes progressive, these individuals become wheelchair dependent and require assistance for activities of daily living and grooming.

Swallowing difficulties often exist but remain underrecognized. The upper one-third of the esophagus has skeletal muscle, and weakness of these groups of muscles often presents as difficulty in swallowing. Hard-textured food may become difficult to chew and swallow. During a routine clinical visit, eliciting this history is important, as is obtaining accurate weight, because it assists in preparing the family and the medical team in planning for tube feeding. As they age, patients with Duchenne muscular dystrophy often need gastrostomy tubes to maintain caloric needs. Likewise, flaccid dysarthria with hypophonia becomes detectable as the disease progresses. It is often noted that weakness of laryngeal muscles may precede a decline in pulmonary function.

Diaphragmatic weakness is an important symptom to consider as the disease progresses. In line with this clinical decline, by age 12 years, the American Thoracic Society recommends that boys with Duchenne muscular dystrophy be evaluated by a pulmonologist 2 times a year.[26] Nocturnal hypoventilation and carbon dioxide retention can cause early-morning headaches and poor sleep quality, both of which have an impact on quality of life. Pulmonary hygiene and sleep hygiene are important anticipatory guidance aspects of the disease that should be regularly reviewed during clinic visits as discussed later in this article.

Developmental delay in Duchenne muscular dystrophy is often underrecognized. Approximately one-third of boys with Duchenne muscular dystrophy meet criteria for intellectual disability, with a full-scale IQ of less than 70.[27,28] Cognitive delay often postpones the diagnosis, in part because the spectrum of cognitive involvement in the disease is not fully appreciated. Speech delay is one of the commonly reported developmental symptoms and is 10 times more common in boys with Duchenne muscular dystrophy than in the general population.[29] Therefore, all boys presenting with cognitive symptoms warrant easy screening with a serum creatine kinase level.

KEY POINT

- **Young boys presenting with developmental delay and delayed motor milestones should be tested for dystrophinopathy. Serum creatine kinase is the first diagnostic testing that can help.**

BECKER MUSCULAR DYSTROPHY

Boys affected by the milder and variable skeletal muscle phenotype are often referred to as having Becker muscular dystrophy (CASE 5-2). Historically, boys with dystrophinopathy who are still ambulating at age 15 years have been described to have the Becker muscular dystrophy phenotype. Muscular symptoms in patients with Becker muscular dystrophy may vary from isolated muscle cramps to moderate to severe skeletal muscle weakness. The severity of cardiac involvement can help distinguish Duchenne muscular dystrophy from Becker muscular dystrophy. Patients with Becker muscular dystrophy tend to have more severe cardiac involvement, including cardiomyopathy, compared with those with Duchenne muscular dystrophy. No relationship exists between the severity of skeletal muscle weakness and cardiac symptoms in Becker muscular dystrophy. Most physicians would defer treatment with corticosteroids in a patient with Becker muscular dystrophy; however, a course of corticosteroids can be considered in those with severe weakness. Performing quantitative muscle-strength testing before and after commencement of corticosteroids can help guide treatment.

INTERMEDIATE PHENOTYPE

An intermediate phenotype is increasingly recognized in dystrophinopathy, particularly when a subject with a documented *DMD* mutation may not follow a stereotypical pattern of decline in motor function. For example, a boy with an out-of-frame *DMD* mutation predicting a Duchenne phenotype may continue to walk past age 12 to 14 years and may fall into an intermediate phenotype. This spectrum in severity of symptoms is, in part, due to genetic modifiers that influence the age at loss of ambulation, steroid responsiveness, and cardiac severity. The three genetic modifiers reported in humans that have a bearing on clinical care are *SPP1* (osteopontin), *LTBP4*, and *CD40*.[30–36]

A single nucleotide polymorphism in the promoter of *SPP1* is associated with an earlier loss of ambulation in patients with Duchenne muscular dystrophy.[30] Furthermore, a difference in the median age at the loss of ambulation was detected based on *SPP1* genotypes; those with the rare *SPP1* allele who were treated with glucocorticoids lost ambulation earlier than those with the common *SPP1* allele, showing that patients with Duchenne muscular dystrophy carrying the *SPP1* minor allele respond poorly to glucocorticoids.[31] The *LTBP4* genotype in patients with Duchenne muscular dystrophy confers a protective effect, resulting in a later median age at the loss of ambulation.[31–33] Further, the *LTBP4* genotype shows a trend toward a delayed onset of cardiomyopathy.[34] Last, the minor allele in *CD40* locus (rs1883832) is also associated with an earlier age at the loss of ambulation.[35]

CARDIAC INVOLVEMENT IN DYSTROPHINOPATHY

The spectrum of cardiac symptoms in dystrophinopathy includes sinus tachycardia, dilated cardiomyopathy, and, rarely, pericardial effusion and cardiac tamponade.[37] Resting sinus tachycardia is an early and consistent finding and may be seen even in young boys with dystrophinopathy.[38] Overt signs of cardiac failure, such as exertional dyspnea, chest pain, orthopnea, and lower extremity edema, may or may not be present. As noted earlier, patients with Becker muscular dystrophy may experience more severe cardiac symptoms compared with individuals with Duchenne muscular dystrophy. Cardiac dysfunction can be seen in 15% of patients with Becker muscular dystrophy before age 16 and in as many as 75% by age 40.[39]

Nonspecific changes on ECG can be seen in dystrophinopathy; reported findings include a shortened PR interval, right ventricular hypertrophy changes, and deep Q waves in inferolateral leads.[40] The use of noninvasive imaging has expanded the recognition of subclinical cardiac symptoms. Findings can include abnormalities of wall motion, subendocardial fibrosis, decreased mitral systolic wave velocity, and systolic and diastolic dysfunction. Several neuromuscular clinics are adopting cardiac MRI as an additional resource to evaluate cardiac function, and it is increasingly becoming the imaging modality of choice. Late gadolinium enhancement on cardiac MRI is a sign of fibrosis and can be seen in dystrophinopathy (FIGURE 5-3).

For patients with dystrophinopathy, routine cardiac surveillance should begin in childhood. Understanding of the natural history of changes in dystrophinopathy has shown the value of treatment during the presymptomatic stages of cardiac involvement. Routine cardiac surveillance should begin in childhood, with a baseline echocardiogram at the time of diagnosis or at 6 to 7 years of age. In boys older than 10 years, an annual echocardiogram is recommended. The use of angiotensin-converting enzyme inhibitors or angiotensin-receptor blockers is recommended in boys older than 10 years[41] and can be considered in boys with dystrophinopathy who are younger than 10 years who exhibit cardiac dysfunction

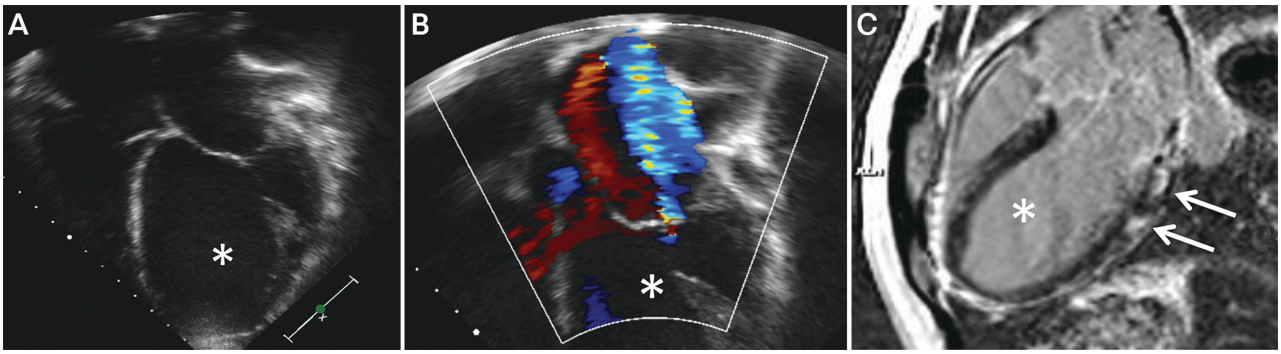

FIGURE 5-3

Cardiac imaging in Duchenne muscular dystrophy. *A*, Two-dimensional apical four-chamber echocardiogram image showing a dilated left ventricle (*asterisk*) associated with decreased systolic function. *B*, Color Doppler image of a dilated left ventricle (*asterisk*) inflow demonstrating associated mitral regurgitation (*blue color jet*). *C*, Cardiac MRI apical four-chamber image showing late gadolinium enhancement (*white arrows*) in the lateral wall of the left ventricle (*asterisk*). Cardiac MRI images are shown in coronal view.

Courtesy of Christopher Spurney, MD.

based on clinical examination and laboratory evidence. In older patients with dystrophinopathy, the placement of defibrillators is advised when the ejection fraction falls to less than 50%. The choice of a left ventricular assistance device in those for whom optimal medical management has failed can be challenging and requires a multidisciplinary team approach. Likewise, heart transplantation is an option in dystrophinopathy but needs to be considered on a case-by-case basis.

PULMONARY INVOLVEMENT

Cardiopulmonary failure is one of the most important causes of morbidity and early death in dystrophinopathy. The diaphragm is the largest skeletal muscle in the body, so it is not surprising that it is affected in dystrophinopathy. Natural history and prospective studies[42–44] have shown that lung function begins to decline in the early to middle teenage years. Nocturnal hypoventilation is one of the earliest manifestations of pulmonary compromise and can manifest on oximetry-capnometry studies as an oxygen saturation less than 95% on room air or an increase in end-tidal or transcutaneous partial pressure of carbon dioxide. A decline in pulmonary function presents as a weak cough, atelectasis, and recurrent respiratory infections. As the disease progresses and chest wall compliance decreases, worsening dyspnea and the other symptoms mentioned can worsen the restrictive lung disease (FIGURE 5-4).[45]

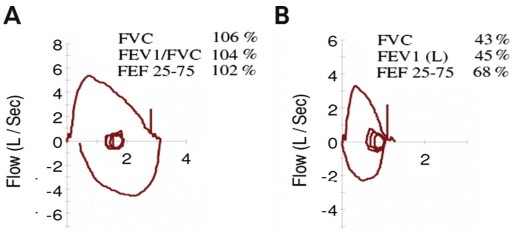

FIGURE 5-4

Pulmonary function test in an asymptomatic individual with dystrophinopathy (*A*) and severe decreases in forced vital capacity, expiratory volume, and force consistent with respiratory muscle weakness in a boy with dystrophinopathy (*B*).

FEF 25-75 = forced expiratory flow of 25% to 75% of lung volume, FEV1 = maximal amount of air that can be forcefully exhaled in 1 second, FVC = forced vital capacity.

Courtesy of Geovanny Perez, MD.

In dystrophinopathy, the decline in vital capacity is approximately 8% every year.[46] Loss of ambulation has a tremendous impact on pulmonary symptoms and their management. During the early nonambulatory phase when pulmonary function is just beginning to decline, anticipatory guidance includes the use of a self-inflating manual ventilation bag or mechanical insufflation-exsufflation device for daily use. Patients with a peak cough flow of less than 270 L/min are begun on assisted cough, which includes manual exercises and devices that help in clearing secretions from the lungs. During periods of illness and when peak cough flow decreases to less than 160 L/min, patients are placed on assisted cough daily. When the vital capacity declines to less than 50% of predicted or when the absolute maximal inspiratory pressure is less than 60 cm H_2O, nocturnal assisted ventilation is initiated. Some patients with dystrophinopathy will extend their assisted ventilation into the day as symptoms become worse. The use of noninvasive ventilation 24 hours a day is an indication for tracheostomy, although that decision is dependent on the patient and his medical team.[47]

BRAIN INVOLVEMENT

One of the overlooked comorbidities in dystrophinopathy is cognitive involvement. The spectrum of intellectual disabilities in dystrophinopathy ranges from lower IQ to attentional difficulties and learning disorders.[48] Other neurodevelopmental disorders, such as autism spectrum disorder and obsessive-compulsive disorder, are 4 times more likely in those with dystrophinopathy compared with the general population.[49,50] Epilepsy and social difficulties are other comorbid conditions that can occur in dystrophinopathy.[51,52] Although IQ has often been used as a marker of cognitive ability, even those with a normal IQ can have problems with working memory, a skill that is important for executive function and learning.[53,54] As with other chronic medical conditions, individuals with dystrophinopathy experience more psychosocial challenges.[55] Thus, anticipatory guidance includes screening for depression and anxiety during routine medical visits and psychosocial support for families, including the availability of a social worker as an additional resource.

PATHOLOGIC CHANGES IN DYSTROPHINOPATHY

Skeletal muscle biopsy is a powerful diagnostic accessory and is readily accessible for diagnosis of many neuromuscular diseases, including dystrophinopathy. Despite the widespread availability of targeted genetic testing to diagnose dystrophinopathy, muscle biopsy continues to be an important tool to help with diagnosis and, in a research setting, allows for evaluation of dystrophin restoration with treatment. When the clinical and genetic phenotypes are not congruent, the absence of dystrophin on skeletal muscle biopsy makes a strong argument that the disease is a severe phenotype. Dystrophin is completely absent in Duchenne muscular dystrophy and may be present in smaller amounts in Becker muscular dystrophy. The absence of dystrophin on muscle biopsy is considered a gold standard in the diagnosis of Duchenne muscular dystrophy. Likewise, the restoration of dystrophin by therapy (read-through and exon-skipping therapies) allows for assessment of the success of treatment. A recent review of skeletal muscle pathology in the era of new therapeutic interventions highlights the value of muscle biopsy.[56]

Irrespective of the clinical phenotype, pathologically characteristic dystrophic changes can be seen on muscle biopsy, including evidence of muscle fiber degeneration in the form of necrosis, evidence of muscle fiber regeneration

(internalization of myonuclei), and an increase in connective tissue. Several laboratories across the United States have standardized protocols to stain for dystrophin using a panel of dystrophin antibodies and have critical internal quality controls (FIGURE 5-5, FIGURE 5-6, and FIGURE 5-7).

The practice of performing a routine skeletal muscle biopsy to make the diagnosis of dystrophinopathy is declining as more sophisticated genetic testing is becoming increasingly available. One issue, especially in the pediatric population, is parental concern regarding the safety of this invasive procedure, but muscle biopsy can be safely performed in individuals with suspected dystrophinopathy. The author's institutional experience has been to perform open muscle biopsies, the removal of a small piece of tissue, with the patient under anesthesia, although several other institutions perform needle biopsies that are of high quality. Once biopsied, skeletal muscle tissue has to be processed carefully to minimize artifacts. The deltoid or vastus lateralis muscles are commonly chosen for biopsy.

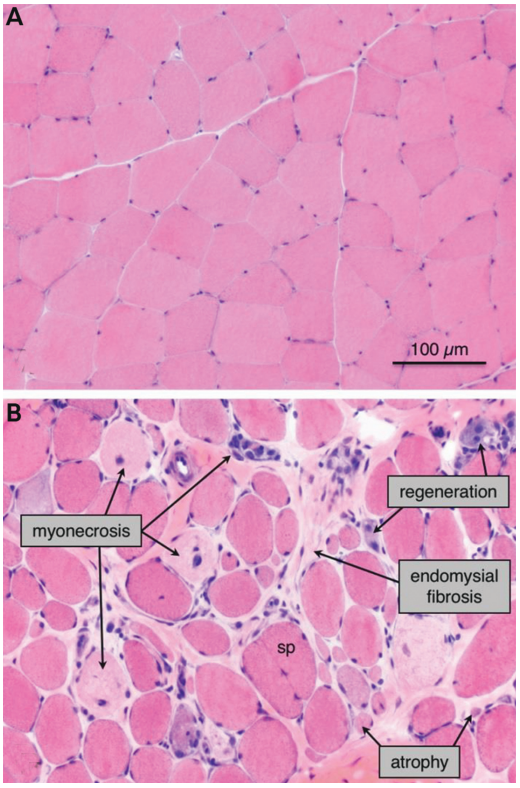

FIGURE 5-5
Dystrophic histopathology. Normal muscle (*A*) is compared with muscle of patient with Duchenne muscular dystrophy (*B*). The common dystrophic features of myonecrosis, regeneration, endomysial fibrosis, and atrophy, are annotated in the figure. Hypertrophy and internally placed nuclei often lead to split fibers (sp). The size marker shown (*A*) applies to both panels in the figure.
Courtesy of Steven Moore, MD, PhD, The University of Iowa.

CLINICAL AND LABORATORY EVALUATION OF DYSTROPHINOPATHIES

The use of quantitative muscle strength assessment and myriad laboratory studies (eg, imaging, muscle biopsy, and genetic approach) allow for a systematic evaluation of dystrophinopathies.

Timed Function Tests

Timed function tests are commonly used to help in the assessment of physical ability in people with dystrophinopathies. Since the ability to walk is one of the critical functions affected in dystrophinopathy, a concerted effort has been made to develop a reliable, sensitive, and valid outcome measure to assess ambulation. Of the timed function tests, the 6-minute walk test is one of the most commonly used, both in the outpatient clinic setting and as a primary outcome measure in clinical trials.[57–62] The test is performed indoors, along a flat straight surface (usually a corridor) with a 25-meter (82-foot) test area. Safety cones are usually

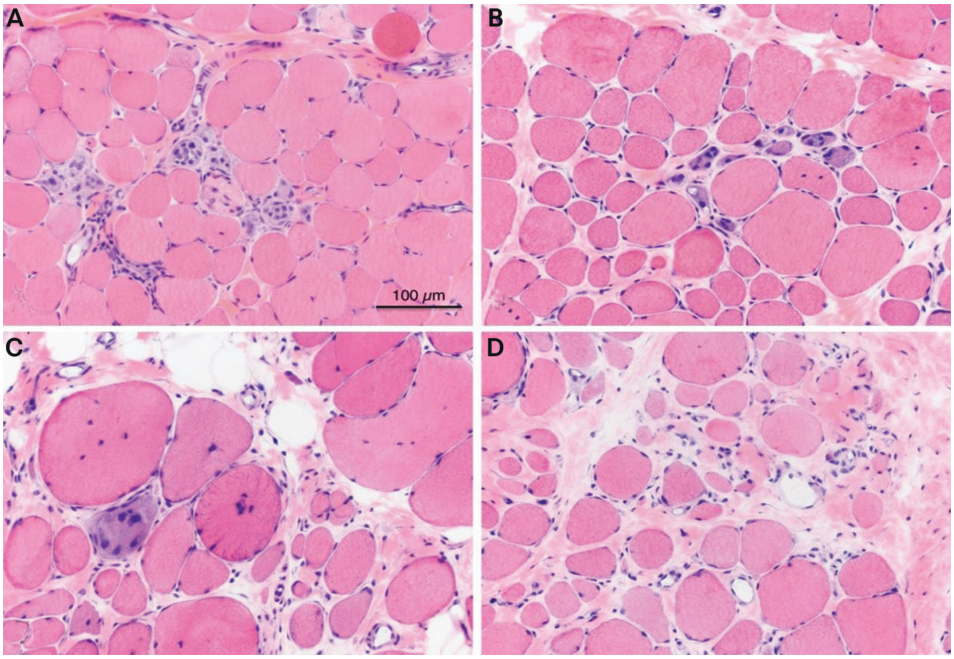

FIGURE 5-6

Progression of pathologic severity in dystrophinopathy. Early in the progression of muscle pathology (*A*), myonecrosis and regeneration without significant atrophy, hypertrophy, or endomysial fibrosis are observed in Duchenne muscular dystrophy. Later (*B*, *C*, and *D*), gradually greater degrees of severity are manifested by muscle fiber atrophy and hypertrophy, internally placed nuclei, endomysial fibrosis, and fatty replacement. The size marker shown (*A*) applies to all panels in the figure.

Courtesy of Steven Moore, MD, PhD, The University of Iowa.

placed at both ends of the test area, and the path to walk is highlighted with arrows taped to the floor. Both walking distance and walking velocity over a 6-minute period are obtained. Boys as young as 4 years of age can be assessed using the 6-minute walk test. While this test may be the most popular clinical outcome measure, other timed function tests are available, including time from a supine position to standing (at least 5 seconds or at least 10 seconds), time to climb four-step stairs, and hand-to-mouth function.[63] These timed function tests are not only important prognostic tools but provide clinically meaningful assessment of disease progression.[63]

Skeletal Muscle Ultrasonography

Noninvasive imaging, such as skeletal muscle ultrasonography, is becoming an increasingly attractive tool to complement other diagnostic testing for dystrophinopathy. Skeletal muscle ultrasonography can be conducted at the bedside and is relatively inexpensive, painless, and easy to use in the pediatric population. It provides quantifiable measures, allows for serial testing, and may be a potential biomarker for clinical trials. Muscle echointensity, which is affected by skeletal muscle fibrosis and fat, disease stage, and disease severity, allows for a quantification of skeletal muscle health in patients with dystrophinopathy.[64,65] FIGURE 5-8 shows the progressive atrophy of the biceps in a young boy with dystrophinopathy as detected by muscle ultrasonography.

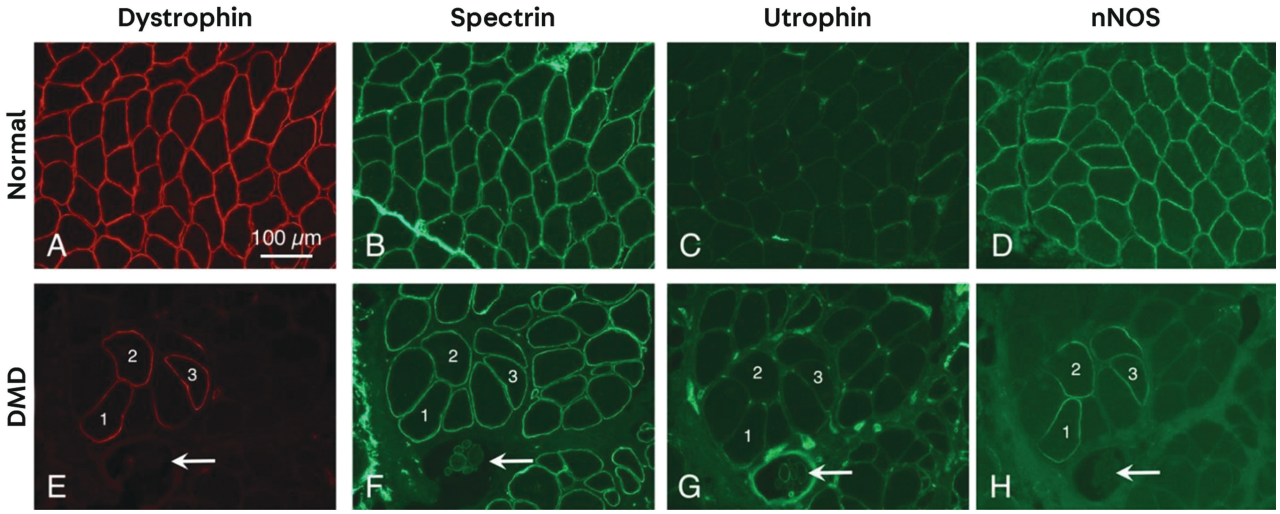

FIGURE 5-7

Immunofluorescence abnormalities. Normal muscle (*A* through *D*) is contrasted with muscle affected by Duchenne muscular dystrophy (DMD) (*E* through *H*). Dystrophin is absent from most muscle fibers in the DMD biopsy (*E*); only revertant fibers (numbered), which spontaneously correct their mutation, express dystrophin at the sarcolemma. Intrafusal muscle fibers within the DMD muscle spindle (*arrow*) are dystrophin negative. Uniform sarcolemmal expression of spectrin (*F*) demonstrates that the dystrophin-negative fibers are not necrotic. Utrophin, normally present only in endomysial capillary endothelium (*C*), is expressed at the sarcolemma of many DMD muscle fibers (*G*). The capsule of muscle spindles normally expresses utrophin. Roughly paralleling dystrophin, neuronal nitric oxide synthase (nNOS) is not expressed at the sarcolemma in most DMD muscle fibers (*H*). Revertant fibers express nNOS, as well as dystrophin. The DMD images are from serial cryosections; numbers identify the same muscle fibers in each image. The size marker shown (*A*) applies to all panels in the figure.

Courtesy of Steven Moore, MD, PhD, The University of Iowa.

Skeletal Muscle Imaging

As treatment options for dystrophinopathy are becoming more available, including exon-skipping, sophisticated imaging techniques using MRI not only aid in identifying more deep-muscle pathology, but also function as a biomarker that can help identify fibrosis and other pathologic characteristics of dystrophin deficiency. Specifically, the measurement of skeletal muscle fat, fibrosis, and inflammation can be used to evaluate the progression of the disease[66–68]; changes in these parameters make it possible to evaluate the potential efficacy of therapeutic agents in dystrophinopathy.

Genetic Testing

The availability of genetic testing for dystrophinopathies has greatly improved patient care. Genetic testing helps confirm the diagnosis and aids in prognostication and care planning. For example, information regarding out-of-frame versus in-frame genetic mutation can help families plan for change in functional status and cardiac surveillance. Genetic confirmation allows for eligible patients to receive specific therapy or facilitate participation in experimental clinical trials. Also, targeted genetic testing helps in female carrier testing, which is important because dystrophinopathies are X-linked disorders; thus, female carrier testing can help families with reproductive planning.

Commercial genetic testing is available to identify mutations in *DMD*. The first step in ordering genetic testing for dystrophinopathies is to perform

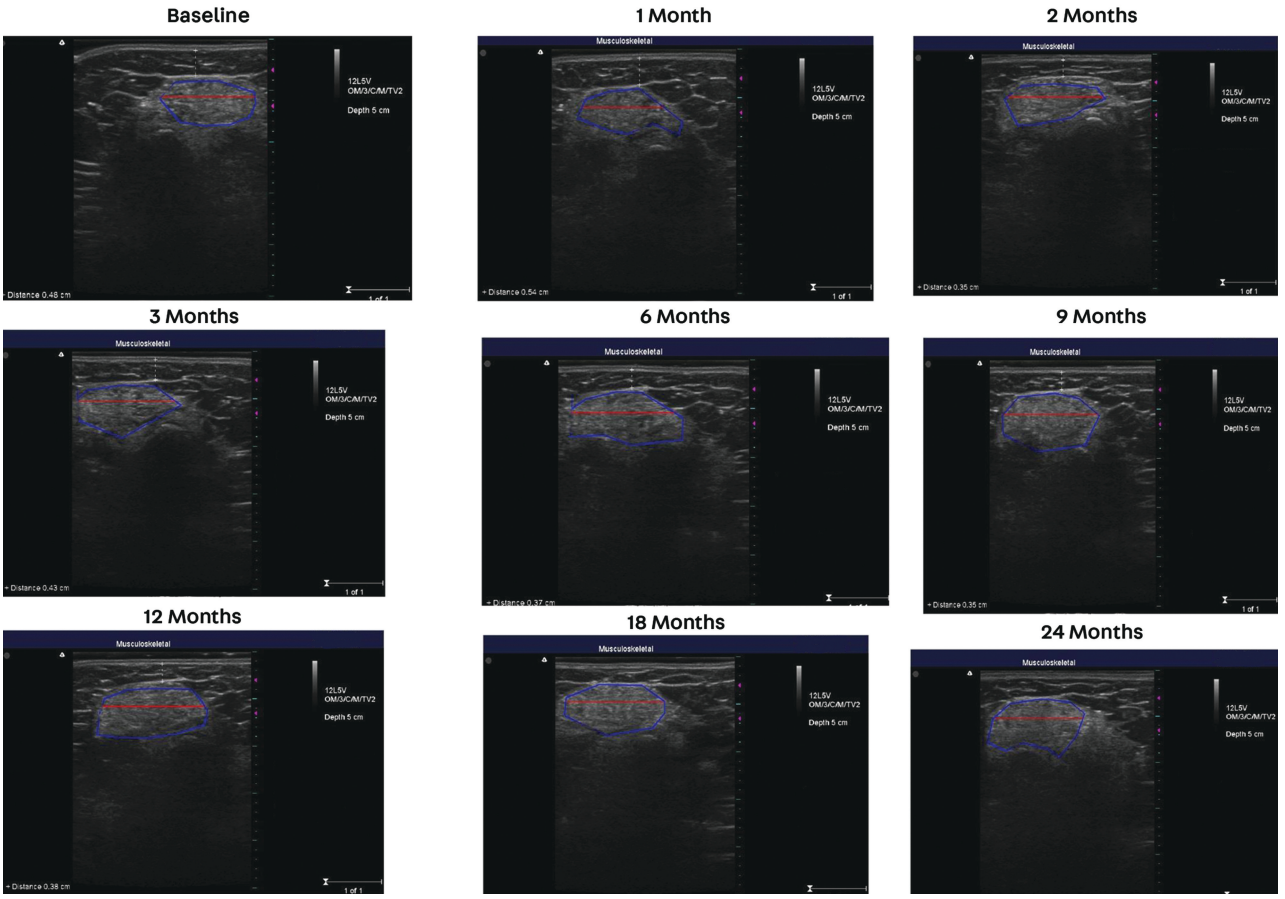

FIGURE 5-8

A 12-year-old boy with dystrophinopathy with serial skeletal muscle ultrasonography of the biceps from baseline up to 2 years. The *blue outline* marks the region of interest (biceps muscle in this case), and the *red line* indicates the level above the section from which the echointensity of the muscle is calculated. The figure tracks the change in the echointensity of biceps, which can be due to deposition of endomysial fat, connective tissue development, or muscle fiber atrophy.
Courtesy of Seward Rutkove, MD.

deletion/duplication analysis of *DMD* that detects the presence of deletions of one or more exons or of duplications of exons. Should initial deletion/duplication analysis be negative or no pathogenic variant is detected, the next step is to perform sequence analysis of *DMD* coding regions. This step can help detect the presence of a nonsense mutation or small frameshift mutation. If sequencing of *DMD* coding regions yields no result, the next step would be to perform whole-exome testing. Next-generation sequencing may be performed to help identify a splicing mutation or complex rearrangement.[69]

Carrier Testing

The rate of manifesting carriers (women carriers of the mutation who experience severe skeletal muscle weakness) is reportedly one in 1 million. Many carriers do experience myalgia and may have a high creatine kinase level. In one study, fewer than 20% of 129 women who were carriers were found to have mild to moderate skeletal muscle weakness.[70] Women who are carriers can develop cardiomyopathy, underscoring the critical importance of carrier testing. The risk of developing cardiomyopathy appears to increase with age; a recent review of care series of

dystrophinopathy carriers as detected by echocardiography or cardiac MRI found that the mean age of women with cardiomyopathy was 38.2 years, whereas the mean age of women without cardiomyopathy was 20.9 years.[71] The spectrum of cardiac manifestations in carriers of dystrophinopathy includes late gadolinium enhancement, left ventricular dilation, dilated cardiomyopathy, and overt cardiac failure. Because of the largely asymptomatic nature of cardiac manifestations, the standard recommendations include an evaluation by a cardiologist and imaging (echocardiography or cardiac MRI) periodically.[47]

Carrier testing allows women who are carriers and their families to engage in reproductive planning. Options available include working with a reproductive endocrinologist, a preimplantation genetic diagnosis, and prenatal testing through chorionic villus or amniotic fluid sampling. A family history of dystrophinopathy in either a brother or maternal uncle is often helpful to elicit to allow for more informed genetic counseling. The absence of an affected family member does not rule out the possibility of de novo mutations that can occur in approximately one-third of women who have boys with dystrophinopathy.

MANAGEMENT

The improvement in the clinical course of dystrophinopathies due to therapy and standardization of ambulatory care in this population is summarized below.

Disease-Modifying Therapy in Dystrophinopathies

Natural history studies in dystrophinopathies have provided valuable information regarding disease course and efficacy of therapy, particularly corticosteroids.

CORTICOSTEROIDS. Unambiguous data show that oral corticosteroids begun between ages 4 and 7 years in boys with Duchenne muscular dystrophy alter the natural history of the disease.[63,72,73] The objective advantages of corticosteroids include prolonging the ability to walk by 1.5 to 2 years, preservation of upper extremity strength, reduced risk of scoliosis, and cardiopulmonary benefits. Commonly used corticosteroids include prednisone (0.75 mg/kg/d) and deflazacort (0.9 mg/kg/d); maximum daily doses in boys who weigh more than 40 kg (88 lb) are 30 mg for prednisone and 36 mg for deflazacort. At least three regimens are accepted: daily prednisone or deflazacort, 10 days on and 10 days off prednisone or deflazacort, and weekend-only doses of prednisone. Although most clinicians recognize that corticosteroids are indeed beneficial in improving skeletal muscle health outcomes, significant side effects are associated with the long-term use of corticosteroids; thus, it is not surprising that great variation exists in prescribing the optimal dose of corticosteroids in this population. In the largest, prospective natural history study of Duchenne muscular dystrophy, more than 14 regimens of steroid use were reported.[74] The FOR DMD (Finding the Optimal Dose for Duchenne Muscular Dystrophy) trial was designed to attempt to address the lack of standardization of corticosteroid regimens, and data from this trial will be informative of side effects of the three regimens most commonly used.[75]

EMERGING THERAPIES FOR DYSTROPHINOPATHIES. Broadly, treatment strategies in dystrophinopathy can be divided into increasing dystrophin protein expression or altering effects of downstream pathologic processes. Both of these strategies can be either mutation specific or mutation agnostic.

Currently, two drugs, mutation-specific eteplirsen and mutation-agnostic deflazacort, are approved by the US Food and Drug Administration (FDA) for

KEY POINTS

- Genetic testing is readily available and should be actively pursued to establish a diagnosis of dystrophinopathy and guide therapy as more mutation-specific therapies are becoming available.
- Cardiomyopathy is a frequent complication of dystrophinopathy, especially in those with the milder but variable phenotype (Becker muscular dystrophy) and in female carriers of dystrophinopathy.
- Corticosteroids begun between the ages 4 and 7 years change the natural history of Duchenne muscular dystrophy.
- At least two drugs have been approved for treatment in Duchenne muscular dystrophy, and new therapeutic agents are increasingly being tested.

dystrophinopathy. Approximately 14% of individuals with dystrophinopathy are amenable to mutation-specific exon-51–skipping therapy, namely eteplirsen. The exon-51–skipping therapy uses antisense oligonucleotides, which are synthetic nucleic acid analogs, to restore the reading frame by "skipping" an exon. This exon skipping is based on the ability of antisense oligonucleotides to bind to complementary sequences in the pre–messenger RNA and allows for exclusion of an exon.

Eteplirsen was the first drug to receive conditional approval by the FDA for Duchenne muscular dystrophy in September 2016.[7] In a study by Mendell and colleagues,[62] 12 boys who were treated with eteplirsen over a 36-month period showed stabilization in the 6-minute walk test compared with a matched historical cohort. The difference in the mean test score was 151 meters between the boys treated with eteplirsen and matched controls. Pulmonary function tests remained stable in these 12 boys during eteplirsen treatment.

Deflazacort, mentioned earlier in the Corticosteroids section, is an oxazolone derivative of prednisone and was the second drug to be approved for Duchenne muscular dystrophy in February 2017. Deflazacort can be used in all subjects with severe dystrophinopathy, regardless of mutation location. One small-molecule drug, which has already been in approved in Europe, is ataluren, which works by a read-through of premature termination codon and is for individuals with a *DMD* nonsense mutation.

The recent developments in therapeutics in dystrophinopathy are elegantly summarized in a review by Guiraud and Davies.[76] The broad categories of these drugs are summarized in TABLE 5-1.

The therapeutic options that are either in clinical trials or in the drug pipeline are providing great excitement in the neuromuscular community with more than 52 studies currently registered on *ClinicalTrials.gov* recruiting participants with Duchenne muscular dystrophy.

TABLE 5-1 **A Brief Summary of Different Drug Targets in Development for Dystrophinopathy and the Associated Agents**

Mechanism	Target Drugs
Gene transfer	Mini-dystrophin (rAAV2.5-CMV-minidystrophin)
	Micro-dystrophin (rAAVrh74.MCK.Micro-Dystrophin)
Exon skipping	Eteplirsen
	Multi-exon skipping (in development)
Stop-codon read-through	Ataluren
Selective androgen receptor modulator	DT-200
Muscle growth/regeneration drugs	Human anti-myostatin antibody, follistatin
Antiinflammatory agents	Prednisone, deflazacort
Inhibitors of nuclear factor-kappa B	Vamorolone
Antioxidant	Idebenone
Antifibrotics/transforming growth factor β drugs	Losartan, halofuginone, fibrinogen

Multidisciplinary Care Teams and Health Care Delivery

As with several other neuromuscular diseases, optimal health care management of individuals with dystrophinopathy and their families includes a cohesive, interdisciplinary, scientific approach. The recently published care considerations in dystrophinopathy from the Centers for Disease Control and Prevention,[77] and developed with consensus from a group of international dystrophinopathy experts and parent advocacy groups, highly recommend an integrated team approach that values support across the life span of the affected individual. In recognizing the multisystem nature of the disease, the care guidelines encourage a tailored strategy from the time of diagnosis to the late nonambulatory stage. Regardless of the disease stage, management of dystrophinopathy as recommended by the care guidelines include addressing the following domains: neuromuscular, rehabilitation, endocrine, gastrointestinal and nutritional management, respiratory, cardiac, bone health, orthopedic, psychosocial, and transition to adult care.[6,47,78] A clinic nurse or clinical coordinator can often serve as the point person for families, thus facilitating continued communication that continues beyond the routine medical visit. Multidisciplinary team composition is variable across academic institutions, but most centers in the United States have a neuromuscular physician or physiatrist as the core team member. The availability of other professionals, such as a cardiologist, pulmonologist, speech therapist, genetic counselor, social worker, equipment specialist, and neuropsychologist, can greatly facilitate the patient experience.

KEY POINT

- **A cohesive, interdisciplinary team is integral for the provision of high-quality medical care for individuals with dystrophinopathy.**

CONCLUSION

Dystrophinopathies are systemic disorders that affect multiple organs, including the skeletal muscles, cardiac muscle, and brain, caused by genetic mutation in the dystrophin gene. Dystrophinopathies vary from the severe phenotype to intermediate and milder phenotypes. Genetic diagnosis is recommended both for prognostication and to assess eligibility for enrollment in clinical trials; carrier testing is recommended for women. Standard care for the dystrophinopathies involves a multidisciplinary team approach for optimal clinical outcomes.

USEFUL WEBSITES

CENTERS FOR DISEASE CONTROL AND PREVENTION
The Centers for Disease Control and Prevention provides valuable information regarding muscular dystrophy.
cdc.gov/ncbddd/musculardystrophy/care-considerations.html

CLINICALTRIALS.GOV
ClinicalTrials.gov has up-to-date information regarding clinical trials on dystrophinopathy.
clinicaltrials.gov

CUREDUCHENNE
CureDuchenne is a nonprofit organization that supports research and advocacy efforts in Duchenne muscular dystrophy.
cureduchenne.org

MUSCULAR DYSTROPHY ASSOCIATION
The Muscular Dystrophy Association is a national nonprofit agency that works to cure muscular dystrophy through research.
mda.org

NEUROMUSCULAR DIVISION, DEPARTMENT OF NEUROLOGY, WASHINGTON UNIVERSITY SCHOOL OF MEDICINE
This website provides a significant amount of information regarding dystrophinopathies that is readily accessible to readers.
neuromuscular.wustl.edu

PARENT PROJECT MUSCULAR DYSTROPHY
Parent Project Muscular Dystrophy is a nonprofit parent advocacy group focusing on Duchenne muscular dystrophy and is a good resource for both families and professionals.
parentprojectmd.org

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