

Advances and Perspectives in Muscular Dystrophies

Corrado Angelini

Department of Neurological and Psychiatric Sciences, University of Padova, Padova, Italy

Abstract

The muscular dystrophies are inherited muscle disorders characterized by weakness and progressive muscle wasting. They can be subdivided in different types, including congenital forms in accordance to distribution of weakness: Duchenne, Becker, Emery-Dreyfuss, limb-girdle, distal, facio-scapulo-humeral, oculofaringeal dystrophy.

In this review we will deal with the most recent advances in dystrophinopathies, limb-girdle muscular dystrophies and congenital muscular dystrophies and future perspectives in their pathogenesis and treatment. Advances in autosomal dominant limb-girdle dystrophies such as Emery- Dreyfuss muscular dystrophies and facio-scapulo humeral dystrophy will also be briefly covered.

Key words: Duchenne muscular dystrophy, sarcolemma, limb-girdle dystrophy.

Basic Appl Myol 12 (1): 17-25, 2002

The identification of the Duchenne muscular dystrophy gene and its protein product dystrophin allowed to discover of a large complex of sarcolemmal glycoproteins associated to dystrophin [22, 36]. The dystrophin associated glycoprotein complex (DAG) spans through the sarcolemma and provides a linkage between actin and laminin $\alpha 2$ (merosin) in the extracellular matrix, appears to be functionally divided in subcomplexes. The DAG is composed by dystrophin (427 KDa), α -dystroglycan and β -dystroglycan, which is the component that binds to merosin [22], by the sarcoglycan (SG) complex composed by five transmembrane glycosylated proteins i.e. α -sarcoglycan, beta, gamma, delta and epsilon-sarcoglycan (Fig. 1). Partial or complete absence of one of these proteins might lead to muscle cell necrosis and initiate a cycle of degeneration-regeneration in muscle, this will result in various types of muscular dystrophies, with a predominant proximal muscle involvement. The DAG complex is at play in several muscular dystrophies but other cytoplasmic, cytoskeleton and nuclear proteins play a role in a number of other dystrophies.

X-Linked Muscular Dystrophies

Dystrophinopathies

They include Duchenne (DMD) and Becker muscular dystrophies (BMD) they also include atypical mild cases with cramps myalgia, myoglobinuria, cardiopathy and their female carriers and animal model(s) (mdx mice, golden retriever dog). Dystrophin is a rod-shaped cytoskeletal protein of 427 KDa, localized to the cyto-

plasmic face of skeletal and cardiac sarcolemma, composed of four structural domains: [1] the amino-terminal domain (N) which has a high homology with the actin-binding region of α -actinin and α -spectrin [2] a series of 24 repeats of 109 aminoacid motif in the form of a triple helix (rod) [3] a cysteine-rich domain, homologous to the calcium binding region of α -actinin [4] the C-terminus domain (C). The C-terminus has no homology with any other identified protein except utrophin. Overexpression of utrophin has been tried as therapeutic strategy in dystrophinopathies, with some success in mdx mice. In a female carrier and a BMD case that underwent heart transplant we found overexpression of utrophin but no functional result [16], since the dilated heart underwent progressive failure. Mutations in the cysteine rich domain and in the first half of C-terminus produce a DMD phenotype, while loss of the last 200 aminoacids of C-terminus produce an intermediate DMD/BMD phenotype. An "in frame" deletion of dystrophin gene results in Becker muscular dystrophy.

Severe dystrophinopathies

Duchenne dystrophy boys show disease onset in early childhood, with difficulties in running and later, climbing stairs, their walking ability is lost around 10,5 years of age, 55% of Duchenne patients have deletion mutations of one or more exons of the gene, approximately 5% have duplications, the remaining patients have point mutations. In DMD the "out of frame rule", demonstrable in most cases, predicts that frame-shift

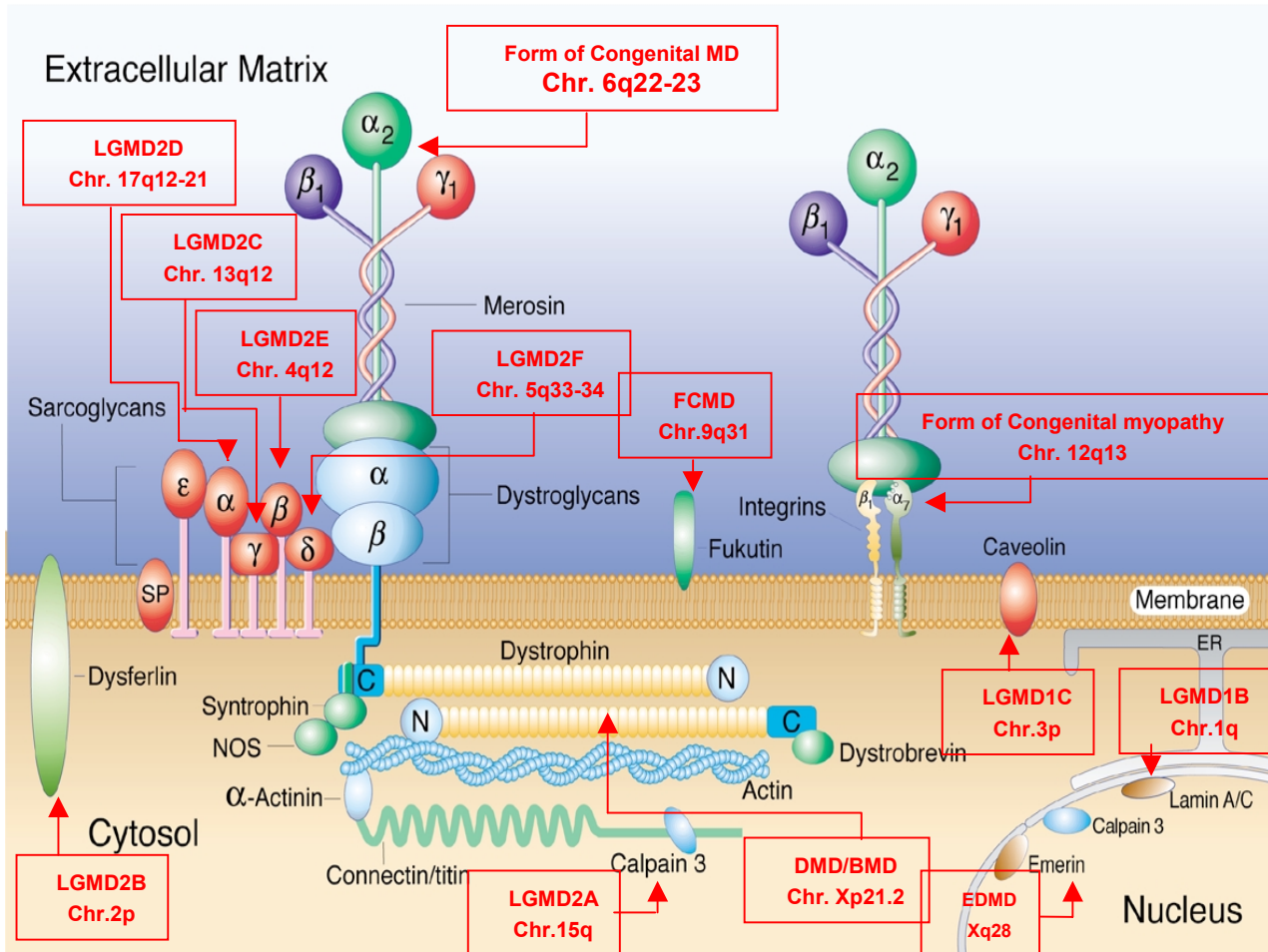


Figure 1. Proteins and muscular dystrophies identified by molecular analyses.

mutations will produce no dystrophin or an unstable dystrophin molecule that is rapidly degraded.

How does the absence of dystrophin lead to muscle cell necrosis in DMD? Understanding this pathogenetic mechanism is crucial for the development of therapies in this devastating disease. Although the exact function of dystrophin remains to be determined, it is likely that mechanical stretch leads to muscle cell necrosis in dystrophin deficient muscle. Immunohistochemistry has shown that all dystrophin associated proteins and nitric-oxide-synthetase (NOS) are reduced in sarcolemma, as a direct consequence of the absence of dystrophin. Secondary events are then caused by the intervention of activated T-

lymphocytes, macrophages and mast-cells, and the dystrophic process is characterized by degeneration/regeneration cycles of muscle fibers. The satellite cells can provide for about 20-25 cycles of regeneration and then muscle undergoes a progressive fibrosis. In regenerating fibers strongly reactive MHC I molecules are expressed at the surface of muscle fibers [13]. The dystrophic process is slowed by steroids, this drug action in DMD patients has been extensively studied in numerous double blind controlled trials. Decreased muscle wasting and decreased T-lymphocytes are found in biopsies of DMD patients treated with steroids. How do steroids work in prolonging ambulation in DMD? The following mechanisms are likely 1) steroids increase muscle mass and muscle strength, 2) steroids decrease muscle catabolism, 3) steroids decrease the number of cytotoxic T lymphocytes, and modulate the secretion of lymphokines.

In a randomized double blind controlled trial with a new steroid: deflazacort (DFC) in 28 DMD patients [2] deflazacort treated patients lost their ambulation at 33.2 ± 9 months while the placebo group lost ambulation 20.5 ± 11 months ($p < 0.05$) after starting the trial. Our treated patients lost ambulation at median age 11.8 years vs 10.5 years in non-treated patients. Variation in steroid

Table 1. X-linked muscular dystrophies.

Muscular Dystrophy	Chromosome location	Deficient protein
Duchenne	Xp 21	Dystrophin (null variant)
Becker muscular dystrophy	Xp 21	Dystrophin (residual protein)
Emery muscular dystrophy	Xq 28	Emerin

response is possible. Some DMD cases benefited more and still are ambulant at 17 years of age. A standard prednisone dose of 0.75 mg/kg/daily has been established by the CIDD collaborative group, its DFC equivalent is 1 mg/Kg/daily or 2 mg/Kg/alternate day. The steroid treatment increases the period of deambulation in carefully followed DMD patients and this effect seems to be related to early age of onset of treatment. In a recent trial deflazacort and prednisone were compared [7], and side effects were strictly monitored i.e. gain in weight, osteoporosis, cataracts. Our DMD patients benefited from both steroid regimens, but there was a significant less increase in weight in deflazacort treated patients.

Mild dystrophinopathy (Becker muscular dystrophy)

In Becker muscular dystrophy DNA deletions or duplications are “in frame”, usually they are clustered in the region of the gene between exons 45-60 in the “distal part of rod domain”, the most common deletions involve exons 45-47. “In frame” deletions in the rod domain give rise to intermediate or milder BMD. In a clinico-genetic study [10] partial intragenic deletion and duplication accounted for 82% of the mutations, of which 77% were deletion and 5% duplications. The dystrophin molecule in BMD is often a truncated protein as predicted from the genetic defect, the size of deletion being inversely proportional to the size of protein produced [8], however in some cases duplications create an enormous dystrophin molecule [1]. There is considerable debate concerning the precise quantity of dystrophin in muscle that produces different clinical phenotypes and in mild BMD cases the dystrophin quantity correlates with disease presentation and progression. Dystrophin quantity correlates also with age of reaching a moderate degree of muscle involvement.

We conducted a multidisciplinary study in order to assess dystrophin expression in a large series of mild X-linked dystrophinopathy patients, with a well-defined clinical phenotype [3]. Our study addressed the prevalence of the various disease courses in a large cohort of mild X-linked muscular dystrophy patients. Our 104 BMD patients were divided in 4 clinical groups, according to clinical severity; the groups were classified as asymptomatic or sub-clinical, benign, moderate and severe. In our series, up to 30% of patients were either asymptomatic or had a sub-clinical phenotype. Cardiomyopathy was also assessed, and dilated cardiomyopathy was found in 47% of sub-clinical and benign cases. Myoglobinuria, cramps and myalgia were also associated with a sub-clinical or benign clinical course [1, 3]. The immunohistochemical pattern of dystrophin labeling and dystrophin amount decreased gradually in the four clinical groups therefore an approximate correlation can be established between dystrophin quantity and disease severity course. Further in 125 mild dystrophinopathies we assessed the prognostic factors in correlation to molecular data [4]. Prognosis was substantially better, showing a stable course in patients with large

deletion or duplication in the proximal rod regions: most such patients have a cramp-myalgia syndrome or hyperCKemia. Our study showed a significant correlation between: [1] patients with a rapid course and low dystrophin amount and severity in immunohistochemical score ($p < 0.05$); [2] deletion or duplication at the 5' end of the gene was associated with a poor prognosis. Most importantly, the combined use of these different molecular techniques might be useful to determine the prognosis of mild X-linked muscular dystrophy.

Dystrophinopathy in females

A mosaic of dystrophin positive and negative muscle fibers is observed in DMD carriers. It is therefore possible to diagnose a as a “sporadic carrier” females that present with a myopathy or heart involvement. These isolated female dystrophinopathy patients were in the past diagnosed as a limb-girdle muscular dystrophy patient or a sporadic cardiomyopathy case prior to the immunohistochemical or biochemical detection of dystrophin abnormalities in their muscle biopsy. It has been demonstrated that these female patients are heterozygous carriers, who carry a random, or non random, inactivation of the X chromosome gene [24]. The precise mechanism by which the partial deficiency of dystrophin leads to muscle fiber degeneration and progressive myopathy is the following: sarcolemmal instability causes muscle degeneration and provokes the high serum CPK in female carriers; after several cycles of muscle degeneration-regeneration regenerative ability might be lost or incomplete. This observation explains the present difficulties of gene or cell therapy in DMD, since female carriers are a natural model of coexistence of dystrophin competent nuclei and dystrophin incompetent myonucle; theoretically both genetic and biochemical normalization should occur in a female dystrophinopathy since normal nuclei are present in variable quantity but in most cases this is not always the case. Therefore to have a successful cellular or gene therapy one should conclude that at least 50% of nuclei have to be dystrophin competent in muscle fibers.

Emery-Dreifuss Muscular Dystrophy (EDMD)

This disorder is characterized by a slow course and the clinical signs: early contractures (before there is any muscle weakness) of the Achilles tendons, elbows, are often associated to a limitation of neck flexion, then progressive weakness in humeroperoneal muscle compartment and cardiomyopathy with conduction block occurs. This X-linked muscular dystrophy is caused by absence of the inner nuclear membrane protein emerin caused by mutation of STA gene at Xq28 but another form of this syndrome has been described due to a heterozygote mutation in laminin A/C [11], another protein of nuclear membrane that interacts with emerin, therefore EDMS is a syndrome with different genetic loci.

Advances and perspectives in muscular dystrophies

Table 2. Dominant limb-girdle muscular dystrophies.

LGMD	inheritance	locus	protein	clinical presentation
LGMD1A LGMD1B Emery-Dreifuss	AD	5q	Myotilin	Onset: 18-35 years. Dysarthria, ankles contractures
Partial lipo-dystrophy	AD	1q11-21	lamin A/C	Heart conduction defects, elbows and ankles contractures
LGMD1C	AD	3p	Caveolin-3	Calf hypertrophy and mild proximal weakness
LGMD1D	AD	7q	-	Proximal weakness

Autosomal dominant muscular dystrophies

The most common autosomal dominant form of muscular dystrophy is facio-scapulo humeral muscular dystrophy (FSH), localized to chromosome 4. This dystrophy derives its name from the muscle groups that are affected: facial and shoulder girdle muscles, later foot extensor and pelvic muscles become involved. It is associated with a subtelomeric deletion of chromosome 4q, with loss of 3.3 kb tandem-repeat unit. Both in FSH and in DMD caspase 3 overexpression correlates with apoptosis and this process might be a possible target for pharmacological treatment [29]. A precise protein defect has not been found in FSH and is likely that the deletion provokes a complex RNA derangement by its positional effect.

An autosomal dominant EDMD is caused by mutations in laminin A/C gene on chromosome 1q21.3. Autosomal dominant limb-girdle muscular dystrophies (LGMD type 1) are an heterogeneous group of disorder localized in different chromosomal loci (Table 2). Several of these disorders are associated with cardiac involvement (LGMD type 1B, 1D). The diagnosis by protein analysis is now only available for caveolin deficiency (LGMD1C): several mutations within the coding sequence of the human caveolin 3 gene (located at 3p25) have been identified. Mutations that lead to a loss of caveolin 3 are responsible for a mild form of dystrophy characterized by familial weakness and hypertrophic calves muscles (LGMD-IC) or sometimes only cause hyperCKemia in patients.

Oculopharyngeal dystrophy

Its onset is in the third decade, this muscle disease affects eye muscles, upper facial muscles and causes weakness of neck and limb-girdle muscles dysphagia is also prominent. The gene locus at 14q codes for poly (A) binding protein in the first exon there is a (GCG)_n triplet expansion. The number of repeats in affected individuals is over 6-8 and allows a diagnostic tool in patients with the above mentioned clinical signs.

Autosomal Recessive Limb Girdle Dystrophies

Autosomal recessive limb-girdle muscular dystrophies (LGMD type 2) are an heterogeneous group of disorders which include at least 9 different genetic entities (Table 3). The first LGMD locus was mapped on chromosome

15q15.1, and the disease associated was called LGMD2A. LGMD2A is due to mutations in the CAPN-3 gene encoding for *calpain-3*, the muscle-specific member of a family of Ca⁺⁺ activated neutral proteases. The mechanism by which calpain-3 mutations result in muscle fiber degeneration is still not well understood. Apoptosis is certainly at play in this disease, but other mechanisms may be due to the biochemical characteristics of calpain-3: i.e. its titin-binding property, autocatalytic activity and its capacity of cleaving fodrin (α -spectrin). Current hypotheses suggest that calpain-3 specifically attaches to a N21 segment of titin (a giant sarcomeric protein) which prevents the attack by ubiquitinary calpains. When calpain-3 is defective or inactive due to the loss of its proteolytic capability, the other calpains digest titin, inducing a myofibrillar disorganisation, studies in myofibrillogenesis show in fact that calpain-3 is required for muscle organisation. Due to its nuclear translocation signal-like sequence, calpain-3 is also possibly involved in the regulation of muscle-specific transcription factors and muscle differentiation, and in repair of plasma membrane in damaged muscle fibers.

A large number of LGMD2A patients world-wide have been so far reported. This disorder is estimated to be the most common form of recessive LGMD, representing about 30-50% of LGMD patients. Its frequency varies in different ethnic groups, ranging from 10% of cases in outbreed US and Caucasian population [9], up to 80% in inbred populations of Basque Country, Turkey or Russia. The disease is clinically characterised by onset from 2 to 40 years, elevated CK levels, selective involvement first of upper-girdle with Erb's type of LGMD, subsequently of lower limb-girdle, and loss of ambulation between 20-30 years after the onset of symptoms (from 5 to 39 years of age). A marked heterogeneity of clinical severity even in intra-family patients has been reported.

LGMD2A is caused usually by single base changes at DNA level, mutations are spread along the CAP3 gene spanning 40 kb, where no mutational hot spots are present. More than 110 different mutations have been so far described, mainly of the missense type. Mutation detection has been firstly done first by direct sequencing in patients selected on the basis of LGMD phenotype, or linkage analysis. Recently, due to the availability of calpain-3 antibodies, LGMD2A diagnosis has been first done by

Advances and perspectives in muscular dystrophies

Table 3. Autosomal recessive limb-girdle dystrophies.

LGMD	inheritance	chromosome	protein	clinical presentation
LGMD2A	AR	15q	Calpain 3	Onset 9-15 years
LGMD2B	AR	2p	Dysferlin	Onset around 20 years.
				Loss of ambulation after 35 years Distal- proximal involvement
LGMD2C	AR	13q	g-sarcoglycan	Onset 3-12 years
				Loss of ambulation 10-20 years
LGMD2D	AR	17q	a-sarcoglycan	Onset and severity variable
LGMD2E	AR	4q	b-sarcoglycan	Early onset and cardiomyopathy
LGMD2F	AR	5q	d-sarcoglycan	Onset and severity variable
LGMD2G	AR	17q	telethonin	Distal involvement, moderate CK elevation, rimmed vacuoles
LGMD2H	AR	9q	TRIM32	Onset 8-27 years, scapular girdle proximal weakness
LGMD2I	AR	19q	FKRP	Onset 2-27. Proximal weakness

protein testing with western blot analysis [15], which can be subsequently followed by mutation detection.

The correlation between the clinical phenotype and genotype are usually the following: an absent protein or homozygous null mutations genotype cause a severe phenotype, whereas patients with missense mutations have variable protein level and relatively milder phenotype. Some LGMD patients have missense mutations but normal level of calpain by western blot. These data are derived from small number of patients in whom a complete analysis was obtained, therefore a study on a larger population of LGMD patients seems useful to better establish a precise genotype-phenotype correlation pattern.

LGMD 2B or Dysferlinopathy

Limb-girdle muscular dystrophy type 2B (LGMD2B), and the distal muscular dystrophy previously described as Miyoshi myopathy (MM), are caused by mutations in *dysferlin* gene, mapped to chromosome region 2p13. Despite the clinical features of LGMD2B and MM are quite different, both phenotypes can be detected among patients belonging to the same family, thus sharing the same mutations [20]. This clinical heterogeneity can not be explained on the basis only of allelic diversity but it might be attributed to additional epigenetic factors resulting in variable dysferlin expression. Myoferlin, a protein homologue to dysferlin, has recently been suggested as a possible modifier gene in dysferlinopathy.

Dysferlin immunolocalizes to the sarcolemma similarly to dystrophin, but it does not associate with dystrophin-glycoprotein complex. Its function and the mechanism by which it causes muscle fiber necrosis is unclear. Dysferlin presents a high degree of sequence homology with the protein Fer-1 in *C. elegans*. Since the mutant worm has an abnormal spermatogenesis, it was argued that mutant fer-1 may be involved in the failure of membrane fusion between the organelles and plasmalemma. By analogy, the first pathogenetic mechanism in dysferlinopathies might be correlated with the abnormal trafficking of vesicles within muscle fibers. The absence of dysferlin might lead to an abnormal

repair of damaged muscle fibers, possibly impairing myotubes fusion and muscle fiber regeneration.

The molecular diagnosis of LGMD2B and the detection of dysferlin deficiency by protein analysis is now easily available, and a number of patients and their muscle biopsy have been diagnosed and analyzed.

We diagnosed over 33 patients with dysferlin deficiency [15] and analyzed 26 muscle biopsies from dysferlinopathy patients, in which we observed a variable inflammatory response, we scored the amount and type of cellular infiltrates, muscle fiber degeneration and regeneration, and severity of histopathological changes. Several studies reported a prominent inflammatory response in dysferlinopathy patients, but the origin of this feature and its role in the development of muscle pathology is still under investigation (Fanin and Angelini, in press). Patients treated with steroids or immunosuppressant drugs do not show benefit on the long term, therefore it seems advisable to try alternative drugs, since this myogenic affection develops in relation to a sarcolemmal damage, where the dysferlin protein seems to be associated with caveolin, but not with DAG complex.

LGMD 2C-2F or Sarcoglycanopathies

The sarcoglycanopathies are a group of recessive limb-girdle muscular dystrophies (LGMD 2C, 2D, 2E, 2F) due to mutations in the genes encoding for the components of the sarcoglycan (SG) complex. Based on molecular and genetic criteria, the sarcoglycanopathies are classified as LGMD2D (α -SG), LGMD2E (β -SG), LGMD2C (γ -SG) and LGMD2F (δ -SG). Private mutations of α and γ sarcoglycan genes due to a founder effect have been found in Northern Italy [14]. The SG complex, composed of 5 glycoproteins (α -, β -, γ -, δ -, ϵ -SG), is a member of the dystrophin-associated glycoprotein (DAG) complex localized to the sarcolemma of muscle fibers, which acts as a link between the extracellular matrix and the cytoskeleton and confers structural stability and protects the sarcolemma from mechanical stress developed during muscle contraction. The pathogenetic mechanism underlying sarcolemmal damage and fiber necrosis in sarco-

Advances and perspectives in muscular dystrophies

glycanopathies is still unclear, but several lines of evidence suggest that the deficiency in any SG subunit results in a loss of the whole SG complex, causing the disruption of the DAG complex. A recent hypothesis is that SG complex masks a metalloproteinase cleavage site of the β -dystroglycan in normal muscle: the loss of SG in sarcoglycanopathies would lead to activation of the cleavage site of β -dystroglycan, causing the disruption of the link between the sarcolemma and the basement membrane.

The clinical phenotype of sarcoglycanopathies is characterized by progressive skeletal muscle weakness, with variable age of onset and disease severity, the latter ranging from a severe Duchenne-like dystrophy to a relatively mild LGMD [6]. Since the SGs are expressed almost exclusively in striated skeletal and cardiac muscle, the development of a cardiomyopathy in these patients is expected. The first studies on primary sarcoglycanopathy patients reported the absence of an overt cardiomyopathy [12], in contrast, recent data suggest that cardiac involvement might represent an important and sometimes life-threatening clinical complication in these disorders [23]. The cardiac involvement is often observed in association with mutations of β -SG and δ -SG genes, whereas patients with mutations in the α -SG gene only rarely display cardiomyopathy, and usually have milder clinical phenotypes [5]. Initially α -sarcoglycan was called adhalin [21, 28] and its clinical phenotype was a severe type of autosomal recessive dystrophy (SCARMD), but nowadays a whole spectrum of clinical presentations have been recognized [6] including asymptomatic hyperCKemia [5].

In the Syrian hamster, the natural animal model of δ -SG gene mutation, a severe muscular dystrophy and cardiomyopathy is caused by the loss of the entire SG complex and by the consequent disruption of the integrity of the DAG complex in the sarcolemma of skeletal fibers and cardiomyocytes. Recent studies demonstrate that β -SG and δ -SG are both expressed in striated muscle and smooth muscle of coronary arteries, suggesting that a perturbation of coronary vascular func-

tion might represent a common mechanisms in the pathogenesis of the cardiomyopathy.

We assessed the frequency and the severity of cardiomyopathy in patients affected with primary β -sarcoglycanopathy, and analyzed the SG expression in skeletal and cardiac muscle tissue. We observed that β -SG gene mutations are responsible for sarcolemmal disruption leading to severe muscular dystrophy; 50% of our LGMD2E patients have an overt cardiac involvement, that in one patient caused a premature death for cardiac failure.

Prenatal diagnosis in α -sarcoglycanopathy is feasible [27] provided that the specific DNA mutations have been found.

Congenital muscular dystrophies: (CMD)

Children with this condition present with weakness and hypotonia at birth. Sometimes in the first few months of life most affected children might be able to stand with support, but few learn to walk.

Congenital muscular dystrophies are heterogeneous conditions, were the following entities have been separated (Table 4):

- classical congenital muscular dystrophy is characterized by weakness, muscle contractures, dystrophic features in muscle; 30-50% of cases of the Caucasian classical congenital muscular dystrophy have a merosin (laminin α) deficiency [31]. Merosin is the heavy chain of the skeletal muscle basement protein laminin and binds the alpha-dystroglycan of DAG complex to the basal lamina. Merosin negative CMD is linked to chromosome 6 [18] and in merosin deficient families prenatal diagnosis by chorionic villi biopsy is possible [25].
- Fukuyama congenital muscular dystrophy in Japan is second only to DMD, but rare elsewhere, it presents with prominent CNS abnormalities i.e. leukoencephalopathy. Most children are mentally retarded and many have epilepsy. This form has been recently linked to chromosome 9 and fukutin is the defective protein [19]; in Japan the gene presents an ancient retrotransposon insert.

Table 4. Congenital muscular dystrophy.

Disease	Locus	Protein	Clinical Presentation
CMD Classic (MDC1A)	6q22-q23	<u>Laminin α2</u> (merosin)	Congenital dystrophy white matter changes
Merosin ? positive CMD	?	Variable phenotype	
MDC1C	19q13.3	<u>FKRP</u>	Congenital muscular dystrophy
Rigid Spine Muscular Dystrophy 1	1p36-p35	<u>SEPN1</u>	Rigid spin, contractions Respiratory insufficiency
Fukuyama Muscular Dystrophy	9q31	<u>Fukutin</u>	Congenital muscular dystrophy Cortical disgenesis, ocular abnormality
Muscle Eye Brain Disease (Santavuori)	1p34-p32	<u>POMGnT1-glycosyl N-transferase</u>	CMD, lissengiria, retinopathy, strabisms
Walker-Warburg	?	?	CMD, ocular signs

- c) a form of congenital muscular dystrophy is due to mutation of a fukutin related protein (FKRP): interestingly a form of mild limb-girdle MD linked to chromosome 19 is allelic and also due to mutations of FKRP; to detect such families one can therefore directly sequence the gene or do a cumbersome linkage analysis study;
- d) some cases of rigid spine syndrome are due to a selenio protein defect (SEPN1) and present associated a restrictive respiratory syndrome;
- e) Muscle-eye-brain disease or Santavuori type of CMD is associated to ocular abnormalities (retinopathy, strabismus, glaucoma) and CNS abnormalities (lissengiria) due to a neuronal migration disorder caused by a mutation in a newly recognized glycosyl transferase: POMGn Ti [33];
- f) a more severe form of CMD with minor ocular signs has been denominated Walker-Walburg syndrome, but its chromosome locus and protein product is still unknown.

Future prospects for diagnosis and treatment

The above advances demonstrate the central role of the DAG complex in several muscle pathological conditions and the importance of an exact diagnosis in individual patients with dystrophy for genetic counselling. These muscle disorders have been called "sarcolemmopathies" i.e. muscular dystrophies with cell membrane defects which include a number of protein defects of this complex of other sarcolemmal protein which directly or indirectly might lead to a dystrophic process (dystrophin, sarcoglycans, merosin, caveolin, integrins, etc.). However numerous defects in other genes producing nuclear or cytoplasmic proteins, not linked to the DAG complex, such as calpain, dysferlin, laminin A/C, emerin, telethonin, TRIM 32 gene mutations result also in limb-girdle muscular dystrophies [17]. Therefore different pathogenetic mechanisms are at play in various types of dystrophies; it is therefore likely that also different types of therapeutic intervention(s) will be required to procrastinate muscle destruction in the various types of muscular dystrophies. The molecular advances have a direct impact on diagnostic accuracy and prenatal counselling.

So far gene replacement has been tried only in two limb-girdle muscular dystrophy 2D patients with α -sarcoglycan deficiency [24]. The results showed that the injection of an adeno associated viral (AAV) vector was well tolerated in the muscle extensor digitorum brevis and α -sarcoglycan was expressed in some muscle fibers. This replication defective non pathogenic vector had low immunogenicity and could transfer the entire cDNA of the sarcoglycan gene. A subsequently gene transfers were stopped due to a catastrophic death in a patient with metabolic disorder that was injected with AAV. An increased efficiency of these vectors and a different choice of muscle might result in functional recovery of affected muscle. An optimistic perspectives is also given by the fact that stem cell therapy is been explored. Researchers have shown that a small proportion of injected stem cells

might be able to replace dystrophin deficient muscle cells in heart and muscle [32] in mdx mouse. Finally it will be interesting to explore further advances drug trials in muscular dystrophies selecting drug according to their possible pathogenetic mechanism(s), this might be an useful a more rapid strategy in giving some benefit to patients, while genetic and stem cell therapy are still in progress.

Acknowledgement

This paper has been supported by Telethon – Italy grant # TF00003Y01 and MURST grant # 2001068328.

Address correspondence to:

Department of Neurological Science, University of Padova, via Giustiniani 5, 35128 Padova, Italy, phone +39 0498213625, Email corrado.angelini@unipd.it.

References

- [1] Angelini C, Beggs AH, Hoffman EP, Fanin M, Kunkel LM: Enormous dystrophin in a patient with Becker muscular dystrophy. *Neurology* 1990; 40: 808-812.
- [2] Angelini C, Pegoraro E, Turella E, Intino MT, Pini A, Costa C: Deflazacort in Duchenne dystrophy: study of long-term effect. *Muscle & Nerve* 1994; 17: 386-391.
- [3] Angelini C, Fanin M, Pegoraro E, Freda MP, Cadaldini M, Martinello F: Clinical molecular correlation in 104 mild X-linked muscular dystrophy patients: characterization of sub-clinical phenotypes. *Neuromusc Disord.* 1994; 4: 349-358.
- [4] Angelini C, Fanin M, Freda MP, Martinello F, Miorin P, Melacini P, Siciliano G, Pegoraro E, Rosa M, Danieli GA: Prognostic factors in mild dystrophinopathies. *J Neurol Sci* 1996; 142: 70-78.
- [5] Angelini C, Fanin M, Menegazzo E, Freda MP, Duggan DJ, Hoffman EP: Homozygous α -sarcoglycan mutation in two siblings: one asymptomatic and one steroid responsive mild limb-girdle muscular dystrophy patient. *Muscle & Nerve* 1998; 21: 769-775.
- [6] Angelini C, Fanin M, Freda MP, Duggan DJ, Siciliano G, Hoffman EP: The clinical spectrum of sarcoglycanopathies. *Neurology* 1999; 52: 176-179.
- [7] Bonifati DM, Ruzza G, Bonometto P, Berardinelli A, Gorni K, Orcesi S, Lanzi G, Angelini C: A multicenter double-blind randomized trial of deflazacort versus prednisone in Duchenne muscular dystrophy. *Muscle & Nerve* 2000; 23: 1344-1347.
- [8] Bushby KMD, Gardner-Medwin D, Nicholson LVB, Johnson MA, Huggerty ID, Cleghom NJ, Harris JB, Bhattacharya SS: The clinical genetic and dystrophin characteristics of Becker muscular dystrophy. *J Neurol* 1993; 240: 105-115.
- [9] Chou FL, Angelini C, Daentl D, Garcia C, Greco C, Hausmanowa-Petrusewicz I, Fidzianska A, Wessel H, Hoffman EP: Calpain III mutation analysis of a

- heterogeneous limb-girdle muscular dystrophy population. *Neurology* 1999; 52: 1015-1020.
- [10] Comi PG, Prelle A, Bresolin N, Moggio M, Bardon A; Gallanti A; Vita G, Toscano A, Ferro MT, Bordon A: Clinical variability in Becker muscular dystrophy. Genetic, biochemical and immunohistochemical correlation. *Brain* 1994; 117: 1-14.
- [11] Di Barletta R, Ricci E, Galluzzi G, Tonali P, Mora M, Morandi L, Romorini A, Voit T, Orstavik KH, Merlini L, Trevisan C, Biancalana V, Housmanowa-Petrusewicz I, Bione S, Ricotti R, Schwartz K, Bonne G, Toniolo D: Different mutations in the LMNA gene cause autosomal dominant and autosomal recessive Emery-Dreifuss muscular dystrophy. *Am J Hum Genet* 2000; 66: 1407-1412.
- [12] Duggan DJ, Gorospe JMR, Fanin M, Hoffman EP, Angelini C: Mutations in the sarcoglycans genes in myopathy patients. *N Engl J Med* 1997; 336: 618-624.
- [13] Fanin M, Angelini C: Regeneration in sarcoglycanopathies expression studies of sarcoglycans and other muscle proteins. *J Neurol Sci* 1999; 165: 170-177.
- [14] Fanin M, Hoffman EP, Angelini C, Pegoraro E: Private beta- and gamma- sarcoglycan gene mutations: evidence of a founder effect in Northern Italy. *Hum Mutat* 2000; 16 (1): 13-17.
- [15] Fanin M, Pegoraro E, Matsuda-Asada C, Brown RH Jr, Angelini C: Calpain-3 and dysferlin protein screening in patients with limb-girdle dystrophy and myopathy. *Neurology* 2001; 56: 660-665.
- [16] Fanin M, Melacini P, Angelini C, Danieli GA: Could utrophin rescue the myocardium in Duchenne patients? *J Mol Cell Cardiol* 1999; 31: 1501-1508.
- [17] Frosk P, Weiler T, Nylen E, Sudha T, Greenberg CR, Morgan K, Fujiwara TM, Wrogemann K: Limb-girdle muscular dystrophy type 2H associated mutation in TRIM 32, a putative E-ubiquitin ligase gene. *Am J Hum Genet* 2002; 70: 663-672.
- [18] Hillaire DA, Leclerc S, Faure, Topaloglu H, Chianilkulchai N, Guicheney P, Grinas L, Legos P, Philpot J, Evangelista T, Routon MC, Mayer M, Pellissier JF, Estournet B, Barois A, Hentati F, Feingold N, Beckmann JS, Fardeau M: Localization of merosin negative congenital muscular dystrophy to chromosome 6q2 by homozygosity mapping. *Hum Mol Genet* 1994; 3: 1657-1659.
- [19] Kobayashi K, Nakahori Y, Miyake M, Matsumura K, Kondo-Iida E, Nomura Y, Segawa M, Yoshioka M, Saito K, Osawa M, Hamano K, Sakakihara Y, Nonaka I, Nakagome Y, Kanazawa I, Nakamura Y, Tokunaga K, Toda T: An ancient retrotransposon insertion causes Fukuyama-type congenital muscular dystrophy. *Nature* 1998; 394: 388-392.
- [20] Liu J, Aoki M, Illa I, Wu CY, Fardeau M, Angelini C, Serrano C, Urtizberea JA, Hentati F, BenHamida M, Bohlega S, Culper EJ, Amato AA, Bossie K, Oeltjen T, Bejaoui K, McKennaYasek D, Hosler BA, Schurr E, Arahata K, Dejong PJ, Brown RH: Dysferlin, a novel skeletal muscle gene, is mutated in Miyoshi myopathy and limb girdle muscular dystrophy. *Nature Genetics* 1998; 20: 31-36.
- [21] Matsumura K, Tomé FMS, Collin H, Azibi K, Chaouch M, Kaplan JC, Fardeau M, Campbell KP: Deficiency of the 50K dystrophin associated glycoprotein in severe childhood autosomal recessive muscular dystrophy. *Nature* 1992; 259: 320-322.
- [22] Matsumura K, Campbell KP: Dystrophin-glycoprotein complex: its role in the molecular pathogenesis of muscular dystrophies. *Muscle & Nerve* 1994; 17: 2-15.
- [23] Melacini P, Fanin M, Duggan DJ, Freda MP, Bernardinelli A, Danieli GA, Bardutta A, Hoffman EP, Dalla Volta S, Angelini C: Heart involvement in muscular dystrophies due to sarcoglycan gene mutations. *Muscle & Nerve* 1999; 22: 473-479.
- [24] Mendell JR, Stedman H, Moore SA, Allamand V, Campbell KP, Wall C, Stolle C, Periquet I, Novak V, Wilson JW: Gene transfer trial for limb-girdle muscular dystrophy type 2D alpha-sarcoglycan deficiency. *Neurology* 2002; 58 (suppl 3): A169.
- [25] Muntoni F, Sewry C, Wilson L, Angelini C, Trevisan CP, Brambati B, Dubowitz V: Prenatal diagnosis in congenital muscular dystrophy. *Lancet* 1995; 354: 591.
- [26] Pegoraro E, Shinke RM, Arahata K, Hayashi Y, Stern H, Marks H, Glasberg MR, Carroll JE, Taber JW, Wessel HB, Bauserman SC, Marks WA, Toriello HV, Higgins JV, Appleton S, Schwartz L, Garcia CA, Hoffman EP: Detection of new paternal dystrophin gene mutations in isolated cases of dystrophinopathies in females. *Am J Hum Genet* 1994; 54: 989-1003.
- [27] Pegoraro E, Fanin M, Angelini C, Hoffman EP: Prenatal diagnosis in β -sarcoglycan muscular dystrophy. *Neuromusc Disord* 1999; 5: 323-325.
- [28] Roberds SL, Leturca F, Allamand V, Piccolo F, Jeanpierre M, Anderson RD, Lim LE, Lee JC, Tomé FMS, Romero NB, Fardeau M, Beckman JS, Kaplan JC, Campbell KP: Missense mutation in the adhalin gene linked to autosomal recessive muscular dystrophy. *Cell* 1994; 76: 625-633.
- [29] Sandri M, El Meslemani AH, Sandri C, Schjesling P, Vissing K, Andersen JL, Rossini K, Carraro U, Angelini C: Caspase 3 expression correlates with skeletal muscle apoptosis in Duchenne and facio-scapulo human muscular dystrophy. A potential target for pharmacological treatment? *J Neuropath Exp Neurol* 2001; 60 (3): 302-312.
- [30] Tinsley JM, Blake DJ, Zuellig RA, Daries KE: Increasing complexity of the dystrophin-associated protein complex. *Proc Natl Acad Sci* 1994; 91: 8307-8313.
- [31] Tomé FMS, Evangelisti T, Leclerc A, Sunader Y, Mande E, Estoumet B, Bavois A, Campbell KB,

Advances and perspectives in muscular dystrophies

- Fardeau M: Congenital muscular dystrophy with merosin deficiency. *CR Acad Sci* 1994; 317: 351-357.
- [32] Torrente Y, D'Angelo MG, Li Z, Del-Bo R, Corti S, Mericskay M, DeLiso A, Fassati A, Paulin D, Comi GP, Scarlato G, Bresolin N: Trans placental injection of somatic derived cells in mdx mouse for the correction of dystrophin deficiency. *Hum Mol Genet* 2000; 9: 1843-1852.
- [33] Yoshida A., Kobayashi K., Manya H., et al.: Muscular dystrophy and neuronal migration disorder caused by mutations in a glycosyl transferase, POMGnT1. *Developmental Cell* 2001; 1: 717-724.