Muscular dystrophies and the dystrophin–glycoprotein complex

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Efforts to understand the function of dystrophin, the protein product for the Duchenne muscular dystrophy gene, resulted in the purification of the dystrophin–glycoprotein complex. Over the past year several novel components of this complex have been identified. Recent studies have extended the number of muscular dystrophies associated with the oligomeric complex to six genetically distinct diseases, including three new forms of limb–girdle muscular dystrophy and one form of congenital muscular dystrophy.

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Abbreviations

BMD Becker muscular dystrophy
CMD congenital muscular dystrophy
DGC dystrophin–glycoprotein complex
DMD Duchenne muscular dystrophy
LGMD Limb–girdle muscular dystrophy

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Introduction

The term muscular dystrophy covers a diverse group of inherited disorders characterized by progressive muscle weakness and wasting in which the primary defect becomes symptomatic in skeletal muscle. Recent studies have demonstrated that there is marked clinical similarity among patients with genetically different diseases, suggesting that the gene products may participate in a common functional structure or biochemical cascade.

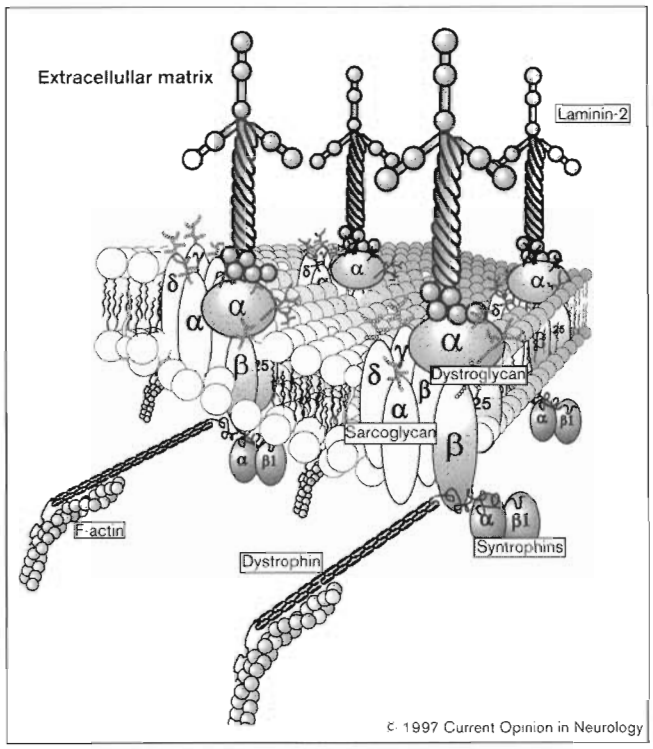
Accumulated patient data indicate that the clinical spectrum of the limb–girdle muscular dystrophies (LGMDs) ranges from severe childhood-onset phenotypes to late-onset cases with moderate weakness in adulthood. This marked heterogeneity in disease severity resembles the clinical spectrum found in patients with Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD). Recent studies also showed that variability of clinical severity and age of onset is found in patients with congenital muscular dystrophies (CMDs) [1••].

The most prevalent and best characterized form of muscular dystrophy, DMD, is caused by mutations in the dystrophin gene on chromosome Xp21 [2]. The isolation of a complex of proteins and glycoproteins associated with dystrophin [3] provided the molecular basis to study a new group of potential candidate proteins involved in muscular dystrophy. In striated muscle cells the dystrophin–glycoprotein complex (DGC) serves as a link between the extracellular matrix and the subsarcolemmal cytoskeleton [4] (Fig. 1). So far studies have shown that at least six proteins with primary genetic defects in muscular dystrophies are affiliated with the DGC (Figs 2–4) [5,6••–9••,10•]. This review focuses on the recent findings about muscular dystrophies caused by mutations in the DGC components.

Dystrophin–glycoprotein complex

In the sarcolemmal DGC (Fig. 1), dystrophin serves as the cytoskeletal linker between the actin-cytoskeleton and dystroglycan, a transmembrane component consisting of α- and β-subunits (156 and 43 kDa) [11•]. Recently direct interaction between dystrophin and β-dystroglycan has been demonstrated by an in-vitro binding study [12••] and the interaction sites were narrowed down to the C-terminal 15 amino acids of β-dystroglycan and a large C-terminal region of dystrophin, encompassing the second half of hinge 4 and the cysteine-rich domain. The

Figure 1. **The molecular organization of the dystrophin–glycoprotein complex at the sarcolemma in normal human skeletal muscle**



heterotrimeric basement membrane protein laminin-2 is the extracellular ligand for α-dystroglycan at the sarcolemma [11•]. A subcomplex of four single transmembrane glycoproteins, called the sarcoglycan complex, is closely associated with the DGC. It consists of the 50 kDa α-sarcoglycan (adhalin), and the recently characterized β-, γ-, and δ-sarcoglycan components [5,6••–8••,13•,14••]. Identifying the different proteins was complicated by the fact that β-sarcoglycan (43 kDa) has the same molecular weight as β-dystroglycan, and both γ- and δ-sarcoglycan are 35 kDa proteins with high homology. Syntrophins have been reported to directly bind to the dystrophin C-terminus [15] and a hydrophobic 25 kDa protein is also tightly associated with the DGC [4]. The structure of the complex, which is best described in skeletal muscle, shows tissue-specific variability and even compositional differences within a single cell type.

There are a growing number of proteins reported to be associated with the DGC, including the adapter protein Grb2, caveolin, and the phosphoglucomutase-related protein [16–19]. However, at present the exact association of these proteins with the DGC and their role in muscular dystrophy is unclear. It has also been shown [20,21•,22] that the subsarcolemmal localization of neuronal nitric oxide synthase is mediated by its interaction with dystrophin and syntrophin. However, whether the displacement of the enzyme from the muscle fiber membrane to the cytosol in DMD muscle contributes to disease progression via an altered free radical metabolism is not understood.

Human and murine homologs of the 87 kDa *Torpedo* electric organ derived post-synaptic protein have now been characterized at the neuromuscular junction and at the sarcolemma [23•,24•]. Several dystrobrevins, as multiple isoforms of this protein are collectively referred to, are closely associated with syntrophins and dystrophin and might be components of the DGC or a similar complex with utrophin [23•–25•,26,27]. The involvement of the dystrobrevin gene on chromosome 18q12 [28] and its products in muscular dystrophies remains to be elucidated.

Dystrophin: structure and function

Regions of functional importance in the dystrophin molecule have been studied for several years by correlating mutations in its gene with clinical and biochemical patient data [29–32]. Although the precise function of dystrophin and its many isoforms is not yet defined, such studies coupled with other biochemical findings [12••,15] suggested a critical role for the C-terminus of dystrophin in muscle fiber viability. However, in many patients with mutations in the dystrophin gene (Fig. 2), expression levels of the mutated protein are too low to perform proper structure–function studies. The significance of the C-terminal domain of dystrophin for maintenance of DGC integrity was demonstrated [33••] by structure–function correlation in transgenic *mdx* mice expressing dystrophin constructs bearing deletions in its C-terminus. In these animals expression of the transgene often results in wild-type levels and therefore the phenotype is not caused by reduced levels of a mutated protein but by the specific mutation. Transgenic mice with deletions in the cysteine-rich domain of dystrophin showed severe muscle fiber degeneration, reduced levels of β-dystroglycan and the sarcoglycan complex, but an unaffected expression and localization of syntrophin. These data suggested that the cysteine-rich domain is most critical for the dystrophin–dystroglycan interaction and thereby for the preservation of the entire DGC in the sarcolemma.

Patients with mutations in the NH_2-terminal actin-binding or the rod domain of dystrophin have also been reported with severe and early onset phenotypes. It was proposed that their phenotype might be a result of the low expression level of an otherwise functional molecule [34,35]. Studies with transgenic *mdx* mice expressing dystrophin deleted for the actin-binding sites encoded by exons 3–7 support this concept [36•]. Expression of the truncated protein at or above normal dystrophin levels resulted in a mild phenotype with relatively few of the

dystrophic features found in *mdx* mice. Interestingly, the uniformity of protein expression, derived from a dystrophin mini-gene lacking exons 17–48, was more important than the overall level of its expression [37]. The studies with transgenic animals address important issues which must be considered when designing gene therapy protocols for muscular dystrophy patients.

The relatively moderate functional deficits in transgenic *mdx* mice deleted for the NH_2-terminal actin binding sites might be caused by the fact that dystrophin contains multiple actin-binding sites and can still attach to the actin cytoskeleton. Recent studies on the interaction of dystrophin with F-actin identified a novel F-actin binding site near the middle of the dystrophin rod domain [38••]. Because several experiments [38••] demonstrated that dystrophin in the context of the DGC was unable to induce cross-linking of actin filaments, a model was proposed (Fig. 1) in which dystrophin binds F-actin in a manner analogous to that of actin side-binding proteins. Taken together, these results indicate that cytoskeletal attachment of dystrophin might not just be a function of the NH_2-terminal domain, but rather performed in concert between the NH_2-terminal domain and the rod-domains. Transgenic *mdx* mice expressing normal levels of a C-terminal 71 kDa nonmuscle isoform of dystrophin (Dp71) lack both of those actin-binding domains but are sufficient to restore the DGC localization to the sarcolemma. However, these animals still have a dystrophic phenotype [39,40]. The absence of all actin binding sites on the Dp71 molecule might result in the failure of this molecule to rescue the dystrophic *mdx* phenotype.

Limb–girdle muscular dystrophies

Most of the recent progress in unraveling the complexity of muscular dystrophies was achieved in the heterogeneous group of autosomal recessive LGMDs. The existence of this group as a separate pathophysiological entity had been questioned for a long time because of the overlap of symptomatology with BMD patients, manifesting carriers of dystrophin mutations, and patients with spinal muscular atrophy, mitochondrial and metabolic myopathies. However, mutations in five different genes have been shown to cause autosomal recessive LGMD [5,6••–9••,41], and gene loci and products in autosomal dominant disorders have been determined as well (Table 1).

When mutations in the α-sarcoglycan gene on chromosome 17q were first shown to be responsible for patients with LGMD2D [5], defects in β- and γ-sarcoglycan, transmembrane proteins of 43 and 35 kDa, became likely candidates in other LGMDs. This possibility seemed even more likely because all three proteins were reduced in the sarcolemma of LGMD2D patients (Fig. 2). However, surprise came in the characterization of LGMD2A which maps to chromosome 15q15.1-q21.1 in families from La Réunion, the Amish of northern Indiana, and Brazil. It is caused by mutations in a gene encoding an enzyme, calpain 3, not a structural protein [41]. Additionally

Table 1. Muscular dystrophies and gene locations

Disease	Mode of inheritance	Gene locus	Gene product
Duchenne/Becker muscular dystrophy	XR	Xp21	dystrophin
Emery–Dreifuss muscular dystrophy	XR	Xq28	emerin
Limb–girdle muscular dystrophy (LGMD)			
Bethlem myopathy	AD	21q22	α1(VI) collagen
Bethlem myopathy	AD	21q22	α2(VI) collagen
Bethlem myopathy	AD	2q37	(α3(VI) collagen)
LGMD1A	AD	5q22	?
LGMD2A	AR	15q15	calpain 3
LGMD2B	AR	2p13	?
LGMD2C*	AR	13q12	γ-sarcoglycan
LGMD2D*	AR	17q12	α-sarcoglycan
LGMD2E*	AR	4q12	β-sarcoglycan
LGMD2F*	AR	5q31	δ-sarcoglycan
Distal muscular dystrophy			?
Distal myopathy	AD	14	
Miyoshi myopathy	AR	2p13	
Congenital muscular dystrophy (CMD)			
'Classical' or 'pure' CMD*	AR	6q22	laminin α2 chain
Fukuyama-type CMD	AR	9q31	?
Facioscapulohumeral muscular dystrophy	AD	4q35	?
Scapuloperoneal muscular dystrophy	AD	12q21	?
Oculopharyngeal muscular dystrophy	AD	14q11	?
Muscular dystrophy with epidermolysis bullosa	AR	8q24	plectin

This table only includes disorders which have been mapped to a chromosome. AD, autosomal dominant; AR, autosomal recessive; XR, X-linked recessive.

Figure 2. **The molecular organization of the dystrophin–glycoprotein complex at the sarcolemma in Duchenne muscular dystrophy**

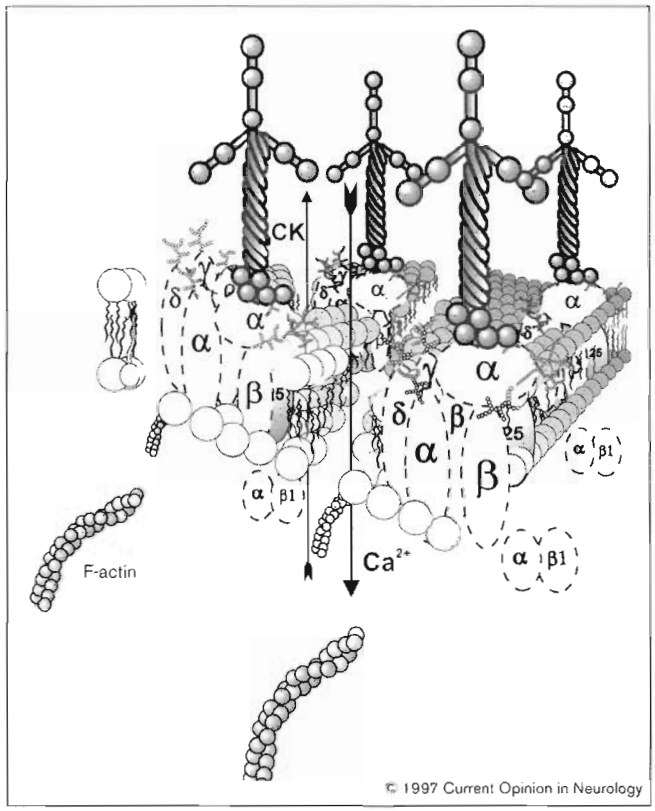
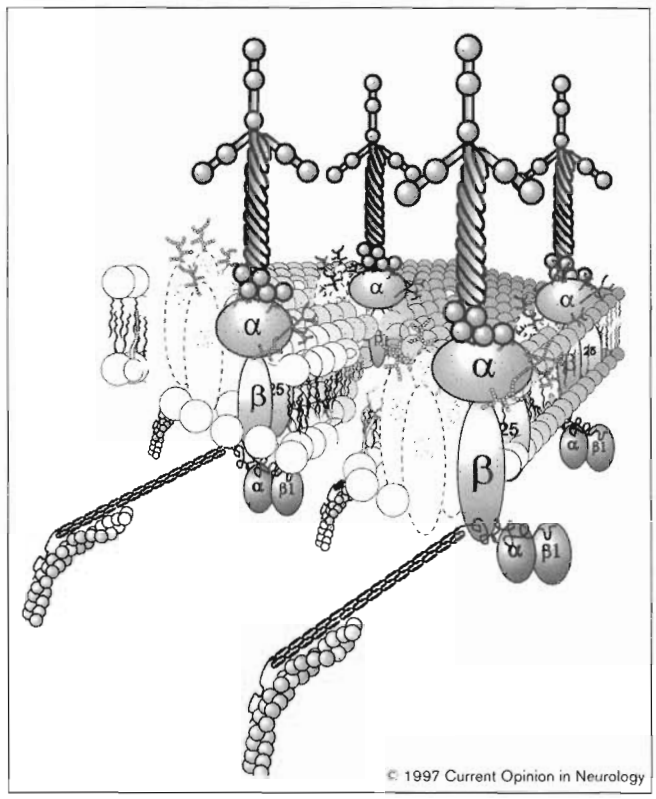


Figure 3. **The molecular organization of the dystrophin–glycoprotein complex at the sarcolemma in limb–girdle muscular dystrophy with sarcoglycan deficiency**



it was unexpected to find genetic heterogeneity among the Amish families, a highly interrelated community with common ancestry. This implicated the existence of another LGMD locus [42], which was subsequently identified when two independent groups demonstrated [6••,7••] mutations in the β-sarcoglycan gene on chromosome 4q to be responsible for autosomal recessive LGMD2E. Immunofluorescence analysis of muscle tissue from affected LGMD2E patients again showed combined reduction of α-, β-, and γ-sarcoglycan (Fig. 3). Segregation of a LGMD phenotype with chromosome 13q was described in 1992 [43] in a number of families from northern Africa. These patients also displayed a sarcoglycan deficiency in their skeletal muscle, which made the γ-sarcoglycan gene a strong candidate for the disease. The γ-sarcoglycan gene was mapped to human chromosome 13q and mutations in this gene were ascertained to cause LGMD2C [8••]. At the time of this report, all of the known sarcoglycans were associated with an autosomal recessive LGMD. When a second DGC-associated 35 kDa sarcoglycan was identified, encoded by a gene at 5q33 [13•,14••], it rapidly became a candidate for another sarcoglycan deficiency, LGMD2F [43]. Mutation screening in the affected families eventually confirmed mutations in the δ-sarcoglycan gene to be causative for this disease [9••].

How can physicians or diagnostic laboratories distinguish between the four sarcoglycan deficient LGMDs if linkage data are not available? So far there does not seem to be a phenotypic difference between the disorders and the clinical severity in the patients is variable [44–46]. Immunofluorescence analysis or immunoblotting do not allow a reliable diagnosis, as primary defects in a single sarcoglycan affects the expression of all sarcoglycans. Mutation assays for genomic DNA have recently been established for the sarcoglycan genes and a variety of mutations have been characterized [48,49,50•,51]. A genotype–phenotype correlation for distinct mutations is not yet applicable. It was shown that patients with the same mutation had different phenotypes [46] and missense mutation can be as severe as null mutations [49,51]. Interestingly patients with autosomal recessive LGMD2A and 2B seem to have a general mild phenotype.

Besides the fact that the sarcoglycans are tightly associated with dystroglycan and dystrophin, not much is known about cytoplasmic or extracellular binding partners. The increasing knowledge about the sarcoglycan genes and the proteins they encode will provide tools for a more specific diagnostic approach to the LGMDs and

will further our understanding of the function of the sarcoglycan complex in muscle cells.

Congenital muscular dystrophies

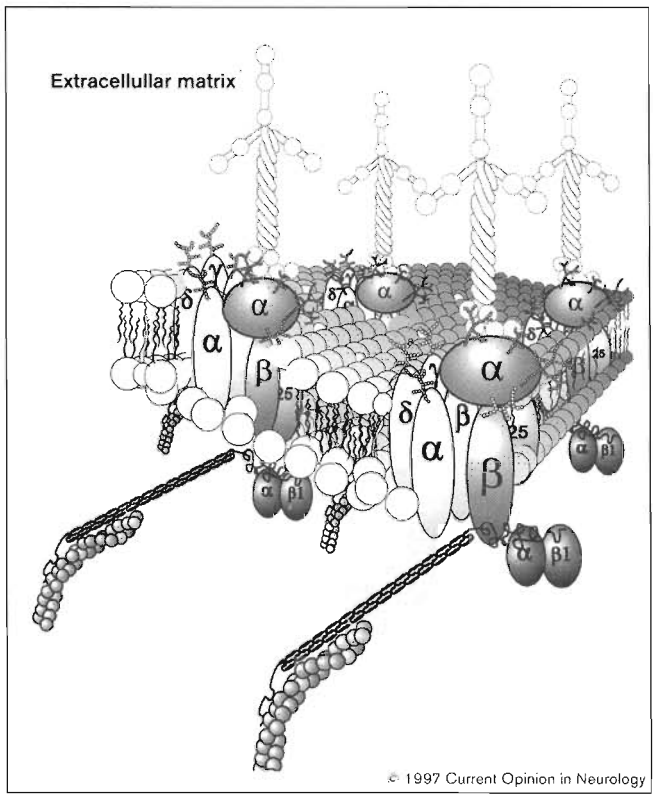
The CMDs have been classified into several subtypes according to the involvement of structural changes in the central nervous system, mental retardation, and ocular defects, in addition to skeletal muscle pathology [52].

Mutations in the *LAMA2* gene, encoding the α2 chain of laminin-2, have been characterized in one form of CMD linked to 6q [10•,53,54•] (Fig. 4). Identification of the primary cause of this disorder provided the first genetic and biochemical approach in the diagnoses of CMDs. Applying these tools it now becomes clear that the clinical term CMD, used for infants presenting with muscle weakness at birth or certainly within the first few months of life, does not cover all laminin α2 chain deficient patients. Patients with laminin α2 chain deficiency in skeletal muscle, and white matter changes in brain magnetic resonance imaging, but late onset of clinical symptoms illustrate the broad spectrum of disease severity [1••]. The characterization of several mild CMD cases [1••] also suggests that the application of only a C-terminal antibody for immunohistochemical screening will not detect patients with a truncated N-terminus. Progress in prenatal diagnosis of laminin α2 chain deficient CMD cases was made by direct trophoblast staining from chorionic villous samples with antibodies against the α2 chain or by haplotype studies for linkage analysis. Normal expression of the laminin α2 chain in fetal trophoblast tissue was used as an indication for a healthy fetus in affected families [55]. Consistency of these results would facilitate prenatal diagnosis given the large size of the gene (10 kb) and lack of any identified mutation hot spots. Recently, deficiency of α actinin3, a type 2 fiber specific isoform of the α actinin family of actin-binding proteins, was demonstrated in skeletal muscle of laminin α2 positive CMD patients [56]. Mutation analysis in affected patients has to confirm the specificity of this finding.

Other muscular dystrophies

A common pathogenic feature in several muscular dystrophies is the interruption of the link between the extracellular matrix and the cytoskeleton. The disruption can occur in the subsarcolemmal cytoskeleton, as seen in DMD/BMD (Fig. 2), at the level of the sarcolemma, as found in several forms of LGMD (Fig. 3), or in the extracellular matrix, described in CMD with laminin α2 chain deficiency (Fig. 4). Recent findings in two additional forms of muscular dystrophy illustrate that perturbation of this linkage is an important pathogenic mechanism leading to muscle cell necrosis

Figure 4. **The molecular organization of the dystrophin–glycoprotein complex at the sarcolemma in congenital muscular dystrophy with laminin α2 chain deficiency**



CK, creatine kinase.

[57•,58•,59•,60,61••]. Patients with autosomal recessive epidermolysis bullosa and muscular dystrophy underline the importance of cytoskeleton–membrane attachment. Affected patients have mutations in a gene on chromosome 8q24, encoding plectin. Plectin is an intermediate filament-associated protein expressed in hemidesmosomes within basal keratinocytes and in the sarcolemma. Plectin deficiency presumably leads to defective anchorage of the cytoskeleton to transmembrane complexes in an analogous way, as proposed for dystrophin in the DGC [57•–59•].

A similar disease mechanism as in laminin α2 deficient CMD patients is suggested for patients with autosomal dominant Bethlem myopathy bearing mutations in the α chains of type VI collagen on chromosome 21q (α1 and α2) and very likely 2q (α3) [60,61••]. The microfibrillar type VI collagen is believed to play a role in bridging cells with the extracellular matrix. In this myopathy the structural link between the actin-cytoskeleton and the basal lamina seemed to be extended one step beyond, from laminin into the surrounding endomysial connective tissue [61••].

Conclusion

Experience with muscular dystrophies arising from defects in the DGC clearly demonstrate the importance of intact molecular links between the internal environment of the cell and its external surroundings for muscle fiber viability (Figs 1–4). Understanding precise gene defects will advance our knowledge not only of the pathophysiology of muscular dystrophies, but also of the role of DGC's in the dynamic processes responsible for development and maintenance of muscle fiber integrity. Whether the sarcolemmal DGC primarily serves as a mechanical link to maintain membrane integrity during cycles of contraction and relaxation or plays other roles is yet to be clarified. Considering the different cell types and structures in which components of the DGC are expressed makes a simplistic model unlikely. Understanding the fundamental biology and pathophysiology of the various players in the DGC will certainly give us a better idea of the mechanisms leading to muscle degradation and necrosis in muscular dystrophies. Ultimately this insight may facilitate rational design of treatments for these diseases as was recently shown by different genetic approaches [62,63•].

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