CHAPTER 5

Muscle Pathology

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During the past decade, advances in molecular genetics have revealed the cause of the most common forms of hereditary myopathies, including Duchenne and Becker muscular dystrophy (DMD and BMD) and myotonic dystrophy. Similar studies are being conducted for other forms of muscular dystrophy, and rapid progress is to be expected in our understanding of the pathogenesis of these disorders. Concomitant advances in muscle morphology have been applied to the investigation of muscle biopsies. This has led to important observations of the cellular localization of normal and mutated gene products, contributed to a better understanding of the pathogenesis of muscle cell dysfunction, and provided new approaches for the diagnosis of muscular dystrophy.

This chapter is a signpost for the new directions that have been taken in the evaluation of muscle pathology, emphasizing the application of immunologic probes for the diagnosis of muscular dystrophy. The metabolic disorders, including the mitochondrial myopathies and distal myopathies, inflammatory myopathies, and other acquired myopathies, are discussed in Chapters 9, 10, and 12 of this book.

MUSCULAR DYSTROPHIES

The muscular dystrophies comprise a group of hereditary myopathies characterized by progressive muscle weakness and wasting. The muscle pathology shows signs of degeneration, regenerative changes, and proliferation of connective tissue but no distinctive morphologic abnormalities.

Duchenne Muscular Dystrophy

DMD is the most common childhood dystrophy, affecting approximately 1 male in 3,500. It is inherited as an X-linked recessive trait, and about one third of cases are due to new mutations (1). It begins in early childhood, usually before the age of 4 years. Muscle biopsy shows various degrees of the following changes, depending on the stage of the disease: increased variation in fiber size, large hypercontracted fibers, focal areas of degenerating and regenerating fibers, increased numbers of internal nuclei, and infiltration of fat and connective tissue. The diagnosis is based on clinical features, muscle biopsy, analysis of serum creatine kinase (CK) levels, and evidence of a mutation in the DMD gene, which can be based either on the analysis of DNA or the gene product, dystrophin. Mutations in the DMD gene cause a shift in the translational reading frame of the dystrophin messenger RNA, a nonsense mutation, and therefore a failure of protein synthesis (2).

Dystrophin is a large (427 kDa) cytoskeletal protein localized to the plasma membrane of the muscle fibers. The protein is tightly associated through its cysteine-rich and C-terminal domains to a large group of dystrophin-associated proteins. These proteins have been divided into three complexes: dystroglycan, syntrophin, and sarcoglycan complexes. The dystroglycan complex consists of α-dystroglycan and β-dystroglycan. The syntrophin complex consists of α-syntrophin and β-syntrophin. The sarcoglycan complex consists of at least four proteins: α-sarcoglycan, β-sarcoglycan, δ-sarcoglycan, and τ-sarcoglycan. In muscle fibers, interactions between cytoskeletal actin filaments and dystrophin and between α-dystroglycan and the α2-chain of laminin have been identified, suggesting that one function of the dystrophin-glycoprotein complex is to provide a link between the cytoskeleton and the extracellular matrix (2–5). Also, it should be noted that mutations in at least five components of the dystrophin-based membrane cytoskeleton (dystrophin, α-sarcoglycan, β-sarcoglycan, τ-sarcoglycan, and δ-sarcoglycan) each cause an
inherited muscular dystrophy with very similar histopathologic features (6).

DNA analysis detects about 65% of patients with DMD, whereas immunologic analysis of the protein allows the detection of all patients. Immunohistochemistry of frozen muscle sections using antibodies against dystrophin shows no staining in DMD patients, whereas in sections of normal muscle, dystrophin is localized at the sarcolemma (Fig. 1). Similarly, immunoblot analysis of muscle extracts shows a severe defect of dystrophin with concentrations less than 3% of normal (Fig. 1).

Because of the lack of specific therapy, female carriers must be detected to prevent new occurrences. Identification of some, but not all, carriers can be achieved by direct DNA analysis, the analysis of polymorphic DNA sequences linked to the DMD gene, or dystrophin immunohistochemistry of muscle biopsies (7). Prenatal diagnosis is carried out by direct DNA analysis of amnioncyes or chorionic villi.

Becker Muscular Dystrophy

This is an allelic form of DMD that is about ten times less common than DMD. The clinical manifestations and distribution of weakness are similar to those of DMD, but the onset is usually after age 5 years. Most patients are still able to walk beyond the age of 12 years. As in DMD, the serum CK concentration is greatly increased even before weakness becomes manifest. Muscle biopsy shows fiber splitting, occasional hypercontracted fibers, segmental myonecrosis, and proliferation of connective tissue.

BMD is due to mutations in the dystrophin gene, but mutations in this disorder do not cause a frame-shift of the coding sequence. The result is synthesis of shorter dystrophin molecules, which may be only partially functional and more susceptible to degradation (5). In agreement with these molecular defects, the immunohistochemical stain for dystrophin in muscle biopsies is present but shows low intensity and appears discontinuous (Fig. 1). Immunoblot shows qualitative and quantitative alterations of the protein with dystrophin molecules that are smaller than normal (Fig. 1).

Familial X-linked Myalgia and Cramps

This disorder is considered a variant or milder form of BMD. Onset varies, and the cramps, which are usually exercise induced, may begin in early childhood or adoles-

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**FIG. 1.** Dystrophin analysis in Du-
chenne/Becker muscular dystrophies
(DMD/BMD). Top, left: Western blot con-
taining a sample from a normal control (N),
two brothers with BMD (B1 and B2), and
samples from two brothers with DMD (D1 and D2). The control shows a normal dystrophin (Dys.); the protein is absent in the brothers with
DMD, and in the brothers with BMD a
mutated protein is seen migrating at a
lower position. The corresponding im-
umnostains on frozen sections show a
normal pattern in the control (N, top
right), low intensity immunostain in the
brothers with BMD (B1 and B2, mid-
dle), and a markedly reduced immuno-
reaction in the DMD brothers (D1 and
D2, bottom). ×110.
ence. Affected patients show elevated resting serum CK levels, calf hypertrophy, and no muscle weakness. There is no history of progressive deterioration of muscle function, and muscle biopsy findings do not show changes consistent with a dystrophic process (7).

Demonstration of a mutation or in-frame deletion in the dystrophin gene is provided by DNA analysis or by direct study of the protein by immunoblotting that shows a smaller dystrophin band (Fig. 2).

**Emery-Dreifuss Dystrophy**

This rare form of X-linked childhood muscular dystrophy is distinguished from BMD by the presence of contractures of the elbow, ankle, and neck and by cardiomyopathy that causes conduction block. The muscle disorder is slowly progressive and rarely disabling, but sudden death attributable to heart conduction disturbances is not uncommon. The serum CK concentration is only mildly elevated, and the electrocardiogram shows various degrees of atrioventricular block. The muscle biopsy usually shows nonspecific myopathic features, including variation in fiber size, muscle fiber necrosis, and endomysial and perimysial fibrosis. The gene for Emery-Dreifuss dystrophy is located on the distal end of the long arm of the X chromosome. The protein encoded by the gene has been called emerin and has been localized to the nuclear membrane of the muscle fibers (9,10). The diagnosis is based on clinical features, evidence of a mutation in the Emery-Dreifuss dystrophy gene, or the immunologic and immunohistochemical analysis of the gene product, emerin (Fig. 3).

**Congenital Muscular Dystrophy**

Congenital muscular dystrophy (CMD) is a heterogeneous group characterized by flaccidity and profound muscle weakness at birth. In addition, contractures or joint deformities and a variable involvement of the central nervous system (CNS) are usually present. Four autosomal recessive forms have been identified on the basis of differential involvement of the CNS. Fukuyama type CMD, muscle-eye-brain disease, and Walker-Warburg type CMD are dominated by signs of CNS involvement. The last form, occidental CMD, is characterized by skeletal muscle involvement in the absence of clinically evident CNS manifestations.

The morphologic hallmark of these clinically heterogeneous syndromes is a marked increase in connective tissue, so suggesting that abnormalities of components of the extracellular matrix might be involved in the pathogenesis of these disorders. The demonstration that laminin, a component of the basal lamina, was a ligand for α-dystroglycan later prompted studies of whether one of the laminin subunits could be involved in CMD. Laminin is a heterotrimer of one heavy chain and two light chains, and the α2-chain (merosin) is a 400-kDa isoform found in muscle, in some regions of the CNS, and in Schwann cells of the peripheral nervous system (11). In 1994, a systematic study of several French patients with typical CMD showed that the laminin α2-chain was deficient in about half of the patients (12). Other studies supported this observation in muscle of patients with CMD from other Western countries (Fig. 4) (13). Because the chromosomal location of the laminin α2-chain was known (chromosome 6q2), genetic studies confirmed the localization of the CMD gene to the 6q2 locus (14). Later, mutations (splice site and nonsense) in the laminin α2-chain gene were reported in patients with the Occidental form of CMD (11,15). The gene for Fukuyama type CMD has been localized to chromosome 9q31-33 (16).

**Facioscapulohumeral Dystrophy**

This autosomal dominant (AD) disorder becomes apparent in adolescence and progresses slowly. The expression of the disease varies greatly among different patients and in

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![FIG. 2. Dystrophin analysis in familial X-linked myalgia and cramps. a: Dystrophin immunohistochemistry shows a few fibers with discontinuous dystrophin stain at the sarcolemma (white circles). ×200. b: Western blot shows a normal dystrophin (Dys.) in two controls (N1 and N2) and a mutated protein migrating at a lower position in the patient (P) with myalgia and cramps.](image-url)
subsequent generations, from a virtually asymptomatic condition to a severe, although rarely disabling, weakness. The serum CK concentration is only slightly increased, and muscle biopsy abnormalities are not very remarkable, often consisting of scattered atrophic fibers. In some families, however, muscle biopsy shows striking inflammatory changes. The gene for facioscapulohumeral dystrophy has been localized on the long arm of chromosome 4. Available genetic markers can be used for genetic counseling and prenatal diagnosis; however, until flanking markers are found, the accuracy of diagnostic testing is limited (17).

Limb Girdle Muscular Dystrophy (LGMD)

This is the least well-defined of all muscular dystrophies, and it probably includes different diseases, which may explain the variation in age at onset and severity in different patients. Typically, it begins in the second or third decade, with slowly progressive weakness, generally affecting one limb girdle first and then spreading to the other. Serum CK concentrations vary widely from normal to grossly increased. Electromyography (EMG) and muscle biopsy are helpful to rule out more specific disorders that may be clinically indistinguishable from LGMD: structurally defined congenital myopathies, metabolic myopathies, and spinal muscular atrophy.

Recently, genetic linkage studies and molecular mutation investigations of candidate genes have begun to elucidate the underlying cause of some forms of LGMD. In a new nomenclature, the dominantly inherited LGMDs have been labeled LGMD 1, with the letters indicating the specific loci: LGMD 1A has been mapped to chromosome 5q and LGMD 1B has been localized to chromosome 1q11-21. All recessively inherited LGMDs have been labeled LGMD 2 and the following loci have been assigned: LGMD 2A (chromosome 15q), LGMD 2B (chromosome 2p), LGMD 2C (chromosome 13q12), LGMD 2D (chromosome 17q21), LGMD 2E (chromosome 4q12), and LGMD 2F (chromosome 5q33).

Mutation studies of candidate genes in recessively inherited LGMD have identified the proteins that, when mutated, are responsible for five of these disorders: LGMD 2A is caused by calpain 3 deficiency, LGMD 2C by δ-sarcoglycan deficiency, LGMD 2D by α-sarcoglycan deficiency, LGMD 2E by β-sarcoglycan deficiency, and LGMD 2F by γ-sarcoglycan deficiency (5,18).

FIG. 4. Congenital muscular dystrophy (CMD) with laminin α2-chain deficiency. a: A normal control shows immunoreaction for laminin α2-chain at the sarcolema of all fibers. b: A patient with CMD shows lack of laminin α2-chain at the sarcolema of all fibers. c: The same patient shows immunoreaction for dystrophin at the cell surface of the muscle fibers. ×110.