



Review

Molecular etiopathogenesis of limb girdle muscular and congenital muscular dystrophies: Boundaries and contiguities

Michela Guglieri, Francesca Magri, Giacomo P. Comi*

Centro Dino Ferrari, Dipartimento di Scienze Neurologiche Università degli Studi di Milano, I.R.C.C.S. Ospedale Maggiore Policlinico, Milano, Italy

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Abstract

The muscular dystrophies are a heterogeneous group of inherited disorders characterized by progressive muscle wasting and weakness. These disorders present a large clinical variability regarding age of onset, patterns of skeletal muscle involvement, heart damage, rate of progression and mode of inheritance. Difficulties in classification are often caused by the relatively common sporadic occurrence of autosomal recessive forms as well as by intrafamilial clinical variability. Furthermore recent discoveries, particularly regarding the proteins linking the sarcolemma to components of the extracellular matrix, have restricted the gap existing between limb girdle (LGMD) and congenital muscular dystrophies (CMD). Therefore a renewed definition of boundaries between these two groups is required.

Molecular genetic studies have demonstrated different causative mutations in the genes encoding a disparate collection of proteins involved in all aspects of muscle cell biology. These novel skeletal muscle genes encode highly diverse proteins with different localization within or at the surface of the skeletal muscle fibre, such as the sarcolemmal muscle membrane (dystrophin, sarcoglycans, dysferlin, caveolin-3), the extracellular matrix (α 2 laminin, collagen VI), the sarcomere (telethonin, myotilin, titin, nebulin and ZASP), the muscle cytosol (calpain-3, TRIM32), the nucleus (emerin, lamin A/C) and the glycosylation pathway enzymes (fukutin and fukutin related proteins). The accumulating knowledge about the role of these different proteins in muscle pathology has led to a profound change in the original phenotype-based classification and shed new light on the molecular pathogenesis of these disorders.

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* Corresponding author. Dipartimento di Scienze Neurologiche Università degli Studi di Milano, Padiglione Ponti-Ospedale Maggiore Policlinico, Via Francesco Sforza 35, 20122 Milano, Italy. Tel.: +39 2 55033817; fax: +39 250320430.

E-mail address: giacomo.comi@unimi.it (G.P. Comi).

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1. Introduction

The identification of dystrophin, the protein that is absent or markedly reduced in skeletal muscle of Duchenne muscular dystrophy (DMD) patients, was the first step taken towards clarification of the molecular pathogenesis of muscular dystrophies [1]. Actually, the cloning of the gene encoding dystrophin and the finding of its role in skeletal muscle as bridge between cytoplasmic γ -actin and sarcolemmal proteins led to the identification of several dystrophin-associated glycoproteins.

The dystrophin glycoprotein complex (DGC) is a multicomponent complex of transmembrane, cytoplasmic and extracellular proteins that provides a strong mechanical link and mediates interactions be-

tween the intracellular cytoskeleton and the extracellular matrix ([2]—Fig. 1).

A large number of genes involved in muscular dystrophy encode components of the DGC. Actually, the striated muscle contraction requires that the myofibres remain intimately connected with the membrane and the extracellular matrix (ECM); mutations in components of the DGC are thought to lead to loss of sarcolemmal integrity and to render muscle fibres more susceptible to damage [3].

Recent studies suggest that several pathogenetic mechanisms determine muscular dystrophy, not only the loss of structural proteins, but also perturbation of sarcolemma repair mechanisms and enzymatic defects (Table 1). Moreover, it seems that also an involvement of vascular smooth muscle DGC is responsible for

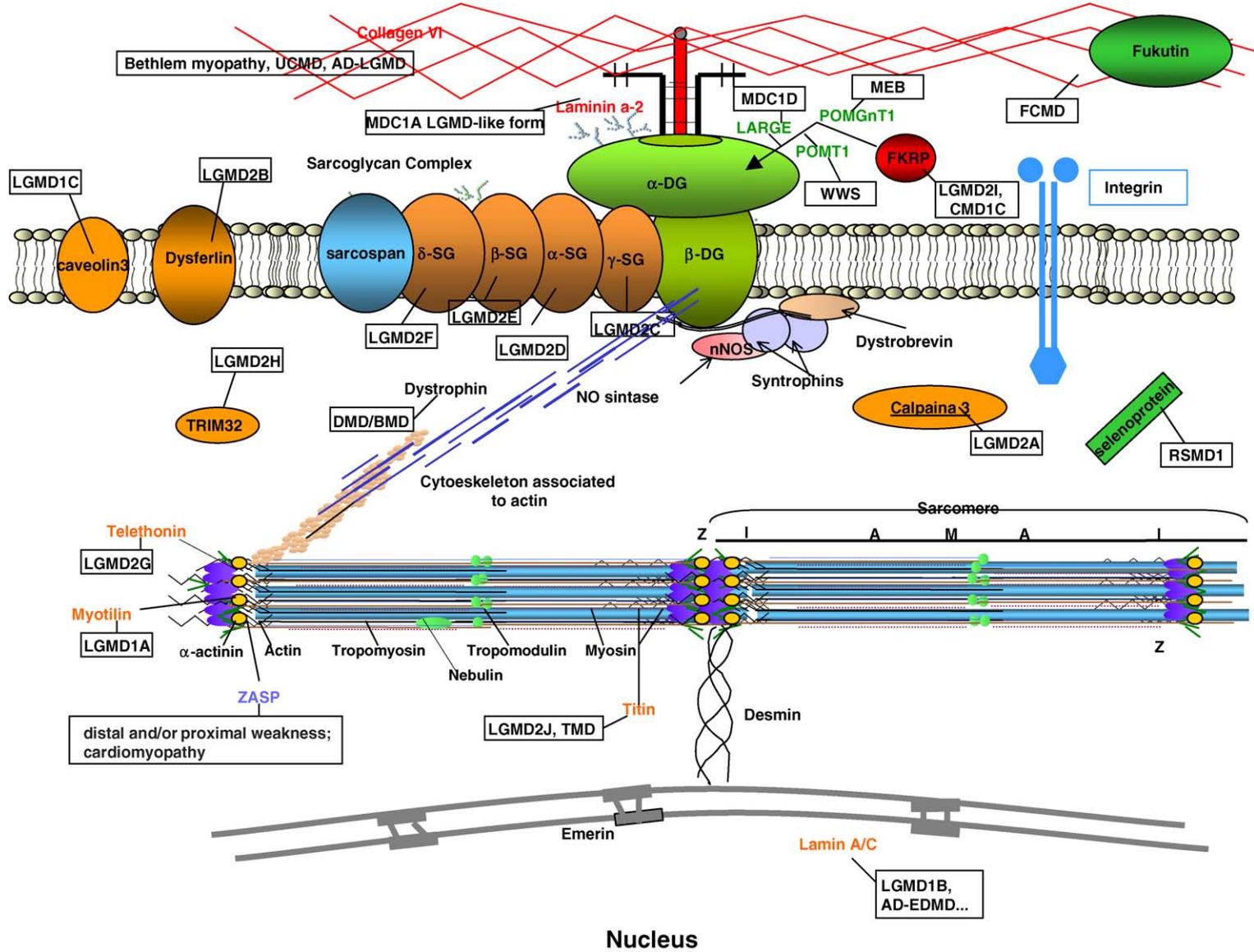


Fig. 1. Schematic diagram illustrating the location of proteins from the extracellular matrix, the sarcolemma, the sarcomere, the cytosol and the nucleus, involved in muscle limb girdle muscular (proteins in orange color) and congenital muscular dystrophies (proteins in green color). Proteins in red color are related to both disorders.

Table 1

Subcellular localization and function of muscular dystrophy proteins

	Subcellular localization	Gene	Gene location	Proposed function	Clinical phenotype
Laminin α 2	Extracellular matrix	LAMA2	6q2	Link α -DG and basal lamina	MDC1A and LGMD-like form with SNC alteration on MRI
Collagen VI	Extracellular matrix	COL6A1; COL6A2; COL6A3	21q22.3; 21q22.3; 2q37	Modulates cell proliferation and prevents apoptosis in fibroblast	Bethlem myopathy, UCMD, AD-LGMD?
Integrin α 7	Transmembrane	ITGA7	12q13	Link extracellular matrix and actin cytoskeleton; muscle differentiation during embryonic development	Mild congenital muscular dystrophy
Putative glycosyltransferase <i>O</i> -linked mannose 1,2 <i>N</i> -acetylglucosaminyltransferase	Golgi apparatus Transmembrane	LARGE POMGnT1	22q12.3–13.1 1p34–p33	Glycosylation of α -DG Glycosylation of α -DG	MDC1D MEB
Mannosyltransferase	Endoplasmic reticulum	POMT1	9q34.1	Glycosylation of α -DG	WWS
Fukutin	Golgi body	Fukutin gene	9q31	Glycosylation of α -DG	FMDC
Fukutin related protein	Golgi apparatus	FKRP	19q13.3	Glycosylation of α -DG	CMD1C, LGMD2I, MEB, WWB
α -Sarcoglycan	Sarcolemmal muscle membrane	ADL gene	17q12–q21.33	Mechano-signalling (stabilization of DCG)	LGMD2D
β -Sarcoglycan	Sarcolemmal muscle membrane	SGCB	4q12	Mechano-signalling (stabilization of DCG)	LGMD2E
δ -Sarcoglycan	Sarcolemmal muscle membrane	SGCD	5q33	Mechano-signalling (stabilization of DCG)	LGMD2F
γ -Sarcoglycan	Sarcolemmal muscle membrane	SGCG	13q12	Mechano-signalling (stabilization of DCG)	LGMD2C
Caveolin-3	Sarcolemmal muscle membrane	CAV3	3p25	Cell signalling (organising the T-tubule system during myogenesis)	LGMD1C
Dysferlin	Sarcolemmal muscle membrane	DYSF	2p13.3–p13.1	Membrane repair	LGMD2B, MM, DMAT
Calpain3	Muscle cytosol	CAPN3	15q15.1–q21.1	Enzymatic activity	LGMD2A; lower motor neuron signs
TRIM32	Muscle cytosol	TRIM32 gene	9q31–q34	Catalyzes transfer of Ub to target protein	LGMD2H
Lamin A/C	Nucleus	LMNA	1q21.2	Stabilization of nuclear membrane, cell differentiation	LGMD1B; AD-EDMD; cardiac conduction system disease; CMT2; mandibuloacral dysplasia, Hutchinson–Gilford progeria syndrome, atypical Werner syndrome
Titin	Sarcomer	TTN	2q24.3	Mechanical role in contraction, muscle elasticity	LGMD2J, TMD, DCM, HCM
Myotilin	Sarcomer	TTID	5q31	Mediates a correct assembly of contractile apparatus	LGMD1A
Telethonin	Sarcomer	TCAP	17q12	Phosphorylation of telethonin in early differentiating myocytes, integrity of sarcomeres	LGMD2G
Selenoprotein	Endoplasmic reticulum	SEPN1	1p36–p35	Enzymatic activity, preventing from oxidant damage	RSMD1, minicore congenital myopathy
ZASP	Sarcomer	ZASP	10q22.3–q23.2	Protein–protein interactions; maintenance of the Z-line during muscle contraction	Distal and/or proximal weakness; cardiomyopathy

Table 2
Clinical Features of LGMD

	Protein	Gene	Secondary proteins deficit	CK levels	Mean age of onset	Muscular groups involved	Cardiac involvement	Respiratory involvement	Rate of progression	Characteristic features
LGMD1A	Myotilin	TTID	Laminin c1	Normal–10×	18–40 years	Pelvic and shoulder girdle; often later distal weakness	Not described	Not described	Slow	Dysarthric speech, tight achilles tendons, rimmed vacuoles
LGMD1B	Lamin A/C	LMNA	Laminin b1 (probable)	normal–20×	4–38 years	Pelvic girdle; often later shoulder girdle	AV conduction defects, less frequently dilatative cardiomyopathy, sudden cardiac death	Not described	Variable, slow	Contractures
LGMD1C	Caveolin-3	CAV3	–	2–25×	<10 years	Pelvic and shoulder girdle	Hypertrophic cardiomyopathy (only in recent studies)	Not described	Slow to moderate	Calf hypertrophy, myalgia and muscle cramps
LGMD1D	?	6q/7q?	–	Normal–4×	15–50 years	Pelvic and shoulder girdle	Dilatative cardiomyopathy	Not described	Slow	Dysphagia
LGMD1E	?	7q31.1/6q22	–	–	Young adult life	Pelvic and shoulder girdle	–	–	Slow	–
LGMD1F	?	7q32.1–q32.2;	–	Normal–20×	1–58 years	Pelvic girdle; often later shoulder girdle and distal weakness	Not described	Described only in some cases	Fast to moderate	–
LGMD1G	?	4p21	–	Normal–10×	30 to 47 years	Pelvic and shoulder girdle; later fingers–toes flexion limitation	Not described	Not described	Slow	Fingers and toes flexion limitation, rimmed vacuoles; possible anticipation
LGMD2A	Calpain-3	CAPN3	–	Normal–50×	2–40 years	Pelvic girdle (glutei and hip adductors) and shoulder girdle	Not described	Described only in some cases	Variable, moderate	Adductor weakness, posterior thigh
LGMD2B	Dysferlin	DYSF	Calpain-3 and caveolin-3	10–150×	20–30 years	Pelvic and shoulder girdles/distal lower limb	Not described	Not described	Slow to moderate	Inflammatory cells in muscle biopsy
LGMD2C	γ-Sarcoglycan	SGCG	Other SG (mild)	5–120×	3–20 years	Pelvic and shoulder girdles	Frequent	Severe	Moderate	–
LGMD2D	α-Sarcoglycan	SGCA	Other SG (mild)	5–120×	3–20 years	Pelvic and shoulder girdles	Rare	Described	Fast	–
LGMD2E	β-sarcoglycan	SGCB	Other SG	5–120×	3–20 years	Pelvic and shoulder girdles	Dilatative cardiomyopathy	Described	Fast	–
LGMD2F	δ-sarcoglycan	SGCD	Other SG	5–120×	3–20 years	Pelvic and shoulder girdles	Dilatative cardiomyopathy	Described	Fast	–
LGMD2G	Telethonin	TCAP	–	Normal–30×	2–15 years	Proximal (4 limbs) and distal (only lower limb)	Described only in half member of 1 family	Not described	Slow to moderate	Distal atrophy of legs; rimmed vacuoles
LGMD2H	TRIM32	TRIM32	–	Normal–20×	15–30 years	Pelvic and shoulder girdle	Not described	Not described	Slow	Late facial muscles involvement; described only in Hutterite
LGMD2I	Fukutin related protein	FKRP	Laminin-a2, a-DG	5–100×	10–40 years	Pelvic and shoulder girdle	Left ventricular dysfunction or dilated cardiomyopathy	Described (diaphragm involvement)	Fast to moderate	Calf hypertrophy
LGMD2J	Titin	TTN	Calpain-3	Normal–2×	5–20 years	Pelvic and shoulder girdle; distal impairment described	Not described	Not described	Fast	Described only in Finns

muscle and cardiac damage in some limb girdle muscular dystrophies.

These recent findings improved our understanding and classification of these disorders, although some aspects remain unknown, for example the same protein loss may be involved in many clinical phenotypes with different age of onset, rate of progression and severity, as it occurs in dysferlinopathies [4].

Limb girdle muscular dystrophies (LGMD) and congenital muscular dystrophies (CMD) are two heterogeneous genetic disease groups differing in clinical

severity and age of onset [5,6]. Onset of symptoms of muscular involvement is at birth or within the first 6 months of life in CMD, whereas in LGMD muscle weakness and wasting can occur in late childhood, adolescence or even adult life (Tables 2 and 3). However recent studies outline the existence of an overlap between forms of LGMD and CMD due to alterations in essential components of the molecular bridge between sarcolemma and ECM [7].

Here we will follow essential features of skeletal muscle structure and function to describe both

Table 3
Clinical features of CMD

	Protein	Gene	CK levels	Secondary protein deficit	Mean age of onset	Muscular groups involved	Cardiac involvement
MDC1A	Merosin	LAMA2	+++	Integrin alpha7	At birth; described cases with late onset	Limb girdle and axial muscles	Sporadic (left ventricular hypokinesia, subclinical cardiomyopathy)
MDC1B	?	Not known locus1q42		Merosin	Early; possible also delayed onset	Limb girdle	Not described
MDC1C	Fukutin related protein	FKRP	+++	Merosin	At birth; described in adulthood in milder forms	Generalised	Frequent dilatative cardiomyopathy
MDC1D	LARGE	LARGE	++	–	At birth	Generalised	Mild cardiomyopathy described in mice
UCMD 1 2 3	Collagen VI	COL6A1, COL6A2, COL6A3	Normal–+	–	Early	Generalised	Not described
Bethlem Myopathy	Collagen VI	COL6A1, COL6A2, COL6A3		–	Prenatal but rarely in adulthood	Generalised when present	Rare (hypertrophic cardiomyopathy)
Integrin α 7 deficiency	Integrin α 7	ITGA1	Normal	–	At birth	Generalised	Not described
WWS	POMT1	POMT1	+++	–	At birth	Generalised	Not described
MEB	POMGnT1	POMGnT1	+++	–	Neonatal	Generalised	Not described
FMDC	Fukutin	FCMD	+++	Merosin and dystrophin-associated proteins	At birth	Generalised	Dilatative cardiomyopathy
RSMD1	Selenoprotein N,1	SEPN1	Normal–+	–	Childhood	Proximal and axial muscles	Rare right ventricular hypertrophy

LGMD and CMD. For classification and nomenclature, we will adopt names and acronyms as used in OMIM (Online Mendelian Inheritance in Man): in particular, the CMD abbreviation will be retained whenever describing clinical and/or laboratory findings of congenital muscular dystrophies, while the acronym MDC followed by type will be used to classify these disorders (for instance: MDC1A stands for muscular dystrophy, congenital, type 1A).

2. The extracellular matrix

The ECM plays an essential role in skeletal muscle because it provides reinforcement to the plasma membrane, contributes to elastic properties of the muscle fibres and defines the process of muscle regeneration. It also regulates cell signalling events by concentrating polypeptide growth factors and presenting them to cell surface receptors. Alterations in the structural link between the sarcolemma and the

Respiratory involvement	Brain involvement	MRI and macroscopical abnormalities	Rate of progression	Characteristic features
30% die in the first year of life for cardiopulmonary complications	Normal cognitive function; 30% epilepsy; rare mental retardation	Abnormal white-matter signal 5% occipital pachigiria or agyria	Severe; mild in late-onset LGMD-like form	Failure to thrive; aspirations pneumonia; motor demyelinating neuropathy
Early (diaphragmatic involvement)	Not described	Not described	Variable	Generalised muscular hypertrophy
Frequent (diaphragm involvement)	Normal cognitive function (mental retardation described only in two patients)	Rare structural alterations	Variable	CK elevated (20–75×); pseudohypertrophy of legs
Not described	Severe mental retardation	White matter abnormalities; neuronal migration defect	Severe	Only one case described
Invariably ventilatory insufficiency in the I or II decade	Not described	Normal	Variable (generally severe)	Proximal joints contractures; distal hyperextensibility; rigid spine; characteristic facial traits; hyperkeratosis
Rare (diaphragmatic paralysis)	Not described	Normal	Relatively mild and slow	Contractures of multiple joints; correlation age of onset/severity
Nor described	Variable	Normal	Mild	Only three cases described
Not described	Mental retardation	Neuronal migration defect	Severe (die <3ys)	Feeding difficulties; blindness and ocular malformations
Not described	Severe mental retardation; common epilepsy	Neuronal migration defect; transient dysmyelination	Severe but slow progression	Ocular involvement; correlation severity of CNS involvement/genotype
Frequent in middle–late tens	Severe mental retardation; epilepsy	Delay of myelination; cerebral and cerebellar cortical dysplasia	Variable, from several to mild	Meanly Japanese population, contractures, ocular involvement in 50%
Frequent (severe restrictive respiratory syndrome)	Not described	Normal	Mild (expect for respiratory failures)	Spinal rigidity and scoliosis; axial muscle weakness; nasal speech; specific muscular pattern at CT or MRI

extracellular matrix were originally identified in CMD; it is now clear that at least a relevant pathogenetic mechanism leading to some forms of CMD and LGMD may overlap.

The ECM consists in glycoproteins organised in a double layer: a diffuse collagen fibril network connected to the sarcolemma by the basal lamina [8].

2.1. Laminin $\alpha 2$

The link between α -dystroglycan (α -DG) and the basal lamina is mediated by laminins. These are proteins of the extracellular matrix composed of three different but homologous laminin chains, one α heavy chain and two light chains, β and γ . In skeletal muscle the predominant forms are laminin 2 (also named merosin) and laminin 4, which are composed by the same α and γ chain ($\alpha 2$ and $\gamma 1$) and two different β chain (respectively $\beta 1$ and $\beta 2$) [9].

The deficiency of $\alpha 2$ chain, due to mutations in LAMA2 gene located on chromosome 6q2, is responsible for MDC1A (muscular dystrophy, congenital, type 1A), which represents more than one third of congenital muscular dystrophies [10]. About 50% of patients with MDC1A show a primary total or partial deficiency of laminin $\alpha 2$ [11], which results in a dramatic perturbation of the basement membrane molecular architecture.

Total laminin $\alpha 2$ absence causes a severe form of CMD clinically characterized by profound hypotonia at birth or in the first few months of life, delayed motor milestones with ability to sit only at the age of 2–3 years and inability to reach independent ambulation. Most children present a motor demyelinating peripheral neuropathy, probably because the merosin is also expressed in Schwann cells [12]. The brain magnetic resonance imaging (MRI) shows abnormal T2-signals of the periventricular and subcortical white matter [13]. Cognitive function is usually normal but mental retardation and epilepsy can be present in a small proportion of patients who show structural brain changes (occipital pachygyria or agyria). Epilepsy is present in about one third of patients without brain abnormalities [14]. Up to 30% of the patients die during the first year of life of cardiopulmonary complications.

Although cases of laminin $\alpha 2$ partial deficiency associated with clinically severe phenotypes have been described [15], usually an incomplete loss of

this molecule causes a milder clinical form [16]. In past years, these relatively more benign conditions were thought to be linked to secondary laminin $\alpha 2$ deficiency, but recent studies have proved the presence of mutations in the LAMA2 gene in some late-onset, milder LGMD-like forms [17]. In these disorders, mutations do not result in complete absence of laminin $\alpha 2$, but in production of truncated proteins or in an increased proteolytic degradation. Also this milder clinical presentation is associated with abnormalities of cerebral and/or cerebellar white matter in T2 weighted MRI, cerebellar hypotrophy, and decreased motor and sensory nerve conduction velocities, which are features of the congenital forms.

2.2. Collagen VI

Collagen VI is a component of the extracellular matrix that does not directly belong to the group of dystrophin-associated proteins and that is expressed in a wide variety of tissues, such as skeletal muscle, skin and cartilage. It is a major cell-adhesive protein that forms a microfibrillar network in extracellular matrix, interacting with several proteins and seems to have a role in cell proliferation and in preventing apoptosis of fibroblasts [8,18]. It is composed of three different polypeptide chains ($\alpha 1$, $\alpha 2$ and $\alpha 3$), encoded by three genes (COL6A1 and COL6A2, located on chromosome 22, and COL6A3 mapping to chromosome 2).

Dominant mutations in genes encoding these molecules underlie the Bethlem myopathy, a relatively mild myopathy with generalised muscular weakness and wasting which present, as distinctive feature, contractures of multiple joints [19]. It presents intra-familial variability and different clinical onset, which range from a prenatal form with diminished foetal movements and congenital torcicolis, to a neonatal one with hypotonia and delayed milestones, a more frequent phenotype with onset in early childhood with limb girdle weakness or joint contractures or a rare adult-onset form [20]. Cardiac abnormalities and pulmonary involvement have been described, but they are supposed to be rare [21]. The progression of muscular symptoms is slow.

The pathophysiological defects and mechanisms leading to the myopathic disorder are not known but collagen VI myopathies could also have an unexpected mitochondrial pathogenesis, as suggested by the

presence of spontaneous apoptosis and ultrastructural alterations of sarcoplasmic reticulum (SR) and mitochondria [18].

Autosomal recessive mutations in all three α -chain genes of collagen VI can cause also a more severe form of MDC, named Ullrich muscular dystrophy (UMDC) or scleroatonic muscular dystrophy, which shares some clinical features, such as contractures, with Bethlem myopathy [22,23]. It is characterized by early onset of generalised muscular weakness, with a slow progression, contractures of multiple proximal joints, distal hyperextensibility [24] and rigid spine. Respiratory insufficiency with tendency toward recurrent lung infections has been described in several patients [25]. The subjects affected by UMDC have a normal intelligence and MRI shows a normal brain development. A variable deficiency of collagen VI is evidenced upon immunohistochemistry analysis of patients' muscle biopsy; the deficiency degree seems to correlate with the clinical severity [25].

A heterozygous in-frame deletion of the COL6A1 gene has been shown to cause a more severe form of UMDC [26] than mutations in COL6A2 and COL6A3. However some studies showed that a dominant mutation might also underlie the phenotype of UMDC [27]. Furthermore collagen VI involvement has been found in a significant part of UMDC (40%); however, the role of this molecule was excluded in a number of cases, suggesting genetic heterogeneity even of this condition [26].

Scacheri et al. [28] described missense mutations in COL6A1 and COL6A2 in patients suffering from an autosomal dominant limb girdle muscular dystrophy phenotype with mild to severe weakness without contractures. Their studies widened the clinical spectrum of Bethlem myopathy and suggested that autosomal dominant limb girdle muscular dystrophy should be studied for possible collagen VI etiology.

2.3. Integrins

Integrins are a large family of cell surface receptors that mediate a physical link between the extracellular matrix and the actin cytoskeleton. They are heterodimeric, transmembrane glycoproteins expressed in skeletal muscle, in particular at the sarcolemma, neuromuscular and myotendinous junction. They are essential for muscle differentiation during embryonic

development and are therefore strong candidate genes for unclassified forms of muscular dystrophies. In adult skeletal muscle, integrin $\alpha 7$ is the major form and its absence leads to destruction of the myotendinous junction, rather than compromised sarcolemmal integrity. Integrin $\alpha 7$ is reduced in several muscular dystrophies and mutations in the gene encoding this protein in human cause a mild congenital muscular dystrophy with normal laminin $\alpha 2$ chain expression [29].

3. The glycosylation pathway

Recently, the identification of a group of disease genes that encode putative glycosylation enzymes has underlined the importance of post-translational modification of proteins as a new area of focus for muscular dystrophy research [30]. The glycosylation-deficient muscular dystrophies appear to involve the *O*-glycosylation pathway and seem to be confined to a small number of proteins, while the congenital multi-systemic disorders of glycosylation are caused by defects in the better characterized and highly conserved *N*-glycosylation pathway.

Five conditions belonging to this group have been identified so far; they are characterized by mutations in proven or putative glycosyltransferase and share an abnormally glycosylated α -dystroglycan (α -DG). The α -DG undergoes to extensive *O*-glycosylation with apparent tissue diversity: defects of this biochemical mechanism have been found to be involved in muscular dystrophies. In fact α -DG is an important highly glycosylated component of DGC, expressed in skeletal muscle and in brain. The entire dystroglycan complex is encoded from a single gene, termed DAG1, located on chromosome 3p21 and it is post-translationally cleaved to produce two interacting subunits, α and β . α -DG is a membrane-associated extracellular glycoprotein which interacts with several matrix molecules, in particular with laminin 2. β -DG, a transmembrane protein, interacts with dystrophin, caveolin-3 and other cytoplasmic proteins involved in signal transduction.

The function of β -DG is unknown, but it has been reported to form a mechanical link between the F-actin cytoskeleton and the extracellular milieu and it is crucial for the structural stability of the plasma membrane. Both subunits undergo glycosylation, but

whereas β -DG has a constant molecular mass, the mass of α -DG varies owing to developmental and tissue-specific glycosylation of the core polypeptide [31]. *O*-glycosylation of α -DG mucin-like domain is essential for binding to ligands [32].

3.1. LARGE

The finding in myodystrophy mouse (myd mouse) of mutations in a gene (LARGE gene) encoding a putative glycosyltransferase [33] was rapidly followed by hypothesis that abnormal glycosylation of α -DG could be implicated in human muscular dystrophies.

The enzymatic activity of the LARGE-encoded protein has yet to be defined and its role in α -DG glycosylation is unclear. The human gene maps to chromosome 22q12.3–13.1. It is the fifth largest human gene and it is ubiquitously expressed particularly in heart, brain and skeletal muscle. The predicted protein product shows 98% amino-acid identity with its murine homologue [34].

Myodystrophy mouse presents a progressive muscular dystrophy with ocular defects and CNS involvement (disruption of the basal lamina and abnormal neuronal migration in the cerebral cortex, cerebellum and hippocampus).

Up to date mutations in the human LARGE gene were described only in a patient with a phenotype of congenital muscular dystrophy (MDC1D) associated to profound mental retardation and brain MRI showing extensive white matter abnormalities and subtle structural changes indicative of a neuronal migration defect. Immunoblotting of a muscle biopsy homogenate with an antibody against the α -DG glycosylated epitope demonstrated a reduced molecular weight form of α -DG, suggesting α -DG hypoglycosylation. Although similar to the myodystrophy mouse, the phenotype of this patient is in many respects considerably milder, reflecting probably different levels of LARGE activity [35,36]. LARGE therefore remains a good candidate for involvement in the whole range of muscular dystrophies.

Aberrant glycosylation of α -DG is the primary cause of several other forms of human congenital muscular dystrophies; also in these dystrophies, α -DG is expressed in a hypoglycosylated form and this is thought to lead to the dystrophic phenotype [37]. Defects in a number of putative glycosyltrans-

ferases were identified as causes of three recessive MDC: muscle–eye–brain disease (MEB), Walker Warburg syndrome (WWB) and Fukuyama congenital muscular dystrophy (FMDC), all characterized by severe muscle weakness and mental retardation [37].

3.2. POMGnT1

POMGnT1, a *O*-linked mannose β 1,2 *N*-acetylglucosaminyltransferase, catalyses the transfer of GlcNAc to *O*-mannose of glycoproteins, such as α -DG [38]. It is thought to have four domains: a N-terminal cytoplasmic tail, a transmembrane domain, a stem domain and a C-terminal catalytic domain, these two last are necessary for enzymatic activity [39].

Mutations in POMGnT1 gene cause loss of enzymatic activity, resulting in failure of *O*-mannosyl glycan synthesis [40], and are responsible for MEB, an autosomal recessive disorders, described for the first time in 1977 in Finland [41], characterized by congenital muscular dystrophy, ocular abnormalities (congenital myopia, congenital glaucoma, pallor of the optic discs, retinal hypoplasia) and brain type II lissencephaly [42]. This one is characterized by extensive neuronal migration disorder leading to pachygyria and polymicrogyria, brain hypoplasia and cerebellar dysgenesis [43] and is responsible for severe mental retardation and epileptic seizures. The patients usually present as floppy infants, but this severe hypotonia in some cases can be followed by progressive spasticity, correlated to the degree of contractures, which hints to a CNS origin of the motor dysfunction [42].

Recent studies, using monoclonal antibody directed against a carbohydrate epitope of α -DG, showed a selective deficiency of this protein in muscle biopsy of MEB patients [44], suggesting that α -DG is a potential target of POMGnT1 and that hypoglycosylation of this protein is a pathomechanism of MEB. *O*-mannosyl glycan is present in mammals in a limited number of glycoproteins of brain, nerve and skeletal muscle [45,46]. Defects of *O*-mannosyl-glycan in MEB patients greatly reduced affinities for α -DG with laminin [30–44].

3.3. POMT1

Marked alterations in the glycosylation of α -DG have been found also in muscle fibres of WWS, a very

severe, recessive form of MDC characterized by ocular and retinal abnormalities and by the same brain defects present in MEB [47]. It is caused by mutations in POMT1 gene which encodes a mannosyltransferase. The *O*-mannosylation is rare in mammals, as well, but it was described in human brain, skeletal muscle and nerve [44]. WWB is genetically heterogeneous and mutations in POMT1 have been identified only in about 20% of WWB patients [48]. Another candidate gene could be POMT2, which encodes a mannosyltransferase localised to the endoplasmic reticulum membrane, but it is expressed at low level in skeletal muscle, thus suggesting that other genes are implicated.

3.4. Fukutin

Fukuyama congenital muscular dystrophy (FMDC) is the second common form of childhood muscular dystrophy in Japan, after Duchenne muscular dystrophy [49]. It is due to mutations in the Fukutin gene, which encodes an enzyme that is likely to be involved in the modification of cell surface glycoproteins, although its function is still not clear [50]. This protein localises to the Golgi body and to secretory granules and appears also to play a role in neuronal migration. Highly glycosylated α -DG was found to be selectively deficient in the skeletal muscle of FMDC patients [50].

Clinically, mutations in the fukutin gene cause a severe muscular dystrophy in association with brain malformations (cerebral and cerebellar cortical dysplasia), profound mental retardation and ophthalmologic abnormalities. A clear correlation between genotype and phenotype seems to exist in this form: in fact, typical mild phenotypes are associated with homozygosity for a founder retrotransposon insertion, while compound heterozygotes tend to have a much more severe clinical presentation, presumably because of adverse effects on fukutin protein function in addition to a reduction in the amount of mRNA [51].

3.5. Fukutin related protein

On the basis of homology to the putative catalytic domain of fukutin, recently a new member of the fukutin protein family, highly expressed in heart and skeletal muscle, was identified, the fukutin related

protein (FKRP). It is localised to the Golgi apparatus and it is involved in modifying cell surface glycoproteins and glycolipids.

Mutations in the FKRP gene have been identified in patients with a severe form of MDC (MDC1C) and in a milder form of autosomal recessive LGMD (LGMD2I) [52]. MDC1C is clinically characterized by early onset, hypotonia after birth, muscle hypertrophy, severe weakness with inability to achieve independent ambulation, involvement also of facial muscle and possible left ventricular impairment; brain or ophthalmic alterations have not been described. The spectrum of LGMD2I phenotype is quite wide; the onset of symptoms, such as proximal muscle weakness, possible cardiac involvement (CI) and respiratory failure [53–56], can occur in childhood, adolescent or adult life. Poppe et al. [57], in a recent multicenter retrospective analysis, observe that CI in these patients is more frequent in male than in female and is characterized by left ventricular dysfunction with or without cardiac symptoms or segmental or global dilated cardiomyopathy. Respiratory failure supervenes often when the patients are still ambulant and it is frequently due to specific diaphragm involvement in contrast with the situation observed in dystrophinopathies or in other LGMDs (sarcoglycanopathies, calpainopathy) in which respiratory involvement occurs later and is often associated with important skeletal weakness, while early diaphragmatic weakness is not a common clinical feature [57–60].

A secondary deficiency of laminin α 2 in immunostaining of muscle biopsy sections is typical of MDC1C patient, but it is not frequently seen in muscle biopsy of LGMD2I patients, where a secondary reduction of calpain-3 and laminin β 1 was observed. The sarcolemma of both types of patients displays a variable reduction of α -DG staining and in its molecular weight on immunoblotting, but not of β -DG. This suggests that the abnormal expression of α -DG is a result of its altered glycosylation and this pathogenetic mechanism is responsible for muscular damage [31,37,61]. It is possible to suppose the existence of a tight correlation between the clinical phenotype and the α -DG expression on immunohistochemistry: patients with the severe phenotype of MDC1C display a profound depletion of α -DG, while LGMD2I patients show a variable expression of protein, proportional to the clinical severity. Both MDC1C and LGMD2I map to

an identical region on chromosome 19q13.3, suggesting that they may be allelic disorders. The FKRP mutations identified in LGMD2I patients are different from those seen in MDC1C and recent studies [62] identified also a correlation between clinical phenotype, α -DG expression and genotype. MDC1C patients are generally compound heterozygote for a null allele and a missense mutation or carry two missense mutations; patients with a relatively severe LGMD2I are compound heterozygotes between a common C826A (Leu276Ile) FKRP mutations and either a missense or a nonsense mutation; subjects with milder form of LGMD2I are almost invariably homozygous for the common FKRP mutation [62].

Interestingly, patients heterozygous for the common C826A mutation and a second different mutation were likely to develop cardiac involvement earlier than homozygous patients [54] though they do not show clear correlation between severity of cardiomyopathy and degree of muscle weakness.

Recently, mutations in FKRP gene have been shown to be responsible also for forms of MEB and WWB [63]. Moreover the muscle of these patients characteristically shows a marked alteration in the glycosylation of α -DG, but not β -DG.

4. Sarcolemmal muscle membrane

The sarcolemma is a highly organised and specialized cellular structure which forms a physical limit and mediates signalling events between the cell and the external environment. The maintenance of its integrity is an essential aspect for muscular function and since it is repeatedly involved in rounds of muscle contraction and relaxation it requires a highly efficient and continuous self-repair.

Mutations in genes encoding sarcolemmal proteins can be found in several patients affected from LGMDs; in particular sarcoglycans, dysferlin and caveolin-3 are the three major proteins involved.

4.1. Sarcoglycan

The sarcoglycan complex includes six transmembrane subunits with at least one glycosylation site. In skeletal and cardiac muscles the major sarcoglycan complex is composed of α , β , γ and δ subunits; β and

δ sarcoglycan forming a core to which α and γ sarcoglycan then bind. In vascular smooth muscle β and δ subunits form a complex with other two sarcoglycans (ϵ and ζ).

Studies in vitro demonstrate that before maturation and targeting to the sarcolemma the subunits must be assembled together to form a complex [64].

The precise function of this complex is not clear. Its extracellular part binds to the small proteoglycan biglycan and, either directly or indirectly through the biglycan, to α -DG [65,66]; on the intracellular side, the SG complex binds to filamin-C/filamin-2, a presumed actin crosslinker that is also closely associated with the Z-disk [67]. It seems to have an important role in stabilization of DCG, similar to sarcosplasm and α -DG at the sarcolemma, but has been described as a mechano-signalling complex, by the binding with γ -filamin.

The sarcoglycanopathies are usually the most severe forms of autosomal recessive LGMD, with early onset in childhood, confinement to wheelchair before the age of 16 years and frequent involvement of cardiac muscle. Nevertheless, milder phenotypes have been described in patients with missense or even nonsense mutations [68–71]. A dilatative cardiomyopathy is most frequent in patients with deficiency of β and δ -SG. Initial evidence suggested the role of the loss of SG complex in vascular smooth muscle (VSM) as an important factor implicated in the cardiac involvement present in LGMD2E and 2F. However, the demonstration of similar focal regions of ischaemic necrosis in γ -SG-deficient mouse models, which have an intact VSM sarcoglycan complex, showed that the pathology must therefore be intrinsic to the myocardium.

A mutation in one of the sarcoglycans results in destabilization of the entire complex and, as a consequence, in abnormal immunostaining for most or all of the sarcoglycans. In the majority of muscle biopsies from patients with a mutation in one of the SG genes, the primary loss or deficiency of any one of the four SGs leads to a secondary deficiency of the whole subcomplex [72–75]. This is frequently seen especially in primary deficiency of β and δ -SG, reflecting their position as the core of the complex; otherwise generally γ -SG deficiency leads to a partial preservation and a primary mutation in α -SG leads to a milder loss of the other three SG. To confirm the diagnosis it is always necessary to perform the genetic analysis.

In patients with primary SG mutations, especially in the gene encoding γ -SG, it is possible to find also a secondary reduction in dystrophin, suggesting that γ -SG may be the subunit that interacts more directly with dystrophin.

It has been described the existence of a correlation between gene mutation and clinical phenotype. Missense changes in both alleles generally cause an important reduction of the primary protein and a variable deficiency of the other SG-complex components; this pattern is usually associated with a severe clinical phenotype in LGMD2E and 2F, and with both mild and severe forms in LGMD2D. The severity in this last group seems to correlate with the residual amount of γ -SG in the muscle, despite the absence of the other three SGs, rather than with the kind of mutation [75].

α -Sarcoglycan (α -SG or adhalin) was the first protein of this complex to be studied. Deficiency of α -SG causes LGMD2D, an autosomal recessive muscular dystrophy, generally without cardiomyopathy. Human adhalin mRNA is most abundant in skeletal muscle and, at lower levels, in cardiac muscle and in lung, but it was not detected in brain. The mRNA present in cardiac muscle is shorter than that from skeletal muscle and lacks the base sequence encoding the transmembrane domain. The finding of lower expression of the α -SG gene in cardiac muscle may explain the less severe cardiac dysfunction in some patients with LGMD2D.

LGMD2E and 2F are caused by deficiency, respectively, of β and δ sarcoglycans and are both associated with cardiac involvement.

β -Sarcoglycan is expressed ubiquitously, although predominantly in muscle, while δ -SG is expressed in skeletal and heart muscle and, at a weaker level, in smooth muscle. δ -Sarcoglycan shows 70% identity at the amino acid level to both the human and rabbit γ -sarcoglycan sequences. The two isoforms of δ -SG in skeletal muscle differ in the C-terminal region [76]. SGCG mRNA has been found exclusively in skeletal and cardiac muscles.

The SGCG protein is a type II single-transmembrane protein with an extracellular C terminus; cytoplasmic and extracellular domains each contain 1 phosphorylation site. It has been reported that the C terminus of γ -SG is critical for the functioning of the entire sarcoglycan/sarcospan complex; its defect is responsible for LGMD2C.

4.2. Caveolin-3

Caveolin-3 (or M-caveolin) is the muscle-specific form of the caveolin protein family [77,78], which also includes caveolin-1 and -2, proteins expressed in most cell types, particularly in adipocytes, fibroblasts, endothelial and epithelial cells [79]. It is the principal integral membrane component of caveolae, small (50 to 100 nm) vesicular invagination of the plasma membrane implicated in cell signalling [80].

Caveolin-3 is expressed in both cardiac and skeletal muscles. It is associated with the dystrophin glycoprotein complex by the binding to the intracellular domain of β -DG. However, most caveolin-3 appears to be not directly associated with the DGC complex [81]. Caveolins act as scaffolding proteins to organise and concentrate specific caveolin-interacting lipids and proteins. It has been shown to directly bind nNOS and recently it was suggested to have a possible interaction with dysferlin [82]. One of the major functions of caveolae is to bring together membrane-based receptor molecules with their intracellular second messenger system; it has additional functions in organising the T-tubule system during myogenesis and in regulating numerous signalling pathways.

The human caveolin-3 gene (CAV3) maps on chromosome 3p25 and mutations in this gene cause muscle disorders, probably by interacting with caveolin oligomerization in a dominant negative manner causing disruption of caveolae formation at the muscle cell plasma membrane.

Four different phenotypes associated with CAV3 mutations have been described: limb girdle muscular dystrophy-1C (LGMD-1C), rippling muscle disease (RMD), and distal myopathy (DM), as well as idiopathic and familial hyperCKemia (HCK) [83–86].

Different clinical phenotypes are associated with the same CAV3 gene mutation in different families, suggesting that phenotypic heterogeneity cannot be ascribed to differences in expression levels of muscle proteins neither to the mutation; probably modifying factors are involved.

Caveolin-3 is expressed also in cardiac muscle. In the cardiac muscle of a patient with caveolinopathy, a normal or relatively decreased expression of caveolin-3 with preservation of caveolar structure had been observed [87]. These data suggest that some factors operating in preserving the caveolin-3 expression in

heart, in particular caveolin-2, may be involved. Nevertheless, a recent study has described a caveolin-3 mutation associated with hypertrophic cardiomyopathy [88]. Therefore, also cardiac involvement might depend upon mutation type or, more likely, variable genetic backgrounds.

4.3. Dysferlin

Dysferlin is a plasma membrane protein with an ubiquitous distribution, but most strongly expressed in skeletal muscle, heart and kidney. It is a member of the ferlin family, a large family of proteins with a conserved structure characterized by the presence of a single C-terminal transmembrane domain and multiple C2 domains which act as calcium-sensor mediating membrane fusion and vesicle trafficking events [89]. In skeletal muscle it is localised to the sarcolemma, as well as in cytoplasmic vesicles.

Dysferlin is the first identified member of the membrane repair machinery in skeletal muscle. Earlier studies showed that membrane repair requires the accumulation and fusion of vesicles near the site of membrane disruption, a process which is essential to avoid plasma fibre degeneration. More recently, it has been observed that dysferlin plays a role in vesicle trafficking and membrane fusion in muscle cells, binding its first C2 domain to phospholipids in a calcium-dependent manner [90]. Indeed Bansal et al. [91] demonstrated that isolated dysferlin-null muscle fibres are defective in calcium-dependent releasing of disrupted membranes and proposed a role of this protein in a new model of membrane repair [92]. According to this model, dysferlin facilitates vesicle docking and fusion with the plasma membrane by interacting with other dysferlin molecules and unknown proteins. In particular it has recently been demonstrated that dysferlin co-localises and interacts with annexin A1 and A2, two widely expressed phospholipid binding proteins. Consequently, a role for annexins in vesicle fusion during dysferlin-mediated membrane repair has been proposed [93].

Although dysferlin is not an integral component of the DGC, its distribution is altered in DGC-linked muscular dystrophies, in which its expression is reduced on the plasma membrane and increased in cytoplasmic vesicles [94]. This suggests that a func-

tional association between dysferlin and DGC may be possible. On the other side in muscle fibres of dysferlinopathic patients a structurally stable sarcolemma and a normal expression of DGC on muscle fibres were demonstrated. Also a possible association between dysferlin and caveolin-3 has been recently described [82], but it does not seem to be a constant finding [87–91].

Three distinct phenotypes, which differ in weakness distribution at onset, are associated with mutations in the gene encoding dysferlin: limb girdle muscular dystrophy 2B (LGMD2B), Miyoshi myopathy (MM) and distal anterior compartment myopathy (DMAT).

The former is a relatively mild disease with a late onset in the second or third decade and a predominantly proximal slowly progressive involvement of the pelvic and shoulder girdles [95], while the others present with a predominantly distal pattern.

MM is characterized by an early involvement of the posterior compartment of the lower limb [96] and DMAT differs from MM for a rapidly progressing onset in the anterior tibial muscles [97]. In these latter forms proximal lower and upper limb weakness often develops as the disease progresses. Although dysferlin is expressed also in cardiomyocytes, in dysferlin-deficient patients there is no evidence for cardiac muscle dysfunction.

Muscle biopsy of LGMD2B patients is often characterized by the expression of major histocompatibility complex class I (MHC-I) and the presence of inflammatory cells (more macrophages than T cells, and not T cytotoxicity); this infiltrating cells are always present although their number is considerably variable [98]. Mutations in dysferlin gene can determine both a partial or complete loss of protein.

One interesting and unclear feature is the heterogeneity of presentation of dysferlinopathy: the same mutation underlies all the clinical presentations in the same pedigree [99] and the same clinical presentation can be caused by different kind of mutation. The cause of this heterogeneity is unknown and could be genetic and/or environmental.

Future work is required to understand the function of each of these proteins in the membrane repair pathway. Our current knowledge suggests that there might be several other types of muscular dystrophies which will show similar defects.

5. The muscle cytosol

5.1. Calpain-3

LGMD2A, the most frequent form of recessive LGMD, is the first example of progressive muscular dystrophy caused by an enzymatic defect, although the mechanism whereby calpain mutations cause muscular dystrophies remains to be defined [100].

Calpain-3 is the skeletal muscle-specific member of the calpain family, a group of intracellular calcium-activated nonlysosomal cysteine proteases expressed in a ubiquitous or tissue-specific manner [101]. They are involved in a variety of signalling pathways, inducing irreversible modifications through limited proteolysis of specific targets [102,103]. Calpain-3 is a multidomain protein, characterized by three exclusive sequence inserts (NS, IS1, IS2) and four domains; domain I has regulatory role, domain II is the proteolytic module, domain III has a C2-domain like Ca^{2+} -binding function (probably involved in Ca^{2+} -dependent translocation of calpain to the membrane), and domain IV binds Ca^{2+} ions.

The precise function of calpain-3, as well as the identification of its substrates and its activation mechanism, remains unknown. Several molecules with regulatory functions have been reported to be substrates for calpains in cell culture, but various lines of research suggested that calpains may be involved in cytoskeletal or myofibrillar protein degradation [100,104,105]. It is unlikely that calpain-3 is implicated in myoblast fusion, but it might act in the disassembly of myofibrils during early stages of turnover [105] or it can cause a post-fusion defect in muscle maturation [106].

Taveau et al. [100] propose that calpain-3-mediated cleavages modify muscle properties, and enable it to display efficient physiological response to external and/or internal stimuli. It may be a regulatory enzyme which would act on a pathway involving transcription factors controlling survival genes. The importance of calpain-3 in muscle homeostasis has been pointed out by the observation that its deficiency is associated with a perturbation of the antiapoptotic pathway of $\text{I}\kappa\text{B}\kappa/\text{NF-}\kappa\text{B}$ in skeletal muscle [107–109].

Although calpain-3 substrates are unknown, some studies [110] demonstrated that it binds to filamin-C and, through this protein, to γ - and δ -sarcoglycans, Z-

disc proteins myotilin and myozenin. In this way calpain-3 participates in the processes of cytoskeleton re-modelling, being implicated in myoblast fusion and repair [67,111,112]; its deficiency leads to disruptions of the regulation of protein–protein interactions at the sarcolemma or Z-line of sarcomere. Calpain cleavage of annexins may be critical to patch formation and/or membrane insertion [93].

Clinically the disease caused by calpain-3 deficiency is not usually severe and may be extremely mild. The age of onset is extremely variable: it is reported from 8 to 15 years, with a range from 2 to 40 years, in one study [113], while a most recent analysis performed on 175 genetically confirmed patients showed a range from 2 to 49 years [114]. Pelvic girdle weakness is present and symptomatic from the onset, beginning in the glutei and the hip adductors but even relatively late in the course of the disease. Calf hypertrophy is apparently rare in European patients, but it has been described in several Brazilian families. Classically there is no mental, heart or facial involvement, while respiratory complications have been reported. The rate of deterioration varies between families, but intrafamilial variation is less marked than is frequently reported for other LGMD, such as the sarcoglycanopathies.

The muscle biopsy shows a dystrophic pattern and a variable deficiency of calpain-3 expression on muscle fibres, even if one study [115] describes, in few preclinical cases, an unusual pattern, with isolated fascicles of degenerating fibres in an almost normal muscle. The diagnosis of LGMD2A is currently based on protein analysis, but patients carrying mutations and displaying normal protein expression at the Western blot analysis have also been identified. Fanin et al. [116] described this condition in a considerable proportion (20%) of the LGMD2A population, suggesting that some mutations impair protein activity by affecting interdomain protein interaction, or reduce autocatalytic activity by lowering the Ca^{2+} sensitivity.

Same authors also showed a correlation between the severity of muscle histopathology and the clinical phenotype: patients with an active dystrophic process have severe form of muscular dystrophy, whereas patients with mild pathological changes show a slowly progressive myopathy. A direct correlation between the residual amount of calpain-3 on WB analysis and the severity of the phenotype has not been demon-

strated [117]. The probability of molecular diagnosis of calpainopathy is very high in the presence of characteristic clinical phenotype of LGMD2A and absence of calpain 3 protein in WB analysis. However, when both criteria are missing, the diagnostic probability is lower and the phenotype alone seems to be a more accurate factor than WB analysis to obtain a correct diagnosis [114].

The gene responsible for LGMD2A (CAPN3) maps to chromosome 15q15.1–q15.3. Interestingly, the majority of the identified mutations are concentrated in only six exons (1, 2, 4, 5, 11 and 22) [118]. Genotype–phenotype correlations are difficult except in patients who are homozygous for a mutation: as a general rule, patients with homozygous null mutations tend to have a more severe clinical course than patients presenting missense mutations [117–119].

Secondary reduction of calpain-3 has also been demonstrated in other forms of LGMD, as LGMD2I, LGMD 2B, and in other muscular dystrophies. Recently, Starling et al. [120] identified mutations in the CAPN3 gene in patients with normal serum CK, lower motor neuron signs and a neurogenic pattern on electromyography.

5.2. TRIM32

TRIM32 is a member of the tripartite-motif family, named for the presence of three motives: a B1 box, a coiled-coil domain and a RING-finger domain. The domain structure of the TRIM32 protein, described for the first time by Frosk et al. [121], suggests that it may be an E3-ubiquitin ligase implicated in the ubiquitin–proteasome pathway. It probably catalyses a transfer of ubiquitin to target proteins which are recognized by factors associated with the 26S proteasome.

The gene encoding this protein maps on chromosomal region 9q31q34 [121], in the same region of fukutin gene, responsible for FMDC. To date only a missense mutation (Asp487Asn) has been identified as implicated in a relative mild form of autosomal recessive LGMD (LGMD2H), reported only in Manitoba Hutterites population of North America [122–124]. This mutation occurs in an NHL highly conserved domain, but the pathogenetic mechanism through which mutations in TRIM32 gene lead to muscular damage is still not clear. The effect of the mutation could be the degradation of wrong proteins

or the accumulation of proteins normally degraded in the proteasome system. The restriction of symptoms to muscle tissue could be explained by a NHL domain-mediated interaction with muscle-specific proteins or by a specific muscle toxicity of increased level of this protein.

LGMD2H patients present a variable phenotype, characterized by onset usually in the second or third decade with proximal muscle weakness, slow progression and ambulation preserved until the sixth decade in most patients. No cardiac and systemic involvement has been reported, although some patients present facial muscle weakness as the disease progresses.

6. The nucleus

6.1. Lamin A/C

Lamin A and C are ubiquitously expressed multifunctional intermediate filaments of the inner nuclear membrane, encoded by the same gene (LMNA gene) through alternative splicing of a single transcript [125].

Lamin A/C has so far been shown to be involved in a range of quite divergent but also overlapping phenotypes, including autosomal dominant Emery–Dreifuss muscular dystrophy (AD-EDMD) [126], cardiomyopathy with conduction system disease [127], autosomal recessive axonal polyneuropathy (CMT2) [128], mandibuloacral dysplasia [129], Hutchinson–Gilford progeria syndrome [130,131], and atypical Werner syndrome [132], as well as rare cases of autosomal dominant LGMD (LGMD1B) [133].

The functions of lamin A/C and the mechanism of how mutations in this protein cause clinically distinct and/or tissue-specific diseases are not well understood. Lamins form homo- and heteropolymers which associate with other integral nuclear membrane proteins into a network that supports the nuclear membrane [134,135], providing its stability. By their interaction with chromatin, they maintain the chromatin compartmentation required for differentiation of myoblasts into myocytes [136] and regulate the gene expression mainly during cell differentiation. In conclusion, it seems that the lamin A/C has not only a structural function, but also with other nuclear proteins determines the functional diversity and dynamic properties of the nuclear envelope, regulating cell di-

vision, integrity of nuclei and nuclear pore complex and in chromatin structure [137,138].

LGMD1B is a rare form of dystrophy characterized by cardiac involvement with atrioventricular conduction defects and less frequently dilated cardiomyopathy, which usually manifest at the onset of muscular symptoms and increase with age. Untreated patients may eventually die for sudden cardiac arrest [139,140]. The muscular weakness is mild and slowly progressive and some patients present uncommon contractures.

Mutations in LMNA gene are also responsible for the autosomal dominant form of Emery–Dreifuss dystrophy (AD-EDMD), clinically characterized by a progressive muscular weakness with humero-peroneal distribution, tendon contractures and arrhythmia and/or cardiomyopathy.

LGMD1B and AD-EDMD have several clinical features in common with X-linked EDMD, caused by emerin deficiency. This correlates with the demonstration that lamin A/C and emerin interact in nuclear membrane.

7. The sarcomere

7.1. Titin

Titin, also known as connectin, is the biggest single peptide found in humans and the third most abundant protein after myosin and actin. It is a central sarcomeric myofilament, expressed in heart and skeletal muscle, localised besides the thick myosin and thin actin filaments and spanning half of the sarcomere from Z-line to M-line [141,142].

Titin consists of four structurally distinct regions—the M-line, A-band, I-band and Z-line—that mirror the several parts of the sarcomere and can have different functions [143,144]. In particular they have the ability to bind to many other sarcomere proteins, among them telethonin α -actinin, obscurin, calmodulin, myosin and myosin binding protein C, actin, nebulin and calpain-3. In mature muscle, one of the main tasks of titin is to maintain thick filaments centrally in the sarcomere during cycles of contraction and extension. This is important, because it ensures development of balanced forces between both halves of the sarcomere by myosin [145].

It plays a mechanical role, keeping the contractile element of skeletal muscle in place, and controls the assembly of the contractile proteins actin and myosin and the resting length of the sarcomere during muscle development. In mature muscle, titin is responsible, in particular through its PEVK region, for muscle elasticity [144–146] and consequently for the operating range of sarcomere lengths and tension-related biochemical processes [147]. It has at least two different binding sites for calpain-3 [148] and has been suggested that it has a role in stabilizing it from autolytic degradation.

Titin is responsible for limb girdle muscular dystrophy type 2J (LGMD2J), human skeletal tibial muscular dystrophy (TMD), dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM) [149].

The first is a clinically severe autosomal recessive form of LGMD described in a consanguineous Finnish family, characterized by onset in the first to third decade with progressive weakness of all proximal muscles, determining disability with loss of ambulation within 20 years [149,150]. Some patients develop late distal muscle weakness, but facial muscle involvement or cardiomyopathy has not been described.

TMD is an autosomal dominant late-onset distal myopathy, also observed in Finnish patients, characterized by slowly progressive weakness and atrophy usually confined to the anterior compartment of the lower leg muscle without cardiac involvement.

Titin maps on chromosome 2q24.3. It was suggested that the same mutation in the titin gene, TTN (Leu→Pro in exon Mex6 of the gigantic TTN gene), can cause LGMD2J, when present in homozygous form, and TMD if present in heterozygosis; so the severe LGMD phenotype could be the homozygous manifestation of a dominant gene that in the heterozygous state causes the milder distal myopathy [151]. This mutation affects the ultimate C terminus of protein, functionally located in the M-line of the sarcomere, in contrast to the mutations responsible for cardiomyopathy that were indeed located in the cardiac-specific N2-B region of titin, which occurs only in cardiac isoforms of protein.

In muscle biopsies from a patient with LGMD2J or TMD haplotype, Haravuori et al. [152] found almost complete loss of calpain-3, suggesting that the loss of

calpain-3 is a secondary downstream effect of deficiency of the TMD gene protein and results in phenotypic overlap with LGMD2A.

7.2. Myotilin

Myotilin is a thin filament-associated Z-disc protein, composed of a unique serine-rich NH₂ terminus and a COOH terminus with two Ig-like domains, which show a high sequence homology to Ig-domains of titin [153]. It binds α -actinin, via its N-terminal region, γ -filamin, with its C-terminal domain and F-actin [112–154] and it is expressed in striated muscles, heart and peripheral nerves [153].

The function of myotilin in skeletal muscle is highlighted by the finding of mutations in the human myotilin gene in patients with an autosomal dominant form of limb girdle muscular dystrophy (LGMD1A) [155]. By its interactions with sarcomeric structural components, myotilin plays an indispensable role in stabilization and anchorage of thin filaments, which may be a prerequisite for correct Z-disc organisation. The expression of myotilin starts in relatively late time of muscle differentiation, when the myofibrillar alignment appears, suggesting an important role of this protein for correct assembly of contractile apparatus [112].

LGMD1A has been described only in two families (an Argentinian and a North American of German descendent family), which members presented a typical limb girdle phenotype with autosomal dominant inheritance, later progressing to include distal weakness and associated to dysarthric speech in approximately half of the affected individuals [153–156]. Although myotilin protein is expressed at relatively high levels in the heart, there is no evidence of a cardiac defect in either known pedigree.

Muscle biopsy of affected individuals shows a dystrophic pattern and a large number of rimmed vacuoles: their presence in patients with unlinked myopathies should suggest the diagnosis of LGMD1A [156]. Patches of striking Z-line streaming are similar to those observed in nemaline myopathy. Schroder et al. [157] demonstrated a strong myotilin immunoreactivity of nemaline rods and central core lesions in congenital nemaline myopathy and central core disease, indicating a possible common pathophysiological element in these inherited muscle diseases.

7.3. Telethonin

Telethonin is the first sarcomeric protein shown to be associated with an autosomal recessive LGMD [158]. It is localised to the Z-disc of striated and cardiac muscles, where it interacts with the C-terminal domain of titin [145]. It has been proposed that telethonin is one of the substrates of titin, which phosphorylates its C-terminal domain in early differentiating myocytes [159]. In cardiac myocytes, it has been shown that both titin and telethonin are required for the structural integrity of sarcomeres. Nevertheless, the ultrastructural analysis of muscle of LGMD2G patients with mutations in the telethonin gene shows the maintenance of the sarcomeric architecture, suggesting that muscle degeneration and the clinical phenotype are more likely due to a functional defect than to alteration of sarcomeric structure. On the other hand, the presence of rimmed vacuoles in the muscle fibres from these patients might be accounted for focal sarcomeric degeneration [160]. All other proteins involved in muscular dystrophies are normally expressed in LGMD2G skeletal muscle.

LGMD2G has been described only in four Brazilian families [158]. The age at onset ranged from 2 to 15 years with marked weakness and atrophy in the distal muscles of the legs and proximal involvement of the four limbs. Some patients presented pronounced symmetric or asymmetric calf hypertrophy. Heart involvement was observed only in half member of one family [158], while extraocular and facial muscles were spared in all patients.

Two different mutations were identified in these four families; both gave rise to premature stop codons resulting in truncated proteins lacking the C-terminal domain, which is usually phosphorylated by titin kinase [158].

7.4. ZASP

A recent study identified mutations in the gene that encodes for ZASP, a Z-disk-associated protein, in patients with a pathologically and clinically progressive myopathy, suggesting that zaspopathy can also be classified as a muscular dystrophy [161]. ZASP is a Z-band alternatively spliced PDZ motif-containing protein encoded by a gene (the ZASP gene) consisting of 16 exons and located on chromosome 10q22.3–q23.2

[162]. It is expressed predominantly in cardiac and skeletal muscle [163] where it binds to α -actinin by the PDZ domain at the N terminus and to PKCs by the LIM domain at the C terminus [164]. In muscle ZASP participates to protein–protein interactions and in mouse it has been demonstrated to have an essential role for maintenance of the Z-line during muscle contraction.

Previous studies demonstrated that mutations in ZASP gene cause severe skeletal and cardiac myopathies with fragmented Z-disk in mice; dilated cardiomyopathy is described in humans [165,166].

Recently Selcen et al. [161] described the presence of mutations in this gene also in patients that showed a histological pattern of myofibrillar myopathy (pleomorphic hyaline, granular and amorphous deposits, accumulation of amyloid material) and electronic features of disintegration of the Z-disk. This form is characterized by a late onset and an autosomal dominant inheritance. Clinically the majority of patients analysed in this study present both distal and proximal weaknesses (in most cases distal is more prominent than proximal impairment), but in some patients a singular muscular group involvement has been described. Some patients have a cardiac impairment (paroxysmal supraventricular tachycardia, low ejection fraction, unusual depolarisation, prolonged QT), but none present respiratory failure. Clinical, electromyographic or histological peripheral neuropathy can also be present.

8. LGMD1 D-G

In recent years other four forms of autosomal dominant LGMD have been described (LGMD 1 D-G) [167–171]. The genes and the proteins implicated have not been identified yet, but it is known that they map in different chromosomic regions and that the implicated genes are different from the ones responsible for other AD-LGMD.

9. Unclassified disorders

Among LGMD forms, as well as in congenital phenotypes, there are some conditions in search for a genetic defect. Reliable data about the relative fre-

quency of unclassified LGMD are not available, at the moment. Since different populations seem to have a different distribution of the various LGMD, also the unclassified fraction might be different in different countries [74].

As far as congenital phenotypes are concerned, there is a number of patients with clinical features similar to MDC, in which no mutations in known genes were identified. This group includes: (1) WWS or CMD patients with α -DG depletion, but no mutations in any of the glycosyltransferases identified so far, sometimes with marked inflammatory changes at the muscle biopsy [172]; (2) Rigide Spine Syndrome cases with no mutation in selenoprotein N gene (SEPN1), the gene responsible for rigid spine muscular dystrophy-1 (RSMD1) [173]; (3) autosomal recessive MDC1B, a form observed in a consanguineous family from the United Arab Emirates, which presents severe diaphragmatic involvement responsible for early respiratory failure, rigidity of the spine, intellect and brain imaging normality [174,175]; (4) other MDC associated with cerebellar hypoplasia and megacysterna magna [176]; (5) a variant of UMDC with mental retardation and no mutations in the collagen VI genes.

10. Conclusions

Different pathogenetic mechanisms acting at every relevant biological site of the myofibre may lead to muscular dystrophies with variable age of onset, progression and other tissue involvement. A simplified diagnostic algorithm, based on clinical and molecular findings, is provided in Fig. 2.

The area of overlap between mechanisms leading to LGMD and those leading to CMD is mainly restricted to defects that involve the correct interaction between muscle fibre and extracellular matrix, as it occurs with integrin α 7, collagen VI and α -DG glycosylation defects. The direct link between the cytoskeleton and ECM (in terms of force transmission during muscle contraction and/or as a signalling pathway) is therefore essential to muscle integrity. It should be also noted that the ECM constituents represent the niche for satellite cells [177]: perturbations of these constituents may contribute to a severe reduction of the muscle regenerative capacity, therefore

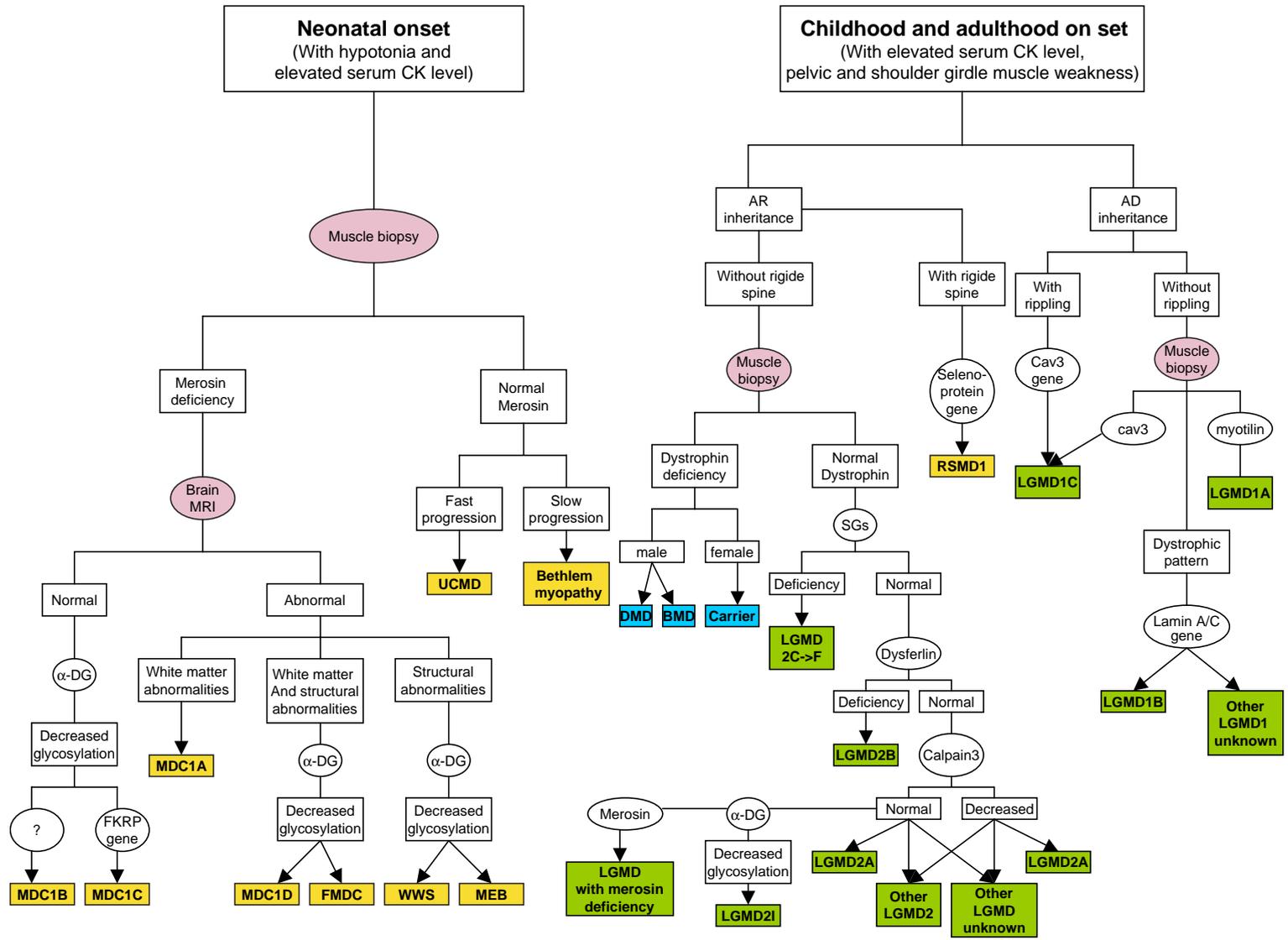


Fig. 2. Diagnostic algorithm of congenital and later-onset muscular dystrophies. For abbreviations, see the text.

resulting in early onset or congenital phenotypes. Since identification of the CMD and LGMD genes can be predicted to be exhaustive within shortly, more work will probably be focused on the pathogenesis of these untreatable disorders.

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References

- [1] Baumbach LL, Chamberlain JS, Ward PA, Farwell NJ, Caskey CT. Molecular and clinical correlations of deletions leading to Duchenne and Becker muscular dystrophies. *Neurology* 1989;39:465–74.
- [2] Beskrovnaya I, Ervasti JM, Leveille CJ, Slaughter CA, Sennett SW, Campbell KP. Primary structure of dystrophin-associated glycoproteins linking dystrophin to the extracellular matrix. *Nature* 1992;355(6362):696–702.
- [3] Matsumura K, Ohlendieck K, Ionasescu VV, et al. The role of the dystrophin-glycoprotein complex in the molecular pathogenesis of muscular dystrophies. *Neuromuscul Disord* 1993;3:533–5.
- [4] Illarioshkin SN, Ivanova-Smolenskaya IA, Greenberg CR, et al. Identical dysferlin mutation in limb-girdle muscular dystrophy type 2B and distal myopathy. *Neurology* 2000;55:1931–3.
- [5] Laval SH, Bushby KMD. Limb-girdle muscular dystrophies—from genetics to molecular pathology. *Neuropathol Appl Neurobiol* 2004;30:91–105.
- [6] Muntoni F, Voit T. The congenital muscular dystrophies in 2004: a century of exciting progress. *Neuromuscul Disord* 2004;14:635–49.
- [7] Kirschner J, Bonnemann CG. The congenital and limb-girdle muscular dystrophies: sharpening the focus, blurring the boundaries. *Arch Neurol* 2004;61:189–99.
- [8] Caldwell CJ, Matthey DL, Weller RO. Role of the basement membrane in the regeneration of skeletal muscle. *Neuropathol Appl Neurobiol* 1990;16:225–38.
- [9] Sewry CA, Chevally M, Tome FM. Expression of laminin subunits in human fetal skeletal muscle. *Histochem J* 1995; 27:497–504.
- [10] Dubowitz V, Fardeau M. Proceedings of the 27th ENMC sponsored workshop on congenital muscular dystrophy. 22–24 April 1994, The Netherlands. *Neuromuscul Disord* 1995; 5:253–8.
- [11] Helbling-Leclerc A, Zhang X, Topaloglu H, et al. Mutations in the laminin alpha 2-chain gene (LAMA2) cause merosin-deficient congenital muscular dystrophy. *Nat Genet* 1995;11:216–8.
- [12] Shorer Z, Philpot J, Muntoni F, Sewry C, Dubowitz V. Demyelinating peripheral neuropathy in merosin-deficient congenital muscular dystrophy. *J Child Neurol* 1995;10: 472–5.
- [13] Philpot J, Topaloglu H, Pennock J, Dubowitz V. Familial concordance of brain magnetic resonance imaging changes in congenital muscular dystrophy. *Neuromuscul Disord* 1995;5: 227–31.
- [14] Deodato F, Sabatelli M, Ricci E, et al. Hypermyelinating neuropathy, mental retardation and epilepsy in a case of merosin deficiency. *Neuromuscul Disord* 2002;12:392–8.
- [15] Nissinen M, Helbling-Leclerc A, Zhang X, et al. Substitution of a conserved cysteine-996 in a cysteine-rich motif of the laminin alpha2-chain in congenital muscular dystrophy with partial deficiency of the protein. *Am J Hum Genet* 1996;58:1177–84.
- [16] Mora M, Moroni I, Uziel G, et al. Mild clinical phenotype in a 12-year-old boy with partial merosin deficiency and central and peripheral nervous system abnormalities. *Neuromuscul Disord* 1996;6:377–81.
- [17] Naom I, D’Alessandro M, Sewry CA, et al. Laminin alpha 2-chain gene mutations in two siblings presenting with limb-girdle muscular dystrophy. *Neuromuscul Disord* 1998;8: 495–501.
- [18] Irwin WA, Bergamin N, Sabatelli P, et al. Mitochondrial dysfunction and apoptosis in myopathic mice with collagen VI deficiency. *Nat Genet* 2003;35:367–71.
- [19] Bethlem J, Wijngaarden GK. Benign myopathy, with autosomal dominant inheritance. A report on three pedigrees. *Brain* 1976;99:91–100.
- [20] Jobsis GJ, Boers JM, Barth PG, De Visser M. Bethlem myopathy: a slowly progressive congenital muscular dystrophy with contractures. *Brain* 1999;122:649–55.
- [21] Haq R, Speer MC, Chu ML, Tandan R. Respiratory muscle involvement in Bethlem myopathy. *Neurology* 1999;52: 174–6.
- [22] Vanegas OC, Bertini E, Zhang R-Z, et al. Ullrich scleroatonic muscular dystrophy is caused by recessive mutations in collagen type VI. *Proc Natl Acad Sci* 2001;98: 7516–21.
- [23] Pepe G, Bestini E, Bonaldo P, et al. Bethlem myopathy (BETHLEM) and Ullrich scleroatonic muscular dystrophy: 100th ENMC international workshop, 23–24 November 2001, Naarden, The Netherlands. *Neuromuscul Disord* 2002;12:984–93.
- [24] Furukawa T, Toyokura Y. Congenital, hypotonic-sclerotic muscular dystrophy. *J Med Genet* 1977;14:426–9.
- [25] Mercuri E, Yuva Y, Brown SC, et al. Collagen VI involvement in Ullrich syndrome: a clinical, genetic, and immunohistochemical study. *Neurology* 2002;58:1354–9.
- [26] Pan T-C, Zhang R-Z, Sudano D, Marie SK, Bonnemann CG, Chu M-L. New molecular mechanism for Ullrich congenital muscular dystrophy: a heterozygous in-frame deletion in the

- COL6A1 gene causes a severe phenotype. *Am J Hum Genet* 2003;73:355–69.
- [27] Baker NL, Morgelin M, Peat R, Goemans N, North KN, Bateman JF. Dominant collagen VI mutations are a common cause of Ullrich congenital muscular dystrophy. *Hum Mol Genet* 2005;14:279–93.
- [28] Scacheri PC, Gillanders EM, Subramony SH, et al. Novel mutations in collagen VI genes: expansion of the Bethlem myopathy phenotype. *Neurology* 2002;58:26593–602.
- [29] Hayashi YK, Chou FL, Engvall E, et al. Mutations in the integrin alpha7 gene cause congenital myopathy. *Nat Genet* 1998;19:94–7.
- [30] Michele DE, Barresi R, Kanagawa M, et al. Post-translational disruption of dystroglycan–ligand interactions in congenital muscular dystrophies. *Nature* 2002;418:417–22.
- [31] Hewitt JE, Grewal PK. Glycosylation defects in inherited muscle disease. *Cell Mol Life Sci* 2003;60:251–8.
- [32] Henry MD, Campbell KP. Dystroglycan inside and out. *Curr Opin Cell Biol* 1999;11:602–7.
- [33] Grewal PK, Hewitt JE. Mutation of Large, which encodes a putative glycosyltransferase, in an animal model of muscular dystrophy. *Biochim Biophys Acta* 2002;1573:216–24.
- [34] Peyrard M, Seroussi E, Sandberg-Nordqvist AC, et al. The human LARGE gene from 22q12.3–q13.1 is a new, distinct member of the glycosyltransferase gene family. *Proc Natl Acad Sci U S A* 1999;96:598–603.
- [35] Grewal PK, Holzfeind PJ, Bittner RE, Hewitt JE. Mutant glycosyltransferase and altered glycosylation of alpha-dystroglycan in the myodystrophy mouse. *Nat Genet* 2001;28:151–4.
- [36] Congman L, Brockington M, Torelli S, et al. Mutations in the human LARGE gene cause MDC1D, a novel form of congenital muscular dystrophy with severe mental retardation and abnormal glycosylation of alpha-dystroglycan. *Hum Mol Genet* 2003;12:2853–61.
- [37] Muntoni F, Brockington M, Blake DJ, Torelli S, Brown SC. Defective glycosylation in muscular dystrophy. *Lancet* 2002;360:1419–21.
- [38] Aruto Y, Kobayashi K, Manya H, et al. Muscular dystrophy and neuronal migration disorder caused by mutations in a glycosyltransferase, POMGnT1. *Dev Cell* 2001;1:717–24.
- [39] Akasaka-Manya K, Manya H, Kobayashi K, Toda T, Endo T. Structure–function analysis of human protein O-linked manose beta1,2-N-acetylglucosaminyltransferase 1, POMGnT1. *Biochem Biophys Res Commun* 2004;320:39–44.
- [40] Haltia M, Leivo I, Somer H, et al. Muscle–eye–brain disease: a neuropathological study. *Ann Neurol* 1997;41:173–80.
- [41] Santavuori P, Leisti J, Kruus S. Muscle, eye and brain disease: a new syndrome. *Neuropadiatrie* 1977;8:550.
- [42] Santavuori P, Somer H, Sainio K, et al. Muscle–eye–brain disease (MEB). *Brain Dev* 1989;11:147–53.
- [43] Dobyns WB, Truwit CL. Lissencephaly and other malformations of cortical development: 1995 update. *Neuropediatrics* 1995;26:132–47.
- [44] Kano H, Kobayashi K, Herrmann R, et al. Deficiency of alpha-dystroglycan in muscle–eye–brain disease. *Biochem Biophys Res Commun* 2002;291:1283–6 [Erratum in: *Biochem Biophys Res Commun* 293 (2002) 1579].
- [45] Endo T, Toda T. Glycosylation in congenital muscular dystrophies. *Biol Pharm Bull* 2003;26:1641–7.
- [46] Endo T. O-mannosyl glycans in mammals. *Biochim Biophys Acta* 1999;1473:237–46.
- [47] Cormand B, Pihko H, Bayes M, et al. Clinical and genetic distinction between Walker–Warburg syndrome and muscle–eye–brain disease. *Neurology* 2001;56:1059–69.
- [48] De Bernabe DB, Van Bokhoven H, Van Beusekom E, et al. A homozygous nonsense mutation in the fukutin gene causes a Walker–Warburg syndrome phenotype. *J Med Genet* 2003;40:845–8.
- [49] Fukuyama Y, Osawa M, Suzuki H. Congenital progressive muscular dystrophy of the Fukuyama type—clinical, genetic and pathological considerations. *Brain Dev* 1981;3:1–29.
- [50] Hayashi YK, Ogawa M, Tagawa K, et al. Selective deficiency of alpha-dystroglycan in Fukuyama-type congenital muscular dystrophy. *Neurology* 2001;57:115–21.
- [51] Kondo-Iida E, Kobayashi K, Watanabe M, et al. Novel mutations and genotype–phenotype relationships in 107 families with Fukuyama-type congenital muscular dystrophy (FMDC). *Hum Mol Genet* 1999;8:2303–9.
- [52] Brockington M, Blake DJ, Brown SC, Muntoni F. The gene for a novel glycosyltransferase is mutated in congenital muscular dystrophy MDC1C and limb girdle muscular dystrophy 2I. *Neuromuscul Disord* 2002;12:233–4.
- [53] Brockington M, Yuva Y, Prandini P. Mutations in the fukutin-related protein gene (FKRP) identify limb girdle muscular dystrophy 2I as a milder allelic variant of congenital muscular dystrophy MDC1C. *Hum Mol Genet* 2001;10:2851–9.
- [54] Poppe M, Cree L, Bourke J, et al. The phenotype of limb-girdle muscular dystrophy type 2I. *Neurology* 2003;60:1246–51.
- [55] Mercuri E, Brockington M, Straub V, et al. Phenotypic spectrum associated with mutations in the fukutin-related protein gene. *Ann Neurol* 2003;53:537–42.
- [56] Dohna-Schwake C, Ragette R, Mellies U. Respiratory function in congenital muscular dystrophy and limb girdle muscular dystrophy 2I. *Neurology* 2004;60:513–4.
- [57] Poppe M, Bourke J, Eagle M, et al. Cardiac and respiratory failure in limb-girdle muscular dystrophy 2I. *Ann Neurol* 2004;56:738–41.
- [58] Philipps MF, Quinlivan RCM, Edwards RHT, Calverley PMA. Changes in spirometry over time as a prognostic marker in patients with Duchenne muscular dystrophy. *Am J Respir Crit Care Med* 2001;164:2191–4.
- [59] Politano L, Nigro V, Passamano L. Evaluation of cardiac and respiratory involvement in sarcoglycanopathies. *Neuromuscul Disord* 2001;11:178–85.
- [60] Pollitt C, Anderson LV, Pogue R. The phenotype of calpainopathy: diagnosis based on a multidisciplinary approach. *Neuromuscul Disord* 2001;11:287–96.
- [61] Michele DE, Campbell KP. Dystrophin-glycoprotein complex: post-translational processing and dystroglycan function. *J Biol Chem* 2003;278:15457–60.

- [62] Brown SC, Torelli S, Brockington M, et al. Abnormalities in alpha-dystroglycan expression in MDC1C and LGMD2I muscular dystrophies. *Am J Pathol* 2004;164:727–37.
- [63] Beltran-Valero de Bernabe D, Voit T, Longman C, et al. Mutations in the FKRP gene can cause muscle-eye-brain disease and Walker-Warburg syndrome. *J Med Genet* 2004;41:61.
- [64] Holt KH, Campbell KP. Assembly of the sarcoglycan complex. *J Biol Chem* 1998;273:34667–70.
- [65] Bowe MA, Mendis DB, Fallon JR. The small leucinerich repeat proteoglycan binds to alpha-dystroglycan and is upregulated in dystrophic muscle. *J Cell Biol* 2000;148:801–10.
- [66] Crosbie RH, Heighway J, Venzke DP, Lee JC, Campbell KP. Sarcospan, the 25-kDa transmembrane component of the dystrophin-glycoprotein complex. *J Biol Chem* 1997;272:31221–4.
- [67] Thompson TG, Chan YM, Hack AA, et al. Filamin 2 (FLN2): a muscle-specific sarcoglycan interacting protein. *J Cell Biol* 2000;148:115–26.
- [68] Lim LE, Duclos F, Broux O, et al. Beta-sarcoglycan: characterization and role in limb-girdle muscular dystrophy linked to 4q12. *Nat Genet* 1995;11:257–65.
- [69] McNally EM, Passos-Bueno MR, Bonnemann CG, et al. Mild and severe muscular dystrophy caused by a single gamma-sarcoglycan mutation. *Am J Hum Genet* 1996;59:1040–7.
- [70] Duclos F, Broux O, Bourg N, et al. Beta-sarcoglycan: genomic analysis and identification of a novel missense mutation in the LGMD2E Amish isolate. *Neuromuscul Disord* 1998;8:30–8.
- [71] Passos-Bueno MR, Moreira ES, Marie SK, et al. Main clinical features of the three mapped autosomal recessive limb-girdle muscular dystrophies and estimated proportion of each form in 13 Brazilian families. *J Med Genet* 1996;33:97–102.
- [72] Vainzof M, Passos-Bueno MR, Pavanello RC, Marie SK, Oliveira AS, Zatz M. Sarcoglycanopathies are responsible for 68% of severe autosomal recessive limb-girdle muscular dystrophy in the Brazilian population. *J Neurol Sci* 1999;164:44–9.
- [73] Hack AA, Groh ME, McNally EM. Sarcoglycans in muscular dystrophy. *Microsc Res Tech* 2000;48:167–80.
- [74] Bonnemann CG. Limb-girdle muscular dystrophies: an overview. *J Child Neurol* 1999;14:31–3.
- [75] Vainzof M, Passos-Bueno MR, Canovas M, et al. The sarcoglycan complex in the six autosomal recessive limb-girdle muscular dystrophies. *Hum Mol Genet* 1996;5:1963–9.
- [76] Nigro V, De Sa Moreira E, Piluso G, et al. Autosomal recessive limb-girdle muscular dystrophy, LGMD2F, is caused by a mutation in the delta-sarcoglycan gene. *Nat Genet* 1996;14:195–8.
- [77] Okamoto T, Schlegel A, Scherer PE, Lisanti MP. Caveolins, a family of scaffolding proteins for organizing “preassembled signaling complexes” at the plasma membrane. *J Biol Chem* 1998;273:5419–22.
- [78] Razani B, Schlegel A, Lisanti MP. Caveolin proteins in signaling, oncogenic transformation and muscular dystrophy. *J Cell Sci* 2000;113:2103–9.
- [79] Scherer PE, Lewis RY, Volonte D. Cell-type and tissue-specific expression of caveolin-2. Caveolins 1 and 2 co-localize and form a stable hetero-oligomeric complex in vivo. *J Biol Chem* 1997;272:29337–46.
- [80] Schlegel A, Volonte D, Engelman JA, et al. Crowded little caves: structure and function of caveolae. *Cell Signal* 1998;10:457–63.
- [81] Crosbie RH, Yamada H, Venzke DP, Lisanti MP, Campbell KP. Caveolin-3 is not an integral component of the dystrophin glycoprotein complex. *FEBS Lett* 1998;427:279–82.
- [82] Matsuda C, Hayashi YK, Ogawa M, et al. The sarcolemmal proteins dysferlin and caveolin-3 interact in skeletal muscle. *Hum Mol Genet* 2001;10:1761–6.
- [83] Minetti C, Sotgia F, Bruno C, et al. Mutations in the caveolin-3 gene cause autosomal dominant limb-girdle muscular dystrophy. *Nat Genet* 1998;18:365–8.
- [84] Betz RC, Schoser BG, Kasper D, et al. Mutations in CAV3 cause mechanical hyperirritability of skeletal muscle in rippling muscle disease. *Nat Genet* 2001;28:218–9.
- [85] Tateyama M, Aoki M, Nishino I, et al. Mutation in the caveolin-3 gene causes a peculiar form of distal myopathy. *Neurology* 2002;58:323–5.
- [86] Carbone I, Bruno C, Sotgia F, et al. Mutation in the CAV3 gene causes partial caveolin-3 deficiency and hyperCKemia. *Neurology* 2000;54:1373–6.
- [87] Cagliani R, Bresolin N, Prella A, et al. A CAV3 microdeletion differentially affects skeletal muscle and myocardium. *Neurology* 2003;61:1513–9.
- [88] Hayashi T, Arimura T, Ueda K, et al. Identification and functional analysis of a caveolin-3 mutation associated with familial hypertrophic cardiomyopathy. *Biochem Biophys Res Commun* 2004;313:178–84.
- [89] Bashir R, Britton S, Strachan T, et al. A gene related to *Caenorhabditis elegans* spermatogenesis factor fer-1 is mutated in limb-girdle muscular dystrophy type 2B. *Nat Genet* 1998;20:37–42.
- [90] Davis DB, Doherty KR, Delmonte AJ, McNally EM. Calcium-sensitive phospholipid binding properties of normal and mutant ferlin C2 domains. *J Biol Chem* 2002;277:22883–8.
- [91] Bansal D, Miyake K, Vogel SS, et al. Defective membrane repair in dysferlin-deficient muscular dystrophy. *Nature* 2003;423:168–72.
- [92] Bansal D, Campbell KP. Dysferlin and the plasma membrane repair in muscular dystrophy. *Trends Cell Biol* 2004;14:206–13.
- [93] Lennon NJ, Kho A, Bacskai BJ, Perlmutter SL, Hyman BT, Brown Jr RH. Dysferlin interacts with annexins A1 and A2 and mediates sarcolemmal wound-healing. *J Biol Chem* 2003;278:50466–73.
- [94] Piccolo F, Moore SA, Ford GC, Campbell KP. Intracellular accumulation and reduced sarcolemmal expression of dysferlin in limb-girdle muscular dystrophies. *Ann Neurol* 2000;48:902–12.
- [95] Kawabe K, Goto K, Nishino I, Angelini C, Hayashi YK. Dysferlin mutation analysis in a group of Italian patients with

- limb-girdle muscular dystrophy and Miyoshi myopathy. *Eur J Neurol* 2004;11:657–61.
- [96] Anderson L, Davison K, Moss J. Dysferlin is a plasma membrane protein and is expressed early in human development. *Hum Mol Genet* 1999;8:855–61.
- [97] Illa I, Serrano Munuera C, Gallardo E, et al. Distal anterior compartment myopathy: a dysferlin mutation causing a new muscular dystrophy phenotype. *Ann Neurol* 2001;49:130–4.
- [98] Confalonieri P, Oliva L, Andreetta F, et al. Muscle inflammation and MHC class I up-regulation in muscular dystrophy with lack of dysferlin: an immunopathological study. *J Neuroimmunol* 2003;142:130–6.
- [99] Weiler T, Bashir R, Anderson LV, et al. Identical mutation in patients with limb girdle muscular dystrophy type 2B or Miyoshi myopathy suggests a role for modifier gene(s). *Hum Mol Genet* 1999;8:871–7.
- [100] Taveau M, Bourg N, Sillon G, Roudaut C, Bartoli M, Richard I. Calpain 3 is activated through autolysis within the active site and lyses sarcomeric and sarcolemmal components. *Mol Cell Biol* 2003;23:9127–35.
- [101] Ueyama H, Kumamoto T, Fujimoto S, Murakami T, Tsuda T. Expression of three calpain isoform genes in human skeletal muscles. *J Neurol Sci* 1998;155:163–9.
- [102] Sorimachi H, Ishiura S, Suzuki K. Structure and physiological function of calpains. *Biochem J* 1997;328:721–32.
- [103] Combaret L, Bechet D, Claustre A, Taillandier D, Richard I, Attaix D. Down-regulation of genes in the lysosomal and ubiquitin–proteasome proteolytic pathways in calpain-3-deficient muscle. *Int J Biochem Cell Biol* 2003;35:676–84.
- [104] Poussard S, Duvert M, Balcerzak D, et al. Evidence for implication of muscle-specific calpain (p94) in myofibrillar integrity. *Cell Growth Differ* 1996;7:1461–9.
- [105] Huang J, Fosberg NE. Role of calpain in skeletal-muscle protein degradation. *Proc Natl Acad Sci U S A* 1998;95:12100–5.
- [106] Spencer MJ, Guyon JR, Sorimachi H, et al. Stable expression of calpain 3 from a muscle transgene in vivo: immature muscle in transgenic mice suggests a role for calpain 3 in muscle maturation. *Proc Natl Acad Sci U S A* 2002;99:8874–9.
- [107] Fardeau M, Hillaire D, Mignard C, et al. Juvenile limb-girdle muscular dystrophy. Clinical, histopathological and genetic data from a small community living in the Reunion Island. *Brain* 1996;119:295–308.
- [108] Baghdiguian S, Martin M, Richard I, et al. Calpain 3 deficiency is associated with myonuclear apoptosis and profound perturbation of the IkappaB alpha/NF-kappaB pathway in limb-girdle muscular dystrophy type 2A. *Nat Med* 1999;5:503–11 [Erratum in: *Nat Med* 5 (1999) 849].
- [109] Richard I, Broux O, Allamand V, et al. Mutations in the proteolytic enzyme calpain 3 cause limb-girdle muscular dystrophy type 2A. *Cell* 1995;81:27–40.
- [110] Guyon JR, Kudryashova E, Potts A, et al. Calpain 3 cleaves filamin C and regulates its ability to interact with gamma- and delta-sarcoglycans. *Muscle Nerve* 2003;28:472–83.
- [111] Takada F, Vander Woude DL, Tong HQ, et al. Myozenin: an alpha-actinin- and gamma-filamin-binding protein of skeletal muscle Z lines. *Proc Natl Acad Sci* 2001;98:1595–600.
- [112] Salmikangas P, van der Ven PF, Lalowski M, et al. O. Furst, Carpen. Myotilin, the limb-girdle muscular dystrophy 1A (LGMD1A) protein, cross-links actin filaments and controls sarcomere assembly. *Hum Mol Genet* 2003;12:189.
- [113] Topaloglu H, Dincer P, Richard I, et al. Calpain-3 deficiency causes a mild muscular dystrophy in childhood. *Neuropediatrics* 1997;28:212–6.
- [114] Saenz A, Leturcq F, Cobo AM, et al. LGMD2A: genotype–phenotype correlations based on a large mutational survey on the calpain 3 gene. *Brain* 2005;128:732–42.
- [115] Vainzof M, de Paula F, Tsanaclis AM, Zatz M. The effect of calpain 3 deficiency on the pattern of muscle degeneration in the earliest stages of LGMD2A. *J Clin Pathol* 2003;56:624–66.
- [116] Fanin M, Nascimbeni AC, Fulizio L, Trevisan CP, Meznaric-Petrusa M, Angelin C. Loss of calpain-3 autocatalytic activity in LGMD2A patients with normal protein expression. *Am J Pathol* 2003;163:1929–36.
- [117] Anderson LV, Davison K, Moss JA. Characterization of monoclonal antibodies to calpain 3 and protein expression in muscle from patients with limb-girdle muscular dystrophy type 2A. *Am J Pathol* 1998;153:1169–79.
- [118] Chae J, Minami N, Jin Y, et al. Calpain-3 gene mutations: genetic and clinico-pathologic findings in limb girdle muscular dystrophy. *Neuromuscul Disord* 2001;11:547–55.
- [119] Richard I, Roudaut C, Saenz A. Calpainopathy: a survey of mutations and polymorphisms. *Am J Hum Genet* 1999;64:1524–40.
- [120] Starling A, de Paula F, Silva H, Vainzof M, Zatz M. Calpainopathy: how broad is the spectrum of clinical variability? *J Mol Neurosci* 2003;21:233–6.
- [121] Frosk P, Weiler T, Nylen E, Sudha T, Greenberg CR, Morgan K. Limb-girdle muscular dystrophy type 2H associated with mutation in TRIM32, a putative E3-ubiquitin-ligase gene. *Am J Hum Genet* 2002;70:663–72.
- [122] Weiler T, Greenberg CR, Nylen E, et al. Limb girdle muscular dystrophy in Manitoba Hutterites does not map to any of the known LGMD loci. *Am J Med Genet* 1997;72:363–8.
- [123] Shokeir MH, Rozdilsky B. Muscular dystrophy in Saskatchewan Hutterites. *Am J Med Genet* 1985;22:487–93.
- [124] Shokeir MH, Kobrinsky NL. Autosomal recessive muscular dystrophy in Manitoba Hutterites. *Clin Genet* 1976;9:197–202.
- [125] Lin F, Worman HJ. Structural organization of the human gene encoding nuclear lamin A and nuclear lamin C. *J Biol Chem* 1993;268:16321–6.
- [126] Bonne G, Di Barletta MR, Varnous S. Mutations in the gene encoding lamin A/C cause autosomal dominant Emery–Dreifuss muscular dystrophy. *Nat Genet* 1999;21:285–8.
- [127] Fatkin D, MacRae C, Sasaki T. Missense mutations in the rod domain of the lamin A/C gene as causes of dilated cardiomyopathy and conduction-system disease. *N Engl J Med* 1999;341:1715–24.
- [128] De Sandre-Giovannoli A, Chaouch M, Kozlov S. Homozygous defects in LMNA, encoding lamin A/C nuclear-enve-

- lope proteins, cause autosomal recessive axonal neuropathy in human (Charcot–Marie–Tooth disorder type 2) and mouse. *Am J Hum Genet* 2002;70:726–36.
- [129] Novelli G, Muchir A, Sangiuolo F. Mandibuloacral dysplasia is caused by a mutation in LMNA-encoding lamin A/C. *Am J Hum Genet* 2002;71:426–31.
- [130] Eriksson M, Brown WT, Gordon LB. Recurrent de novo point mutations in lamin A cause Hutchinson–Gilford progeria syndrome. *Nature* 2003;423:293–8.
- [131] De Sandre-Giovannoli A, Bernard R, Cau P. Lamin A truncation in Hutchinson–Gilford progeria. *Science* 2003;300:2055.
- [132] Chen L, Lee L, Kudlow BA. LMNA mutations in atypical Werner’s syndrome. *Lancet* 2003;362:440–5.
- [133] Muchir A, Bonne G, van der Kooij AJ. Identification of mutations in the gene encoding lamins A/C in autosomal dominant limb girdle muscular dystrophy with atrioventricular conduction disturbances (LGMD1B). *Hum Mol Genet* 2000;9:1453–9.
- [134] Sakaki M, Koike H, Takahashi N, et al. Interaction between emerin and nuclear lamins. *J Biochem* 2001;129:321–7.
- [135] Stuurmann N, Heins S, Aebi U. Nuclear lamins: their structure, assembly, and interactions. *J Struct Biol* 1998;122:42–66.
- [136] Favreau C, Higuier D, Courvalin JC, Buendia B. Expression of a mutant lamin A that causes Emery–Dreifuss muscular dystrophy inhibits in vitro differentiation of C2C12 myoblasts. *Mol Cell Biol* 2004;24:1481–92.
- [137] Moir RD, Spann TP, Goldman RD. The dynamic properties and possible functions of nuclear lamins. *Int Rev Cytol* 1995;162:141–82.
- [138] Dechat T, Gotzmann J, Stockinger A, et al. Detergent-salt resistance of LAP2alpha in interphase nuclei and phosphorylation-dependent association with chromosomes early in nuclear assembly implies functions in nuclear structure dynamics. *EMBO J* 1998;17:4887–902.
- [139] van der Kooij AJ, Ledderhof TM, de Voogt WG, et al. A newly recognized autosomal dominant limb girdle muscular dystrophy with cardiac involvement. *Ann Neurol* 1996;39:636.
- [140] van der Kooij AJ, van Meegeen M, Ledderhof TM, McNally EM, de Visser M, Bolhuis PA. Genetic localisation of a newly recognized autosomal dominant limb-girdle muscular dystrophy with cardiac involvement (LGMD1B) to chromosome 1q11–21. *Am J Hum Genet* 1997;60:891.
- [141] Gregorio CC, Trombitas K, Centner T, et al. The NH2 terminus of titin spans the Z-disc: its interaction with a novel 19-kD ligand (T-cap) is required for sarcomeric integrity. *J Cell Biol* 1998;143:1013–27.
- [142] Trinick J. Titin and nebulin: protein rulers in muscle? *Trends Biochem Sci* 1994;19:405–9.
- [143] Granzier H, Labeit S. Cardiac titin: an adjustable multi-functional spring. *J Physiol* 2002;541:335–42.
- [144] Labeit S, Kolmerer B. Titins: giant proteins in charge of muscle ultrastructure and elasticity. *Science* 1995;270:293–6.
- [145] Horowitz R, Kempner ES, Bisher ME, Podolsky RJ. A physiological role for titin and nebulin in skeletal muscle. *Nature* 1986;323:160–4.
- [146] Ma K, Wang K. Interaction of nebulin SH3 domain with titin PEVK and myopalladin: implications for the signaling and assembly role of titin and nebulin. *FEBS Lett* 2002;532:273–8.
- [147] Itoh-Satoh M, Hayashi T, Nishi H, et al. Titin mutations as the molecular basis for dilated cardiomyopathy. *Biochem Biophys Res Commun* 2002;291:385–93.
- [148] Tskhovrebova L, Trinick J. Titin: properties and family relationships. *Nat Rev Mol Cell Biol* 2003;4:679–89 [Review].
- [149] Hackman JP, Vihola AK, Udd AB. The role of titin in muscular disorders. *Ann Med* 2003;35:434–41.
- [150] Udd B, Kaarianen H, Somer H. Muscular dystrophy with separate clinical phenotypes in a large family. *Muscle Nerve* 1991;14:1050–8.
- [151] Udd B. Limb-girdle type muscular dystrophy in a large family with distal myopathy: homozygous manifestation of a dominant gene? *J Med Genet* 1992;29:383–9.
- [152] Haravuori H, Vihola A, Straub V, et al. Secondary calpain3 deficiency in 2q-linked muscular dystrophy: titin is the candidate gene. *Neurology* 2001;56:869–77.
- [153] Salmikangas P, Mykkanen OM, Gronholm M, Heiska L, Kere J, Carpen O. Myotilin, a novel sarcomeric protein with two Ig-like domains, is encoded by a candidate gene for limb-girdle muscular dystrophy. *Hum Mol Genet* 1999;8:1329–36.
- [154] van der Ven PF, Wiesner S, Salmikangas P, et al. Indications for a novel muscular dystrophy pathway. gamma-filamin, the muscle-specific filamin isoform, interacts with myotilin. *J Cell Biol* 2000;151:235–48.
- [155] Hauser MA, Horrigan SK, Salmikangas P, et al. Myotilin is mutated in limb girdle muscular dystrophy 1A. *Hum Mol Genet* 2000;9:2141–7.
- [156] Hauser MA, Conde CB, Kowaljow V, et al. Myotilin mutation found in second pedigree with LGMD1A. *Am J Hum Genet* 2002;71:1428–32.
- [157] Schroder R, Reimann J, Salmikangas P, et al. Beyond LGMD1A: myotilin is a component of central core lesions and nemaline rods. *Neuromuscul Disord* 2003;13:451–5.
- [158] Moreira ES, Wiltshire TJ, Faulkner G, et al. Limb-girdle muscular dystrophy type 2G is caused by mutations in the gene encoding the sarcomeric protein telethonin. *Nat Genet* 2000;24:163–6.
- [159] Mayans O, van der Ven PF, Wilm M, et al. Structural basis for activation of the titin kinase domain during myofibrillogenesis. *Nature* 1998;395:863–9 [Erratum in: *Nature* 397 (1999) 719].
- [160] Vainzof M, Moreira ES, Suzuki OT, et al. Telethonin protein expression in neuromuscular disorders. *Biochim Biophys Acta* 2002;1588:33–40.
- [161] Selcen D, Engel AG. Mutations in ZASP Define a novel form of muscular dystrophy in humans. *Ann Neurol* 2005;57:269–76.

- [162] Melberg A, Oldfors A, Blomstrom-Lundqvist C, et al. Autosomal dominant myofibrillar myopathy with arrhythmogenic right ventricular cardiomyopathy linked to chromosome 10q. *Ann Neurol* 1999;46:684–92.
- [163] Faulkner G, Pallavicini A, Formentin E, et al. ZASP: a new Z-band alternatively spliced PDZ-motif protein. *J Cell Biol* 1999;146:465–75.
- [164] Zhou Q, Ruiz-Lozano P, Martone ME, Chen J. Cypher, a striated muscle-restricted PDZ and LIM domain-containing protein, binds to alpha-actinin-2 and protein kinase C. *J Biol Chem* 1999;274:19807–13.
- [165] Zhou Q, Chu PH, Huang C, et al. Ablation of Cypher, a PDZ-LIM domain Z-line protein, causes a severe form of congenital myopathy. *J Cell Biol* 2001;155:605–12.
- [166] Vatta M, Mohapatra B, Jimenez S, et al. Mutations in Cypher/ZASP in patients with dilated cardiomyopathy and left ventricular non-compaction. *J Am Coll Cardiol* 2003;42:2014–27.
- [167] Gamez J, Navarro C, Andreu AL, et al. Autosomal dominant limb-girdle muscular dystrophy: a large kindred with evidence for anticipation. *Neurology* 2001;56:450–4.
- [168] Palenzuela L, Andreu AL, Gamez J, et al. A novel autosomal dominant limb-girdle muscular dystrophy (LGMD 1F) maps to 7q32.1–32.2. *Neurology* 2003;61:404–6.
- [169] Speer MC, Vance JM, Grubber JM, et al. Identification of a new autosomal dominant limb-girdle muscular dystrophy locus on chromosome 7. *Am J Hum Genet* 1999;64:556–62.
- [170] Messina DN, Speer MC, Pericak-Vance MA, McNally EM. Linkage of familial dilated cardiomyopathy with conduction defect and muscular dystrophy to chromosome 6q23. *Am J Hum Genet* 1997;61:909–17.
- [171] Starling A, Kok F, Passos-Bueno MR, Vainzof M, Zatz M. A new form of autosomal dominant limb-girdle muscular dystrophy (LGMD1G) with progressive fingers and toes flexion limitation maps to chromosome 4p21. *Eur J Hum Genet* 2005;13:264.
- [172] Hermanova M, Vondracek P, Lukas Z. A unique case of congenital muscular dystrophy. *Cesk Patol* 2004;40:57–62.
- [173] Lescure A, Gautheret D, Carbon P, Krol A. Novel selenoproteins identified in silico and in vivo by using a conserved RNA structural motif. *J Biol Chem* 1999;274:38147–54.
- [174] Muntoni F, Taylor J, Sewry CA, Naom I, Dubowitz V. An early onset muscular dystrophy with diaphragmatic involvement, early respiratory failure and secondary alpha-2 laminin deficiency unlinked to the LAMA2 locus on 6q22. *Eur J Paediatr Neurol* 1998;1:19–26.
- [175] Brockington M, Sewry CA, Herrmann R, et al. Assignment of a form of congenital muscular dystrophy with secondary merosin deficiency to chromosome 1q42. *Am J Hum Genet* 2000;66:428–35.
- [176] Voit T, Parano E, Straub V, et al. Congenital muscular dystrophy with adducted thumbs, ptosis, external ophthalmoplegia, mental retardation and cerebellar hypoplasia: a novel form of CMD. *Neuromuscul Disord* 2002;12:623–30.
- [177] LaBarge MA, Blau HM. Biological progression from adult bone marrow to mononucleate muscle stem cell to multinucleate muscle fiber in response to injury. *Cell* 2002;111:589–601.