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Limb-Girdle Muscular Dystrophies

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SCIENTIFIC BACKGROUND

Limb-girdle muscular dystrophies (LGMD) are a clinically and genetically heterogeneous group of diseases characterized by progressive proximal muscle weakness and wasting. As suggested by the name, the first muscles affected are those of the pelvic and shoulder girdles. Although neck and facial muscles are generally spared, distal muscles could be affected in the late stages of the disease. A marked variability in age of onset, rate of progression, degree of muscle involvement, and clinical severity has been observed in unrelated patients and between affected members of the same family.

The prevalence of LGMD seems to be extremely variable among populations, depending on historical, geographical, and cultural factors. In the Caucasian population, autosomal dominant forms (LGMD1) account for 14% of the total LGMD cases, autosomal recessive forms (LGMD2) for 34%, and sporadic cases for 52% (1).

Fourteen LGMD loci are known at the time of this writing, and a gene-based classification has replaced the former scheme that was based purely on clinical data (Table 17.1). This first section of this chapter summarizes recent data and current ideas concerning the molecular pathogenesis of LGMD in those types for which the defective gene is known. For completeness, the LGMD with unknown genes and their loci are listed in Table 17.1.

Limb-Girdle Muscular Dystrophy Type 1

Five loci are associated with autosomal dominant LGMD, and three genes have been cloned.

LGMD1A, or Myotilinopathy (Locus at 5q31)

Myotilin is a 57-kD integral component of the sarcomere (Fig. 17.1) that is mutated in LGMD1A. Striated muscle sarcomeres are highly organized structures composed of actin and myosin filaments, which slide past each other during muscle contraction. Assembly, stability, and regulation of the sarcomere are ensured by other sarcomeric proteins such as titin, which links myosin filaments to the Z-line, and α-actinin, which cross-links actin filaments at the Z-line. Myotilin has homologies with immunoglobulin-like domains of titin and binds to α-actinin in the Z-line (2). Muscle biopsies from LGMD1A patients display sarcomeric disorganization and disruption resulting in Z-line streaming. These Z-line abnormalities are similar to the rodlike bodies observed in nemaline myopathies, diseases due also to mutations in sarcomeric proteins associated with actin, such as nebulin (3) and α-tropomyosin (4). The

TABLE 17.1. *Limb-girdle muscular dystrophies and related genes*

Disease	Locus	Protein	OMIM
Autosomal dominant			
LGMD1A	5q31	Myotilin	159000
LGMD1B	1q11	Lamin A/C	159001
LGMD1C	3p25	Caveolin-3	601253
LGMD1D	6q23	Unknown	602067
LGMD1E	7q32	Unknown	603511
Autosomal recessive			
LGMD2A	15q15	Calpain-3	253600
LGMD2B	2p13	Dysferlin	253601
LGMD2C	13q12	γ-Sarcoglycan	253700
LGMD2D	17q12	α-Sarcoglycan	600119
LGMD2E	4q12	β-Sarcoglycan	604286
LGMD2F	5q33	δ-Sarcoglycan	601287
LGMD2G	17q12	Telethonin	601954
LGMD2H	9q31	Unknown	254110
LGMD2I	19q13	Unknown	—

OMIM, Online Mendelian Inheritance in Man number; LGMD, limb-girdle muscular dystrophy.

mutation found in the LGMD1A family does not affect the localization or amount of myotilin nor myotilin binding to α-actinin, and the molecular mechanism by which this mutation disrupts the Z-line has still to be determined (2).

LGMD1B, or Laminopathy (Locus at 1q11)

Genetic evidence suggested that the LGMD1B gene could overlap the locus of the autosomal dominant Emery-Dreifuss muscular dystrophy in 1q11 (5,6). Indeed, for both diseases mutations have been found in the lamin A/C (LMNA) gene, which encodes lamins A and C (6). Three additional clinical phenotypes result from mutations in the LMNA gene: nominally autosomal recessive Emery-Dreifuss muscular dystrophy, cardiomyopathy with conduction defect, and familial partial lipodystrophy. There is no obvious correlation between the nature of the mutation and the phenotype; indeed, intrafamilial variability is observed (7,8). Lamins A and C are two proteins of the nuclear envelope (Fig. 17.1), members of the intermediate filaments family. Lamins form dimers through their rod domains and interact with chromatin and integral proteins of the inner nuclear membrane through binding sites located in their rod domains and their carboxyl-terminal globular tails (7). Two of the mutations found in LGMD1B families affect conserved amino acids of the central rod domain of the lamins A and C; the third mutation leads to a truncation of the carboxyl-terminal end (7). The direct effect of these mutations on the assembly of normal nuclear lamina and the nucleus has been experimentally demonstrated (9,10).

LGMD1C, or Caveolinopathy (Locus at 3p25)

Mutations in the caveolin-3 gene have been found in patients affected with LGMD1C, and caveolin-3 is markedly reduced at the sarcolemma of patient muscle biopsies (11). Caveolins are the most abundant structural components of caveolae, 50- to 100-nm vesicular invaginations of plasma membrane involved in vesicle trafficking and, probably, signal transduction processes (for review, see reference 12). In mammals, caveolins 1 and 2 are ubiquitously co-expressed and form heterocomplexes (13), whereas caveolin-3 is found exclusively in smooth and striated muscles (14). Experimental evidence suggests that either absence or overexpression of caveolin-3 results in a myopathy. The co-expression of wild type and mutant forms of caveolin-3 in a heterologous cell system showed that these molecules associate to form heteropolymers that are intracytoplasmically retained (15) and undergo proteosomal degradation (16). Similar effects have been observed in skeletal muscle of transgenic mice expressing a mutant form of caveolin-3, suggesting that mutated caveolin-3 has a dominant negative effect resulting in loss of wild type protein from the sarcolemma and damage of the muscle (17). Caveolin-3–null mice display an autosomal recessive myopathy. Healthy heterozygous littermates have a 50% reduced amount of protein at the sarcolemma, confirming that the muscle can endure the consequent deficit of caveolae (18). On the other hand, an increased number of caveolae and overexpression of caveolin-3 have been observed in Duchenne muscular dystrophy (19) and in its murine model, the *mdx* mouse (20). The hypothesis that an increased level of caveolin-3 directly contributes to the myopathic phenotype

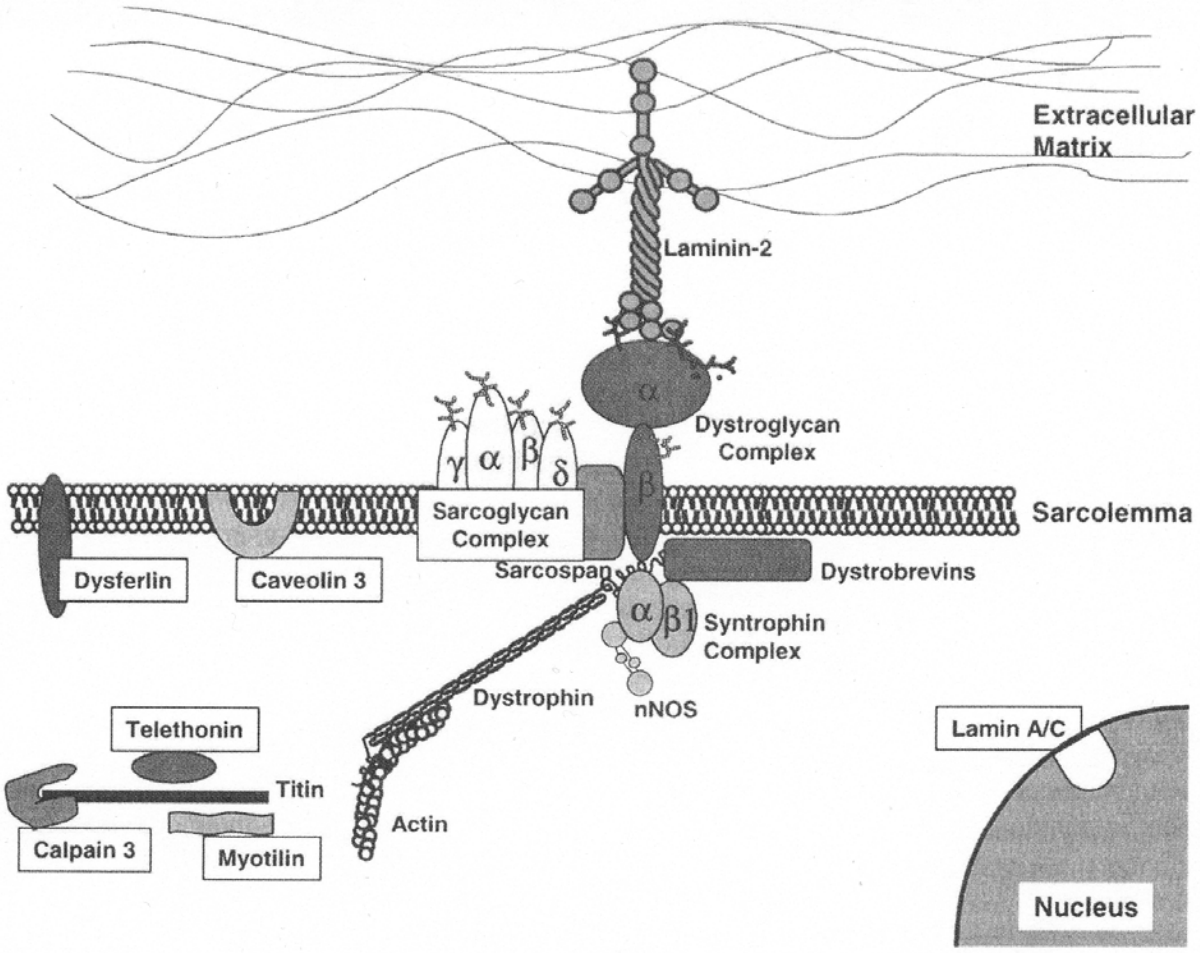

FIG. 17.1. The limb-girdle muscular dystrophy (LGMD) proteins (framed names) and closely associated proteins in skeletal muscle.

has been confirmed in transgenic mice overexpressing caveolin-3 in skeletal muscle (21).

Caveolin-3 is a structural component of the sarcolemma, but indirect evidence suggests a possible role also in signal transduction. Although caveolin-3 is not an integral component of the dystrophin glycoprotein complex (DGC) (see the section on sarcoglycanopathies and Fig. 17.1) (22), an association between caveolin-3 and DGC has been reported (14), and direct interaction between caveolin-3 and β-dystroglycan has been recently suggested (23). Indeed, similar to dystrophin-deficient Duchenne muscular dystrophy (24), a γ-sarcoglycan–deficient LGMD1C biopsy showed a secondary decrease of dystroglycans, DGC integral components, and neuronal nitric oxide synthase (nNOS), a closely DGC-associated signaling protein (25). Furthermore, caveolin-3 directly inhibits nNOS, supporting the hypothesis that caveolins may be involved in signal transduction regulation (26).

Limb-Girdle Muscular Dystrophy Type 2

This larger group of dystrophies is better understood, partly because the genes underlying the autosomal recessive forms of LGMD were the first to be cloned and partly because of the rapid generation of several murine models that have allowed a more thorough analysis of their pathophysiology.

LGMD2A, or Calpainopathy (Locus at 15q15)

Calpain-3 is the sole protein involved in LGMD known to have enzymatic rather than structural properties. This calcium-activated

intracellular protease is a member of the calpain family. In addition to four common catalytic subunits, calpain-3 has three unique regions (NS, ISI, and IS2), which may confer its muscle specificity (27). Calpain-3 is extremely labile *in vitro* because of its autolytic activity (28), whereas it is stable for several hours in human muscle tissue (29). Indeed, *in vivo,* the sarcomeric protein titin specifically binds the calpain-3 IS2 domain, thus inhibiting its proteolytic function.

An interesting hypothesis about the role of calpain-3 in skeletal muscle has recently been proposed based on the assumption that calpain-3, similar to the ubiquitous calpains, may regulate transcription by cleavage of transcription factors or their inhibitors. This idea, reinforced by the dual myofibrillar and nuclear localization of calpain-3, formed the basis of an investigation of apoptotic nuclei in calpain-deficient biopsies (30). The study showed that the transcription factor nuclear factor κB (NF-κB) failed to enter the nucleus where it usually induces the expression of genes involved in the inflammatory responses and cell survival (31). In contrast, its inactivator factor IκBα, a calpain-3 substrate, accumulated in the cytoplasm and nucleus of LGMD2A muscle. Similar results have been found in calpain-3–null homozygous mice (32). Although only very few nuclei had apoptotic features and the relevance of these findings for multinucleate muscle fibers requires further investigation, this study suggests that calpain-3 may be involved in the regulation of the expression of stress-response-related genes in skeletal muscle.

LGMD2B, or Dysferlinopathy (Locus at 2p13)

The term *dysferlinopathy* refers to two different clinical entities: limb-girdle muscular dystrophy type 2B (LGMD2B) and Miyoshi myopathy (MM) (33–35). Whereas LGMD2B involves proximal muscles, MM affects primarily the distal posterior compartment of the legs. There is no clear correlation between the nature and the localization of mutations in the dysferlin gene and the clinical phenotype; indeed, the same mutations have been found in siblings presenting either as LGMD2B or MM (33,36). Dysferlin is widely expressed, but is particularly abundant in striated muscle (33,34). Dysferlin is expressed as early as Carnegie stage 15 or 16 (embryonic age 5 to 6 weeks) of human development, when the limbs start to give rise to different muscle groups (37). Its early developmental expression has spawned the hypothesis that the lack of dysferlin at this critical moment could compromise either the distal or proximal muscles, leading to a pathologic status manifesting years later. It is evident, however, that other genetic or environmental factors may also play a role in determining the distribution of affected muscles and the age of disease onset. This clinical phenotype may also be affected by the presence of similar ferlin proteins. Myoferlin, a protein homologous to dysferlin, shares dysferlin's tissue distribution (38). Thus, it is tempting to imagine that myoferlin compensates for the decrease of dysferlin in nonaffected muscles in LGMD2B and MM. Intriguingly, otoferlin, a product of a second dysferlin-homologous gene, is particularly abundant in the cochlea and has recently been associated with a nonsyndromic form of deafness (39). Taken together, these data suggest that there may exist a family of ferlins in mammals. The lack of any ferlin would lead to disease in the tissue where this specific ferlin is more abundantly expressed when a genetic or environmental factor is interposed and the other ferlins cannot compensate.

The dysferlin primary sequence predicts a homology with the *Caenorhabditis elegans* fer-1 factor, a spermatozoa vesicle–specific protein involved in membrane fusion. The presence of three full and two incomplete C2 domains, known for interacting with negatively charged phospholipids and proteins to trigger the fusion of vesicles to the plasma membrane, suggests a role for dysferlin in muscle membrane fusion. We recently showed that dysferlin is reduced at the sarcolemma and accumulates in the cytoplasm, likely in vesicles, in LGMD2B and MM patients. Although we demonstrated that dysferlin is not an integral component of the DGC, we found similar sarcolemmal reduction and cytoplasmic accumulation of dysferlin in patients with primary disruption of the

DGC (Duchenne/Becker muscular dystrophy and sarcoglycanopathies). Taking these data together, we hypothesized a new model to explain the molecular mechanisms underlying this group of dystrophies. In LGMD2B and Miyoshi myopathy, mutated forms of dysferlin could interfere with the normal fusion of membrane vesicles to the sarcolemma, leading to their accumulation in the cytoplasm. In patients with primary DGC abnormalities, the lack of DGC could jeopardize vesicle-sarcolemma fusion even though dysferlin is properly present in vesicles (40).

LGMD2B muscle biopsies do not display evidence of developmental abnormalities, and patients often report good physical activity until the onset of the disease (41). Additionally, the SJL mouse, a spontaneous animal model of dysferlinopathy, has an increased muscle regeneration capacity (42). Thus, dysferlin does not seem essential for growth or regeneration processes; rather, dysferlin is more likely involved in a membrane repair activity, exacerbated in fibers damaged by extensive use.

LGMD2C to LGMD2F, or Sarcoglycanopathies (α-SG locus at 17q12, β-SG at 4q12, γ-SG at 13q12, δ-SG at 5q33)

The characterization of the sarcoglycans (SGs) derives directly from the purification of dystrophin and the consequent identification of the several components of the dystrophin glycoprotein complex. Several biochemical and genetic experiments provide evidence that the DGC is organized in three subcomplexes: a cytoplasmic complex composed of dystrophin, syntrophins, and dystrobrevins; a sarcolemma-associated dystroglycan (DG) composed of α- and β-DG; and a transmembrane sarcoglycan–sarcospan complex. In skeletal muscle the α-DG links directly to laminin and thus to the extracellular matrix, whereas β-DG associates with the cytoplasmic COOH end of dystrophin, which in turn links to cytoskeletal F-actin at its N-terminus (Fig. 17.1).

Dystrophin mutations are responsible for Duchenne and Becker muscular dystrophy (DMD and BMD, respectively), but no examples of human DG mutations have been reported. Engineered DG-null mice showed their early embryonic lethality (43) and provided a ready explanation for the absence of human examples. Understandably, there are no data available about the direct impact of DG loss in human muscle; however, engineered chimeric mice characterized by the absence of DG in their skeletal muscle display signs of muscular dystrophy (44).

A role for sarcospan in human (45) and mouse (46) muscle pathology is still unclear. In contrast, mutations found in each of the sarcoglycan genes result in a form of LGMD2. Collectively, these have been termed *sarcoglycanopathies* and are classified individually as LGMD2D (or α-sarcoglycanopathy), LGMD2E (or β-sarcoglycanopathy), LGMD2C (or γ-sarcoglycanopathy), and LGMD2F (or δ-sarcoglycanopathy) (Table 17.1). In human skeletal muscle biopsies, a primary defect in one of the sarcoglycans leads to a variable degree of secondary reduction or absence of the other sarcoglycans and sarcospan from the sarcolemma. Attempts have been made to understand how the sarcoglycans organize with respect to each other in forming a complex. For instance, the assembly and trafficking of sarcoglycans has been reproduced in a heterologous cell system (47), showing that although the four wild type molecules are properly glycosylated, assembled, and translocated to the membrane as a single complex, the presence of a truncated sarcoglycan compromises the assembling of the whole complex. This leads to an intracellular retention of the single glycosylated units. This all-or-nothing situation, in which the absence of one sarcoglycan leads to the complete absence of the others, is only sometimes found in human muscles. In very general terms, severe secondary sarcoglycan deficiencies are most often seen in patients with β- or δ-SG mutations, less often seen in patients with α-SG mutations, and rarely in γ-sarcoglycanopathies (48). These immunohistologic patterns could vary depending on the antibodies used for the analysis. Indeed, it has been shown that a patient apparently lacking γ-SG from the sarcolemma had, in reality, a truncated γ-SG at the sarcolemma, revealed by an N-terminal antibody when the sarcolemma was tested with two antibodies directed

against the C-terminus of the protein (45). Based on the residual staining of the four sarcoglycans in muscle biopsies of patients affected with sarcoglycanopathy, Vainzof and colleagues (48) concluded that α-, β-, and δ-SG seem to bind more tightly to each other, whereas γ-SG seems to be more directly associated with dystrophin. In contrast, Chan and co-workers (49) conducted some experiments using immunoprecipitation and *in vitro* cross-linking in C2C12 murine skeletal muscle cell line and concluded that β- and δ-SG are tightly associated with each other and form disulfide bonds with γ-SG, whereas α-SG is the less tightly associated component of the complex.

The analysis of patients' biopsies and the study of animal models for the four sarcoglycanopathies have shed considerable light on the role of the sarcoglycan complex in LGMD. Human and animal muscles with defective sarcoglycans display clear dystrophic phenotypes, sharing features with DMD and BMD. Common characteristics are degeneration and regeneration of the fibers, fatty infiltration, fibrosis, and finally fiber calcification. These features and muscle uptake of Evans blue dye in injected animal models for dystrophin and α-SG deficit suggest that the disruption of the DGC compromises the sarcolemma integrity, regardless of the primary gene defect (50,51). Indeed, adenoviral gene transfer experiments in animal models for sarcoglycanopathies demonstrate that the DGC can be restored at the sarcolemma of transduced fibers, preventing the dystrophic process (52–55). Taken together, these data seem to support the mechanical theory in which the DGC transmits the local stress generated during contraction across the sarcolemma to the extracellular matrix and thus protects the fiber from contraction-induced damage.

However, the clinical evaluation of cardiac disease in a subset of sarcoglycanopathies has led to new insight into the role of the sarcoglycan complex. Dilated cardiomyopathy associated with a muscular dystrophy has been reported in some sarcoglycanopathies (56–59), but whether the cardiac involvement was due to the primary defect in sarcoglycan expression or to other genetic or environmental factors has been unclear for a long time. Dilated cardiomyopathy is now also recognized in Bio 14.6 hamsters (a δ-SG–deficient animal) (60), as well as mice that are null for β-, γ-, and δ-SG but not α-SG (51,53,61–64). Muscle fractionation experiments in α- and β-SG-null mice demonstrate the existence, at least in skeletal muscle, of a second sarcoglycan complex in which α-SG is substituted by its homologous ε-SG (53,61). It is hypothesized that the ε-SG–containing complex may directly compensate for the absence of α-SG and thus prevent the development of the cardiac involvement in α-SG–null mice and human LGMD2D patients.

An alternative fascinating theory has been recently proposed based on the observation of vascular constrictions in β- and δ-SG–null mice, attributed to the loss of the ε-SG–containing complex (53,64). Because α-SG is expressed exclusively in skeletal and cardiac muscle, the ε-SG–containing DGC is normal in the vascular muscle of α-SG-null mice. Accordingly, no vascular constriction has been found in α-SG–null mice. In contrast, the ε-SG complex is absent from the vascular muscle of β- and δ-SG–null mice. This leads to vascular constrictions causing ischemic injuries and large areas of necrosis and fibrosis in both striated muscles and myocardium that may already be compromised by the lack of both α- and ε-SG–containing complexes. In support of this theory, the administration of nicorandil, a vascular smooth muscle relaxant compound, prevented the development of myocardial lesions in treadmill-exercised δ-SG–null mice (64). Additionally, long-term treatment of β- and δ-SG–deficient mice with the vasodilator verapamil abolished vascular constrictions and prevented the development of severe cardiomyopathy (65). These results suggest a possible therapeutic and preventive approach for cardiomyopathies caused by disruption of the sarcoglycan complex.

LGMD2G, or Telethoninopathy (Locus at 17q12)

As already described for myotilin, defects in sarcomeric proteins can lead to LGMD. Telethonin is a 19-kD muscle-specific protein

that localizes at the Z-disk (66) (Fig. 17.1). In early-differentiated myocytes, telethonin is phosphorylated by the kinase domain of titin (67). Mutations found in patients affected by LGMD2G lead to truncated and supposedly inactive forms of telethonin (67,68).

CLINICAL IMPLICATIONS

LGMD encompasses a group of muscle diseases with a wide spectrum of clinical severity, even within specific types of LGMD. The phenotypic variability among unrelated patients is only partly correlated with the gene mutation or mutations, and unknown genetic or environmental factors are likely to play a role. Limb-girdle muscular dystrophies are inherited either as autosomal dominant (LDMD1) or autosomal recessive (LGMD2) diseases. This section introduces each of these major subsets of LGMD, describing common features, and then presents characteristics peculiar to each genetically defined LGMD, with the intention of offering some guidelines for differential diagnosis (Table 17.2).

Limb-Girdle Muscular Dystrophy Type 1

Features common to the autosomal dominant LGMD1 are a young-adult or adult onset, slow progression, rare wheelchair confinement, and nearly normal serum creatine kinase values. LGMD1 patients usually have a less severe phenotype than LGMD2 patients. Autosomal dominant forms of LGMD are less common than the recessive form, with only one or a few families reported for each type.

LGMD1A, or Myotilinopathy

LGMD1A has been described exclusively in a single large North-American family of German origin. A mutation in the myotilin gene (5q31) has been identified in the affected members of this family.

Individuals affected with LGMD1A show proximal lower and upper limb weakness, later progressing to include distal weakness. The mean age at onset is 27 years, although Speer and associates (69) reported an anticipation phenomenon and incomplete penetrance. Approximately half of the patients have a distinctive nasal, dysarthric pattern of speech. Achilles tendon shortening and hyporeflexia are frequently seen, although nerve conduction studies are normal. Creatine kinase (CK) levels range from twofold to ninefold higher than normal. A characteristic histologic feature of LGMD1A biopsies is the presence of a large number of rimmed autophagic vacuoles (similar vacuoles are sometimes observed also in recessive forms of LGMD, such as dysferlinopathies and

TABLE 17.2. *Clinical and histologic differential hallmarks of limb-girdle muscular dystrophies*

	Distinguishing features	
Disease	Clinical	Histologic
LGMD1A	Dysarthric speech	Rimmed vacuoles, rodlike bodies
LGMD1B	Heart conduction disturbance	—
LGMD1C	Onset in childhood	—
LGMD1D	Heart conduction disturbance	—
LGMD1E	—	—
LGMD2A	—	—
LGMD2B	May begin as MM; good physical performance before onset	Rimmed vacuoles; may have inflammation
LGMD2C–2F	Extremely elevated CK levels, maymimic DMD	—
LGMD2G	Distal muscles also affected	Rimmed vacuoles
LGMD2H	Mild phenotype	—
LGMD2I	—	—

LGMD, limb-girdle muscular dystrophy; MM, Miyoshi myopathy; CK, creatine kinase; DMD, Duchenne muscular dystrophy.

LGMD2G). Furthermore, electron microscopic analysis of LGMD1A muscle biopsy reveals extensive and severe Z-line streaming similar to the rodlike bodies observed in nemaline myopathies (2).

LGMD1B, or Laminopathy

LGMD1B has been described in three unrelated families originating from the Netherlands, Surinam, and the Caribbean; a mutation in the lamin A/C gene (1q11) has been found in each family.

Patients display symmetrical weakness of proximal leg muscles before the age of 20, slowly progressing to the arm muscles in the next decade or two. Serum CK activity is normal to slightly elevated. LGMD1B is allelic to autosomal dominant Emery-Dreifuss muscular dystrophy (EDMD-AD), a disease characterized by early contractures of the elbows and Achilles tendons, slowly progressive humeroperoneal muscle wasting and weakness, scapular winging, rigidity of the spine, and heart conduction defects (6). LGMD1B patients develop only mild or late contractures of the tendons, without spine rigidity, distinguishing them clinically from EDMD-AD. In about 60% of LGMD1B patients, cardiac rhythm disturbances or dilated cardiomyopathy follow the neuromuscular symptomatology and worsen with the progression of the disease. The rhythm disturbances may manifest as syncopal attacks and even sudden death. These patients may require a pacemaker (5).

LGMD1C, or Caveolinopathy

An extensive antibody-based screening revealed a severe reduction of caveolin-3 from the sarcolemma of patients from two unrelated families affected with a dominant form of LGMD. Mutation analysis in these patients and their families confirmed the primary involvement of caveolin-3 in this disease, named LGMD1C (locus in 3p25). The main clinical features of caveolin-3–deficient patients are mild to moderate proximal muscle weakness and calf hypertrophy (11). Although the age of onset of the disease is not clear for most of the reported patients, mutations in caveolin-3 gene have been recently found in two asymptomatic children with idiopathic hyperCKemia and a third child with muscle cramps (25,70). Thus, LGMD1C appears to have variable age of onset, and may manifest in childhood with mild symptoms.

LGMD1D

LGMD1D has been linked to a locus in 6q23 in a large, single, American family of French Canadian origin.

Patients present with mild proximal muscle weakness with facial sparing and creatine kinase levels at the upper limit of normal. Dilated cardiomyopathy and cardiac conduction disturbances leading to heart block and sudden death often manifest before the skeletal symptoms, in contrast to the observations in LGMD1B. Affected individuals develop symptoms of muscle disease between 20 and 25 years of age, but remain ambulatory throughout their lifetimes (71).

LGMD1E

LGMD1E has been associated with a locus on chromosome 7q32 in two American Caucasian families (72–74). Symptoms begin in young adulthood with proximal leg weakness and slow progression. Patients do not develop cardiac dysfunction or contractures.

LGMD1F, or Vocal Cord and Pharyngeal Distal Myopathy

Linkage analysis in a single large Caucasian family localized the vocal cord and pharyngeal distal myopathy (VCPDM) locus at 5q31 (75). Because the VCPDM genetic interval overlaps the LGMD1A gene (myotilin) and because VCPDM patients manifest a mild shoulder weakness, this disease is often included among LGMD and classified as LGMD1F. However, VCPDM is a distal myopathy, which begins with lower and upper distal extremity weakness and progresses to involve peroneal muscles

(75). Mutation analysis in VCPDM patients excluded myotilin as the VCPDM gene, indicating that LGMD1A and VCPDM are clinically and genetically distinct entities (2). For these reasons, we feel that the inclusion of VCPDM as one of the types of LGMD1 should be reconsidered, and we do not list this disease in Tables 17.1 and 17.2.

Limb-Girdle Muscular Dystrophy Type 2

Eight genetically distinct subtypes of LGMD2 have been determined worldwide, with regional variations in their relative frequency. It has been estimated that in the Caucasian population, calpainopathies account for 10% to 30% of the LGMD2 cases (75), and dysferlinopathies for 30% (77). Sarcoglycanopathies have been found in 6% to 10% of mild LGMD cases and in 22% of the LGMD with a severe, DMD-like course (78,79).

LGMD2A, or Calpainopathy

Calpainopathies have been described worldwide, and several founder effects have been reported in the Reunion Islands, among the American Amish groups, and in the European Basque country (summarized in reference 80). The calpain-3 gene localizes to 15q15.

The usual presentation of calpainopathy is onset of symmetric weakness of the limb-girdle and trunk muscles in the second decade. Patients show slow progression of weakness, and life expectancy in general is within the normal range (81). Early in the disease, manual testing and muscle imaging such as computed tomographic scanning show a selective atrophy and wasting of hip extensors and adductors. Patients consistently develop heelcord contractures, and calf pseudohypertrophy may be observed (82). At more advanced stages, the weakness extends to quadriceps and lower leg muscles. In the upper limbs, early weakness is limited to the serratus magnus, latissimus dorsi, rhomboid, and pectoral majoris muscles. Contractures around ankles, elbows, hips, and knees are usually mild, but rarely an Emery-Dreifuss–like appearance has been described. Facial muscles are spared, and neck muscle involvement is usually minimal. Cardiac function is generally normal, and a moderate lung vital capacity is found in the majority of the patients. Creatine kinase level is elevated to 10 to 100 times normal, but is usually lower than in the sarcoglycanopathies.

LGMD2B, or Dysferlinopathy

Mutations in the dysferlin gene (2p13) are found in many different populations (33–35), and founder effects have been reported among Libyan and Yemenite Jewish descendants (33,83).

Dysferlin mutations lead to either LGMD2B or Miyoshi myopathy (33,34). Patients with an LGMD2B pattern of involvement present with a hip-girdle weakness. Dysferlinopathy patients with an MM presentation have weakness and wasting primarily affecting the gastrocnemius muscles. Occasionally, patients have a mixed pattern of both proximal and distal lower extremity weakness. Within a single family both the LGMD2B and MM phenotypes may be seen, indicating that the nature of the dysferlin mutation is not the only determinant of clinical phenotype (41,84,85).

Dysferlinopathy usually manifests in the second or third decade of life (there are no reports before age 10 years), and most of the patients walk into their mid-30s or beyond. Patients often describe good motor development and function before the onset of the disease, with normal physical activity at school, in athletic performances, and in military service (41,81). Regardless of the pattern of weakness at onset (which can also be asymmetrical), as the disease progresses most of the leg muscles become equally involved and weakness in the upper extremities develops. Arm weakness predominantly involves the biceps, while shoulder girdle musculature is relatively spared and scapular winging is not observed. Calf hypertrophy is rare among dysferlinopathies, although it sometimes is described as a transient swelling accompanied by pain (41), occasionally present only in one leg (82). Dysferlinopathy patients usually do not develop cardiomyopathy, con-

tractures, respiratory failure, facial weakness, or cognitive impairment. Creatine kinase level may be normal to mildly raised in presymptomatic children and increase abruptly at the onset of clinical disease (41).

Muscle biopsies of LGMD2B and MM patients sometimes show a mild to moderate degree of lymphocytic inflammation (83,84), which can be perivascular or more widespread. Indeed, a common misdiagnosis for LGMD2B is polymyositis. Short-term steroid treatment of three patients did not seem to improve their clinical status (41). The presence of rimmed vacuoles, well recognized in MM biopsies (86), may also lead to the misdiagnosis of inclusion body myositis.

LGMD2C to LGMD2F, or Sarcoglycanopathies

LGMD2D, caused by mutations in the α-SG gene (17q12), is widespread, although most frequently found in Caucasian populations. A few recurrent mutations, in particular R77C (which accounts for roughly 50% of new mutations in this gene), simplify mutation screening (87,88). LGMD2E, or β-sarcoglycanopathy (4q12), is also frequent among Caucasian populations, and founder effects have been reported among the Amish communities (89,90). An 8-bp duplication in the β-SG gene and 1-bp insertion in the γ-SG gene have been recurrently found in families from North Italy (91). LGMD2C, or γ-sarcoglycanopathy (13q12), is particularly common around the Mediterranean basin, where the delT508 founder effect mutation is often present (92). The C283Y missense mutation in γ-SG is found frequently in LGMD2 patients of Gypsy descent (93–95). LGMD2F, or δ-sarcoglycanopathy (5q33), appears to be the least common sarcoglycanopathy and has been reported in five Brazilian (82), one Turkish (96), and one Italian (97) family.

There are no clear clinical observations that distinguish among the four forms of sarcoglycanopathy, although the mildest forms are more often associated with mutations in α-sarcoglycan (81). Sarcoglycanopathies generally manifest earlier in life than the other forms of LGMD. An early age of onset does not necessarily predict a severe phenotype, and the rate of disease progression is extremely variable, even among siblings. In the most severe forms, patients present with weakness between 3 and 5 years old and progress to loss of ambulation before the third decade, with shortened life expectancy. In the milder forms, onset may be in the first, second, or even third decade, and longevity may be reduced or nearly normal. While the earliest reported age of wheelchair confinement is 10 years, the variability toward the mild forms is very marked, and few patients are still ambulant or presymptomatic beyond their 40s (81). Respiratory muscle involvement can be observed in all forms of sarcoglycanopathy (98), and death is frequently related to respiratory failure.

In sarcoglycanopathies, muscle weakness is initially detectable in the pelvic girdle and later in the shoulder with scapular winging. Calf hypertrophy is common, and macroglossia has also been observed (99,100). The disease usually progresses to a diffuse wasting of trunk and distal muscles. The clinical presentation of sarcoglycanopathy is sometimes very similar to that of dystrophinopathy. The pattern of muscle involvement may help to differentiate sarcoglycanopathy from DMD. Shoulder girdle and scapular involvement is usually more prominent in sarcoglycanopathy. Hamstrings are affected earlier in sarcoglycanopathy, whereas quadriceps are affected earlier in DMD. Lordosis and scoliosis seem less prominent and less severe in sarcoglycanopathy than in DMD. Mental retardation has not been observed in LGMD2 (81). Serum values of CK are extremely elevated in sarcoglycanopathy (10 to 100 times), usually higher than in other LGMD forms at a comparable stage of the disease. Although manifest cardiomyopathy seems to be extremely rare among α-sarcoglycanopathies, it is sometimes present in patients with β-, γ- and δ-sarcoglycanopathy. Subclinical cardiac involvement has been observed by electrocardiogram and echocardiogram in about 40% of sarcoglycanopathies (98); therefore, cardiac monitoring is important for these patients. Autosomal dominant forms of dilated cardiomy-

opathy with sparing of skeletal muscle have also been reported to have mutations in the δ-SG gene (101).

LGMD2G, or Telethoninopathy

This form of LGMD has been observed in four Brazilian families and localized to 17q12 (67,68,102).

LGMD2G clinically resembles Kugelberg-Welander disease. It presents as an atrophic myopathy affecting proximal muscles of the upper and lower limbs, along with distal leg muscles. The tibialis anterior is affected before the gastrocnemius (an order of muscle involvement opposite to dysferlinopathies), resulting in foot drop. The disease onset is in childhood, with noticeable progression in the late teens or early 20s. Patients become wheelchair bound in their 30s or 40s. No contractures have been observed, but the cardiac involvement is present in about half of the patients. The serum CK level is mildly increased (3 to 30 times normal) at the onset of symptoms and then decreases with the progression of the myopathy. A characteristic feature of LGMD2G muscle biopsies is the presence of rimmed vacuoles.

LGMD2H

LGMD2H (9q31) seems to be limited to Hutterites living near Manitoba, Canada (81,103).

This relatively mild myopathy manifests in the mid-20s with proximal weakness, back or neck pain, fatigue, difficulty on climbing stairs, waddling gait, and wasting of shoulder girdles. The progression is slow, and patients lose mobility in their 40s. Creatine kinase level is mildly increased in symptomatic patients, but can also be increased up to 15-fold in asymptomatic or presymptomatic members of the family.

LGMD2I

LGMD2I has been identified in a consanguineous Tunisian family, with a markedly variable clinical course among the siblings (104). Linkage analysis pointed to an unknown gene at 19q13. No particular clinical features distinguish LGMD2I from other forms of LGMD, and patients show a variably progressive weakness and wasting of proximal muscles.

Diagnostic Strategy

Although great progress has been made in defining the genetic bases of LGMD, achieving a diagnosis in an individual patient may remain complex, requiring the integration of clinical and histopathologic observation, protein analysis, and genetic studies. Nevertheless, a specific diagnosis is desirable to allow accurate genetic counseling, optimal clinical monitoring and intervention, and, in the future, a specific therapy.

Because LGMD2 overlaps phenotypically with DMD and BMD, a dystrophinopathy should be ruled out in all cases of suspected LGMD, regardless of the sex of the patient. Indeed, a biochemical analysis of 41 biopsies from LGMD patients showed that 31% of male and 13% of female patients had a dystrophinopathy (105). Results were confirmed by mutation identification in the men. Thus, 17% of LGMD patients in this study had a dystrophinopathy. Genomic DNA analysis of DMD and BMD reveals a large deletion or duplication in over two-thirds of the cases and may be used as an initial screen in LGMD patients. For the remaining one-third, point-mutation screening is possible in some research laboratories but is not routinely applied. If no deletion or duplication is detected, dystrophin analysis should be performed on a muscle biopsy by either western blot or immunohistochemistry. By immunohistochemistry, a male with DMD generally would show absence or deficiency of dystrophin and upregulated expression of utrophin, a protein homologous to dystrophin normally confined to the neuromuscular junction in skeletal muscle. BMD may show normal or near normal dystrophin staining, but display a dystrophin of abnormal size by western blot analysis. Female carriers show a mosaic distribution of positive and negative fibers. LGMD patients usually display a normal dystrophin pattern (Fig. 17.2).

Gene-based diagnosis for LGMD is not clinically feasible because phenotypes overlap extensively, particularly for the type 2 limb-girdle

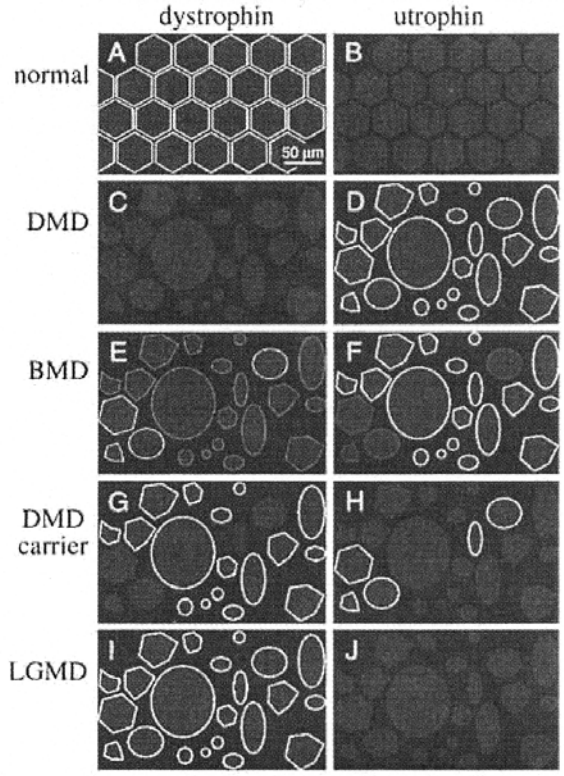

FIG. 17.2. Immunohistochemical patterns of dystrophin and utrophin in Duchenne and Becker muscular dystrophy (DMD and BMD, respectively), Duchenne carrier, and limb-girdle muscular dystrophy (LGMD) skeletal muscle biopsies. Normal adult skeletal muscle expresses dystrophin, but not utrophin at the sarcolemma (**A** and **B**). In DMD, dystrophin is absent and utrophin expression is induced (**C** and **D**). In BMD, dystrophin may be near normal or normal in some fibers and reduced in others (**E** and **F**); utrophin is overexpressed proportionally to the decrease of dystrophin. DMD carriers show a mosaic pattern of dystrophin and utrophin expression (**G** and **H**). LGMD patients have a normal expression of dystrophin and utrophin (**I** and **J**).

muscle dystropies. Mutation screening for the known LGMD genes is possible, but primarily as a research tool, because mutations in each gene are extremely heterogeneous, with very few hot spots outside of rare founder mutations limited to well-defined populations. Thus, DNA-based analysis for LGMD is a very expensive and time-consuming approach.

A strategy to narrow the candidate gene to one or a few of the LGMD genes is thus required. As mentioned, founder-effect mutations have been observed in some populations, such as Amish, Gypsy, Mediterranean, Hutterite, Jewish, and Basque. Therefore, information concerning the ethnic background of the patient may suggest a particular LGMD gene and mutation. For patients outside these populations, a biopsy-based strategy is utilized for diagnosis and possible direct mutation screening. Histopathologic examination alone can suggest the degree of severity of the disease by observation of fiber size variation, internally placed nuclei, fatty infiltration, fibrosis, necrosis, and regeneration, but none of these features is, by itself, predictive of the type of LGMD. A few histologic hallmarks, such as rimmed vacuoles, inclusion bodies, or inflammation, are suggestive of specific subgroups of LGMD (Table 17.2); however, none of these features (by themselves) is diagnostic.

A much more powerful means of reaching a diagnosis or suggesting candidate genes for mutation screening is the use of immunohistochemistry or western blotting. Antibodies directed against most of the proteins involved in LGMD exist, and many are commercially available. LGMD muscle biopsies are generally evaluated by immunohistochemical analysis under the assumption that the absence or deficiency of a protein is indicative of a mutation or mutations in the corresponding gene. This approach was described previously for DMD and BMD, where absence of dystrophin by immunohistochemical analysis indicates a mutation in the dystrophin gene (Fig. 17.2). Analogously, a deficit in sarcoglycans is indicative of mutations in one of the four sarcoglycan genes (Fig. 17.3). However, the sarcoglycans compose a tightly associated complex, with the loss of one protein resulting in secondary deficiencies of the other three. Thus, a prediction of which sarcoglycan gene carries the mutation or mutations frequently is not possible. In some cases, the preferential decrease or absence of one of the sarcoglycans can indicate which gene is most likely mutated (see the example of LGMD2C in Fig. 17.3). In the case of an equal reduction of the four sarcoglycans and without a family history suggesting a founder mutation, the specific mutations are most likely in the

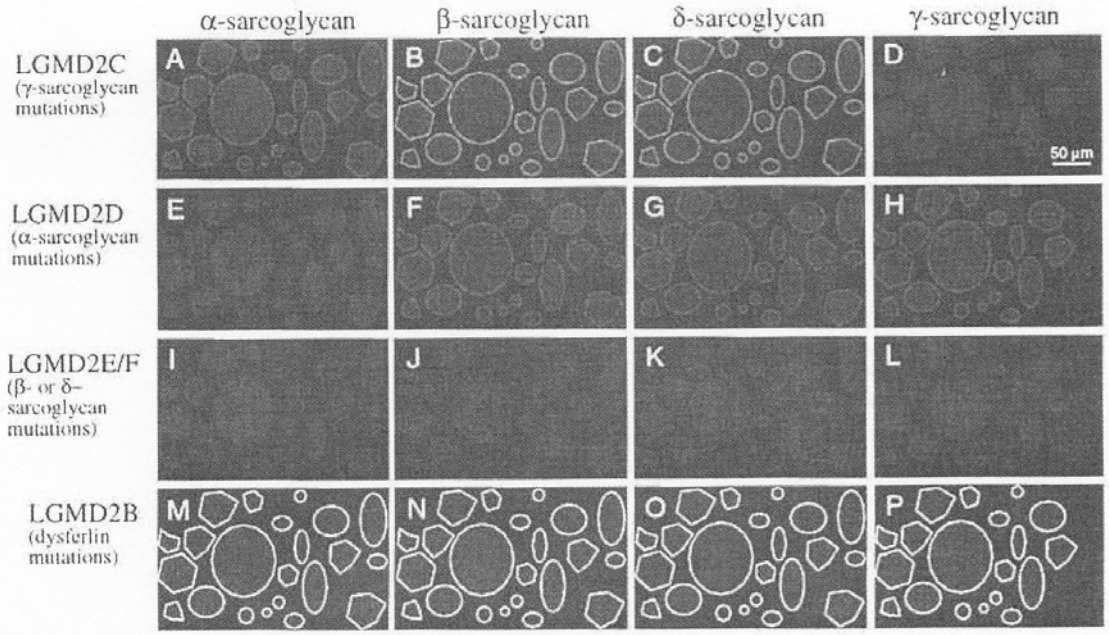

FIG. 17.3. Immunohistochemical patterns of sarcoglycan expression in skeletal muscle biopsies from patients with sarcoglycanopathy (LGMD2C to LGMDC2F) and dysferlinopathy (LGMD2B). Sarcoglycanopathy biopsies have a deficiency or absence of one or more sarcoglycans. Although there is variation from patient to patient, the common expression patterns are illustrated here. Typical LGMD2C patients have no γ-sarcoglycan, variably reduced α-sarcoglycan, and nearly normal β- and δ-sarcoglycan (**A** to **D**). LGMD2D patients are more heterogeneous, but usually have the greatest deficiency in α-sarcoglycan, with the remaining sarcoglycan expression ranging from absent to nearly normal (**E** to **H**). Both LGMD2E and 2F usually have no or nearly no sarcoglycan expression (**I** to **L**). In dysferlinopathy, sarcoglycan expression is normal (**M** to **P**).

α- or β-SG genes, since they are the most frequently mutated among the four. Protein-based screening is extremely useful (for a review, see reference 106) but has its limitations. Indeed, a patient with mutation in γ-SG and normal expression of SGs has recently been found (45). Dysferlin immunohistochemistry must be interpreted cautiously because a patchy or reduced sarcolemmal distribution can be seen in as many as half of sarcoglycanopathies (Fig. 17.4) and in some inflammatory myopathies (40). Secondary abnormalities in proteins such as caveolin-3 have been observed but are less well characterized. Calpain-3 immunohistochemistry is unreliable. To obviate some of these interpretative difficulties, some have advocated multiplex western blot analysis (107). This approach has proved to be especially useful in calpainopathies, but even this protein-based screening has to be interpreted cautiously in the case of decreased calpain. Indeed, while sarcoglycans are normally expressed in calpainopathies and dysferlinopathies, calpain-3 is reduced by western blot analysis in half of dysferlinopathies (108).

Therapeutic Approaches

The management of patients with LGMD consists of supportive care and treatment of complications of the disease, as with other neuromuscular conditions. In the absence of a specific diagnosis that excludes cardiac involvement, LGMD patients should have routine monitoring of cardiac function.

No large pharmacological trials have been conducted for LGMD patients or for any of the several natural or engineered animal models existing for LGMD2. A few scattered clinical experiences report that administration of corticosteroids (prednisone 0.4–0.75 mg/kg daily or in an on/off regimen) to patients affected with

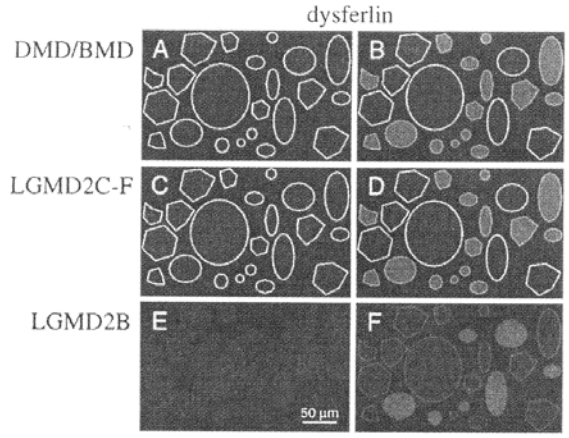

FIG. 17.4. Immunohistochemical pattern of dysferlin in Duchenne and Becker muscular dystrophy (DMD and BMD), sarcoglycanopathies (LGMD2C–2F), and dysferlinopathy (LGMD2B) skeletal muscle biopsies. Dysferlin may be normally expressed in DMD, BMD, and sarcoglycanopathies (**A** and **C**) or variably decreased at the sarcolemma with accumulation in the cytoplasm (**B** and **D**). Dysferlinopathy may have a complete absence of dysferlin (**E**) or a partial decrease in sarcolemmal expression accompanied by cytoplasmic accumulation (**F**).

α- or γ-sarcoglycanopathy resulted in an improvement in muscle pain and muscle strength (97,109,110). The timing and duration of response to corticosteroid treatment for sarcoglycanopathies seems to be similar to that observed for DMD (109,110). Nevertheless, because of the individual variability in the natural course of the disease and the lack of data from a large study, the benefits and risks of steroid treatment for LGMD have to be assessed case by case.

FUTURE DIRECTIONS

For a long time LGMD was considered a diagnosis of exclusion for patients with variably severe proximal muscle disease, particularly for those in whom a dystrophinopathy was ruled out. The recent understanding of the genetic basis of these diseases and the integration of molecular information with clinical data have resulted in a clearer definition of this class of muscular pathologies, with immediate benefits for the patients and their families in terms of diagnosis, prognosis, and genetic counseling.

At present, a main goal is to advance our comprehension of the molecular mechanisms underlying the pathophysiology of LGMD; for this purpose, significant efforts are being made in the generation of animal models mimicking the human disease. Considerable information has already come from the models of sarcoglycanopathy. In particular, data concerning the development of cardiomyopathy in SG-null mice, especially after exercise, suggest that it would be prudent to closely follow the cardiac function of sarcoglycan-deficient patients for whom preclinical signs of heart involvement (59,97,111) or coronary dysfunction (112) have been assessed in the past.

Logically, a major future direction is toward the development of therapies for these patients.

Pharmacological Treatment

The experience accumulated for the treatment of boys with DMD in some ways suggests the avenues of research for LGMD. In this regard, attempts made to locally ameliorate muscle inflammation or sarcolemmal instability by the administration of steroids are not surprising (see the previous section). Similarly, a lot of emphasis was put on the possible treatment of DMD and LGMD after a study showed that aminoglycoside antibiotics suppress the nonsense mutation that causes the truncation of dystrophin in *mdx* mice (113). There should be cautious optimism because more investigations are needed to assess the extent of side effects and therapeutic levels of aminoglycosides, and it must be kept in mind that very few of the known mutations underlying LGMD are nonsense. Other pharmaceutical compounds that may benefit a broader range of LGMD patients must be envisaged.

Most of the other ongoing efforts at therapeutic intervention concentrate on two directions: the replenishment of muscle fibers and gene transfer.

Myoblast Transfer

The ability of myoblasts to incorporate into postnatal skeletal muscle during regeneration has been exploited for myoblast transplantation approaches (reviewed in references 114 and

115). This technique involves delivery of exogenous myoblasts, which contribute to the formation of new muscle fibers during repair and regeneration and therefore result in genetic modification of the host muscle. Clinical trials in children with DMD were met with a lot of enthusiasm, but the results obtained have raised open debates (115). For instance, the low rate of survival of transplanted myoblasts (116), the immunorejection of grafted cells, and the immunoresponse against the therapeutic protein (117) represent some of the major difficulties to overcome in order to successfully achieve myoblast transplantation.

Stem Cell Delivery

Another, more promising avenue of research for delivering new cells to dystrophic muscle is based on the pluripotentiality of stem cells. Recent experiments demonstrate that bone marrow transplantation in *mdx* mice is a source of stem cells that can incorporate into the affected muscle and partially restore dystrophin expression (118). These preliminary results suggest that supplying stem cell populations to the muscle might be a way to regenerate normal muscle fibers.

Gene Transfer

The gene-transfer approach for muscular dystrophies such as Duchenne or sarcoglycanopathies is based on the simple concept that the replacement of the missing protein will restore the sarcolemma integrity and thus protect the muscle from the contraction-induced injuries. Several viral-mediated gene-transfer tests have been performed in animal models for sarcoglycanopathy (for a review see reference 119). The first attempts at gene transfer were made with adenoviral vectors because they can accommodate large amounts of exogenous DNA. Although promising results were obtained, the host immunoresponse to the viral proteins and the inability of the adenovirus to infect mature myofibers pointed to the need for a better vector. New generations of adenovirus have been engineered and are being currently studied. In parallel, adeno-associated viruses have been tested because of their low immunogenicity, possibility of genome integration, and ability to transduce mature myofibers. Unfortunately, the transduction efficiency of adeno-associated virus in muscle is relatively very low.

In summary, the success of the gene-transfer approach lies in the development of better-performing vectors; several groups are working toward this goal. Nevertheless, the preliminary satisfactory attempts at gene transfer in murine models and in nonhuman primates indicate that similar results may be obtainable in human patients (120).

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