

CONGENITAL MUSCULAR DYSTROPHY

Part II: a review of pathogenesis and therapeutic perspectives

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Abstract – The congenital muscular dystrophies (CMDs) are a group of genetically and clinically heterogeneous hereditary myopathies with preferentially autosomal recessive inheritance, that are characterized by congenital hypotonia, delayed motor development and early onset of progressive muscle weakness associated with dystrophic pattern on muscle biopsy. The clinical course is broadly variable and can comprise the involvement of the brain and eyes. From 1994, a great development in the knowledge of the molecular basis has occurred and the classification of CMDs has to be continuously up dated. In the last number of this journal, we presented the main clinical and diagnostic data concerning the different subtypes of CMD. In this second part of the review, we analyse the main reports from the literature concerning the pathogenesis and the therapeutic perspectives of the most common subtypes of CMD: MDCIA with merosin deficiency, collagen VI related CMDs (Ullrich and Bethlem), CMDs with abnormal glycosylation of alpha-dystroglycan (Fukuyama CMD, Muscle-eye-brain disease, Walker Warburg syndrome, MDCIC, MDCID), and rigid spine syndrome, another much rare subtype of CMDs not related with the dystrophin/glycoproteins/extracellular matrix complex.

KEY WORDS: congenital muscular dystrophy, MDCIA, collagen VI related disorders, glycosylation of alpha-dystroglycan, Fukuyama DMC, muscle-eye-brain (MEB) disease, Walker-Warburg syndrome, rigid spine syndrome.

Distrofia muscular congênita. Parte II: revisão da patogênese e perspectivas terapêuticas

Resumo – As distrofias musculares congênitas (DMCs) são miopatias hereditárias geralmente, porém não exclusivamente, de herança autossômica recessiva, que apresentam grande heterogeneidade genética e clínica. São caracterizadas por hipotonia muscular congênita, atraso do desenvolvimento motor e fraqueza muscular de início precoce associada a padrão distrófico na biópsia muscular. O quadro clínico, de gravidade variável, pode também incluir anormalidades oculares e do sistema nervoso central. A partir de 1994, os conhecimentos sobre genética e biologia molecular das DMCs progrediram rapidamente, sendo a classificação continuamente atualizada. Os aspectos clínicos e diagnósticos dos principais subtipos de DMC foram apresentados no número anterior deste periódico, como primeira parte desta revisão. Nesta segunda parte apresentaremos os principais mecanismos patogênicos e as perspectivas terapêuticas dos subtipos mais comuns de DMC: DMC tipo 1A com deficiência de merosina, DMCs relacionadas com alterações do colágeno VI (Ullrich e Bethlem), e DMCs com anormalidades de glicosilação da alfa-distroglicana (DMC Fukuyama, DMC “Muscle-eye-brain” ou MEB, síndrome de Walker Warburg, DMC tipo 1C, DMC tipo 1D). A DMC com espinha rígida, mais rara e não relacionada com alterações do complexo distrofina-glicoproteínas associadas-matriz extracelular também será abordada quanto aos mesmos aspectos patogênicos e terapêuticos.

PALAVRAS-CHAVES: distrofia muscular congênita, merosina, colágeno VI, glicosilação da alfa-distroglicana, DMC Fukuyama, DMC “muscle-eye-brain”-MEB, síndrome de Walker-Warburg, espinha rígida.

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The congenital muscular dystrophies (CMDs) are genetically and clinically heterogeneous hereditary myopathies with a predominant autosomal recessive mode of inheritance that are characterized by congenital hypotonia, delayed motor development and early onset of progressive muscle weakness, as well as dystrophic pattern on muscle biopsy. The clinical course is broadly variable and can comprise the involvement of the brain and eyes¹⁻⁷.

From 1994 and mostly in the first years of the current century a great input in the knowledge of the molecular basis has occurred, and the classification of CMDs has to be continuously up dated. The official journal of the World Muscular Society, *Neuromuscular Disorders*, periodically publishes the revised classification (Table 1)⁸. A computerized version of the classification is accessible at <http://www.musclegenetable.org> and <http://194.167.35.195/>. Most of the different genes involved with the pathogenesis of the CMD subtypes are related to the function of the dystrophin-glycoproteins associated complex (DGC) in the sarcolemma and extracellular matrix and their mutations lead either to defects in the glycosylation of alpha-dystroglycan (alpha-dystroglycanopathies) or to abnormalities of extracellular matrix proteins (MDC1A and collagen VI related disorders)¹⁻⁷. A fourth subtype, rigid spine CMD, is related to a defect of an endoplasmic reticulum protein, selenoprotein N⁹, and recently a new subtype¹⁰ was associated to a defect of a nuclear protein, lamin A/C.

Last month, the first part of this review focused on the clinical and diagnostic aspects of the different subtypes of CMD. Presently, the second part emphasizes the main data on pathogenesis and therapeutic perspectives for the most common subtypes of CMD, i.e. MDC1A, collagen VI related disorders, CMDs caused by defects of glycosylation of alpha-DG, and finally the much rarer rigid spine CMD.

Before reviewing the essential pathogenic data about the most common subtypes of CMD, we summarize the general aspects of the organization of the DGC and extracellular matrix.

DYSTROPHIN-GLYCOPROTEINS ASSOCIATED COMPLEX AND EXTRACELLULAR MATRIX: GENERAL REMARKS ON STRUCTURE AND FUNCTION

The DGC is an assembly of proteins spanning the sarcolemma of skeletal muscle fibers that forms a chain of links between the contractile actin in the cytoskeleton and the extracellular matrix^{11,12} (Fig 1). Defects in the DGC can disrupt these links and alter the basement membrane organization, resulting in sarcolemmal instability and muscle cells apoptose. In addition to the function of stabilizing the sarcolemma, the DGC is also essential in organizing molecules involved in cellular signaling¹².

The first component of the chain of links in the inner cytoskeleton is dystrophin that through the amino-terminus domain binds to actin and through the carboxyl ter-

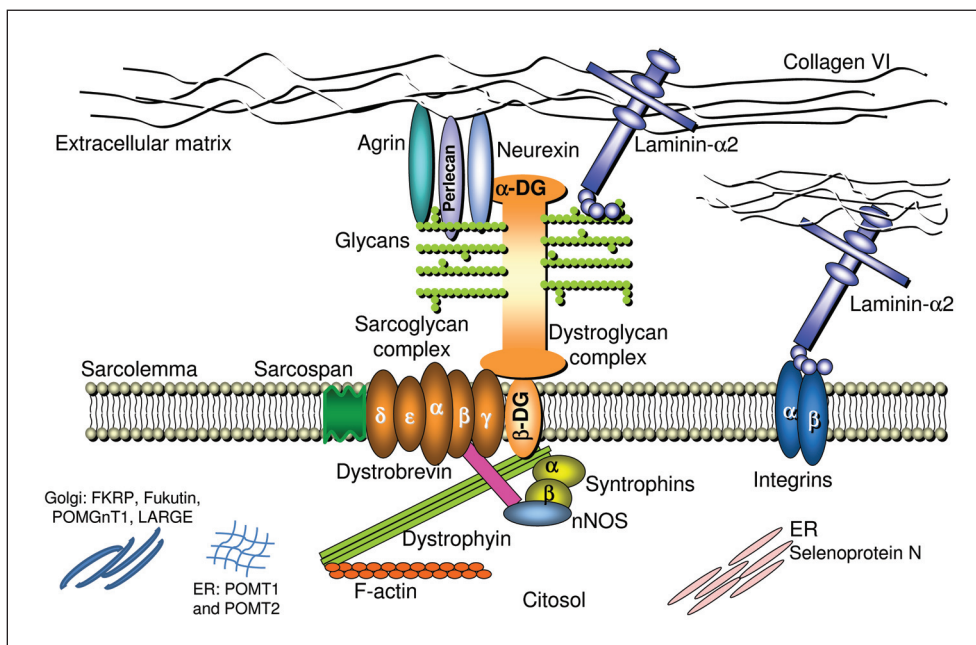


Fig 1. Schematic representation of the main proteins involved in congenital muscular dystrophies, their localization and interactions: laminin alpha-2, integrin alpha-7, collagen VI, alpha-dystroglycan, glycosyltransferases POMT1, POMT2, POMGnT1, fukutin, FKRP and LARGE, and selenoprotein-N. Reproduced with adaptation from Fig.1 of Lisi & Cohn⁶. Abbreviations: see text; ER: endoplasmic reticulum.

Table 1. Classification of congenital muscular dystrophies. Adapted from Gene Table⁵⁸.

Disease phenotype (inheritance)	Gene symbol (chromosome) protein	All allelic disease phenotypes - disease symbols
Merodin deficient CMD – (AR)	LAMA2 (6q22–q23) laminin alpha 2 chain of merodin	Muscular dystrophy, congenital merodin-deficient - MDC1A Muscular dystrophy, congenital, due to partial LAMA2 deficiency
CMD with merodin deficiency – (AR)	? – (1q42)	Muscular dystrophy, congenital, 1B -MDC1B
CMD and abnormal glycosylation of dystroglycan – (AR)	FKRP (19q13.33) fukutin-related protein	Muscle-eye-brain disease -MEB Muscular dystrophy, congenital, 1C -MDC1C Muscular dystrophy, limb-girdle, type 2I -LGMD2I Walker-Warburg syndrome -WWS3
CMD and abnormal glycosylation of dystroglycan – (AR)	LARGE (22q12.3–q13.1) like-glycosyltransferase	Muscular dystrophy, congenital, with severe mental retardation -MDC1D
Fukuyama CMD – (AR)	FCMD (9q31–q33) fukutin	Muscular dystrophy, Fukuyama congenital -FCMD Muscular dystrophy, limb-girdle, type 2M -LGMD2M Walker-Warburg syndrome -WWS
Walker-Warburg syndrome – (AR)	FCMD (9q31–q33) fukutin	Muscular dystrophy, Fukuyama congenital -FCMD Muscular dystrophy, limb-girdle, type 2M -LGMD2M Walker-Warburg syndrome -WWS
Walker-Warburg syndrome – (AR)	POMT1 (9q34.1) protein-O-mannosyltransferase 1	Muscular dystrophy, limb-girdle, type 2K -LGMD2K Walker-Warburg syndrome -WWS
Walker-Warburg syndrome – (AR)	POMT2 (14q24.3) protein-O-mannosyltransferase 2	Muscle-eye-brain disease -MEB Walker-Warburg syndrome -WWS2
Walker-Warburg syndrome – (AR)	FKRP (19q13.33) fukutin-related protein	Muscle-eye-brain disease -MEB Muscular dystrophy, congenital, 1C -MDC1C Muscular dystrophy, limb-girdle, type 2I -LGMD2I Walker-Warburg syndrome -WWS3
Muscle-eye-brain disease – (AR)	POMGNT1 (1p34.1) O-linked mannose beta1,2-N-acetylglucosaminyltransferase	Muscle-eye-brain disease -MEB Walker-Warburg syndrome -WWS
Muscle-eye-brain disease – (AR)	FKRP (19q13.33) fukutin-related protein	Muscle-eye-brain disease -MEB Muscular dystrophy, congenital, 1C -MDC1C Muscular dystrophy, limb-girdle, type 2I -LGMD2I Walker-Warburg syndrome -WWS3
Muscle-eye-brain disease – (AR)	POMT2 (14q24.3) protein-O-mannosyltransferase 2	Muscle-eye-brain disease -MEB Walker-Warburg syndrome -WWS2
Rigid spine syndrome (RSS) – (AR)	SEPN1 (1p36.13) selenoprotein N1	Desmin-related myopathy with Mallory bodies -RSMD1 Minicore myopathy, severe classic form -RSMD1 Muscular dystrophy, rigid spine, 1 -MDRS1 Myopathy, congenital, with fiber-type disproportion -CFTD RSS
Ullrich syndrome – (AR)	COL6A1 (21q22.3) alpha 1 type VI collagen	Bethlem myopathy Ossification of the posterior longitudinal spinal ligaments -OPLL Ullrich congenital muscular dystrophy -UCMD
Ullrich syndrome – (AR)	COL6A2 (21q22.3) alpha 2 type VI collagen	Bethlem myopathy – Ullrich scleroatonic muscular dystrophy -UCMD
Ullrich syndrome – (AR)	COL6A3 (2q37) alpha 3 type VI collagen	Bethlem myopathy – Ullrich congenital muscular dystrophy -UCMD
Bethlem myopathy – (AD)	COL6A1 (21q22.3) alpha 1 type VI collagen	Bethlem myopathy Ossification of the posterior longitudinal spinal ligaments -OPLL – Ullrich congenital muscular dystrophy -UCMD
Bethlem myopathy – (AD)	COL6A3 (2q37) alpha 3 type VI collagen	Bethlem myopathy – Ullrich congenital muscular dystrophy -UCMD
Bethlem myopathy – (AD)	COL6A2 (21q22.3) alpha 2 type VI collagen	Bethlem myopathy Ullrich scleroatonic muscular dystrophy -UCMD –
CMD with integrin deficiency – (AR)	ITGA7 (12q13) integrin alpha 7 precursor	Myopathy, congenital -ITGA7

AD: autosomal dominant; AR: autosomal recessive; CMD: congenital muscular dystrophy.

minus binds to dystroglycan (DG)^{11,12}. Dystrophin changes lead to the X-linked Duchenne and Becker muscular dystrophies, and are not involved in any type of CMD. The following link is dystroglycan (DG) that has two components: beta and alpha-DG. Beta-DG is a transmembrane glycoprotein and alpha-DG is extracellular but with close contact with the peripheral membrane¹¹⁻¹³. Both are encoded by the same gene and then cleaved into two proteins, alpha and beta¹¹⁻¹³. Primary mutations in the gene encoding DG have not been reported and the knockout mouse for DG is embryonically lethal¹⁴.

Alpha-DG is involved in the next step of the chain of links and connects the sarcolemma to the basement membrane. However, for performing this step, alpha-DG needs to be glycosylated¹¹⁻¹⁵ (Fig 2). The glycosylation depends on the biosynthesis of glycans that occurs by the enzymatic activity of glycosyltransferases, and it is necessary for the correct function of many animal proteins, the glycoproteins. Dystroglycan glycosylation is highly conserved during evolution. The covalently addition of sugar chains (glycans), forming a glycoprotein, induces a modification that increases the availability of the protein for ligand interaction with laminin, agrin and perlecan in skeletal muscle, as well as with laminin and neurexin in the brain¹¹⁻¹⁸. The glycoproteins act as biosignals for cell-cell communication, intracellular signaling, protein folding, and targeting of proteins within cells¹¹⁻¹⁵. Each glycosyltransferase has specific expression and localization in tissues or cell type

as well as along the different stages of development, and its specificity is determined by the type of glycan component. The most common type of glycosylation that occurs in mammals' proteins is by N-glycan linkage. However, in a limited number of glycoproteins of brain, nerve, and skeletal muscle, including alpha-DG, the glycosylation is by O-glycan linkage and is named O-mannosyl-glycosylation because mannosylglycans are the specific sugars that promote the interaction between alpha-DG and extracellular matrix ligands. The glycosylation pattern of alpha-DG is specific not only for different tissues but also for different regions of the muscle fiber, such as the sarcolemma and the neuromuscular junction¹⁵. Protein O-mannose beta-1,2-N-acetylglucosaminyltransferase (POMGnT1) that was isolated in 2001¹⁶, is the first human glycosyltransferase which was found to participate in O-mannosyl glycan synthesis in muscle and brain by adding *N*-acetylglucosamine to *O*-linked mannose¹⁶. Soon after, Michele et al.¹⁷ and Moore et al.¹⁸ suggested that, in addition to POMGnT1, fukutin and acetylglucosaminyltransferase-like protein (LARGE) may participate in a similar pathway "that ultimately results in transfers of sugar to dystroglycan". In patients with FCMD or MEB disease and in dystrophic mice they demonstrated that the O-glycosylation of alpha-DG, mediated by different glycosyltransferases, is essential for muscle and brain development and functions. During the O-linked mannose glycosilation the glycosyltransferases add different glycans directly to the hydrox-

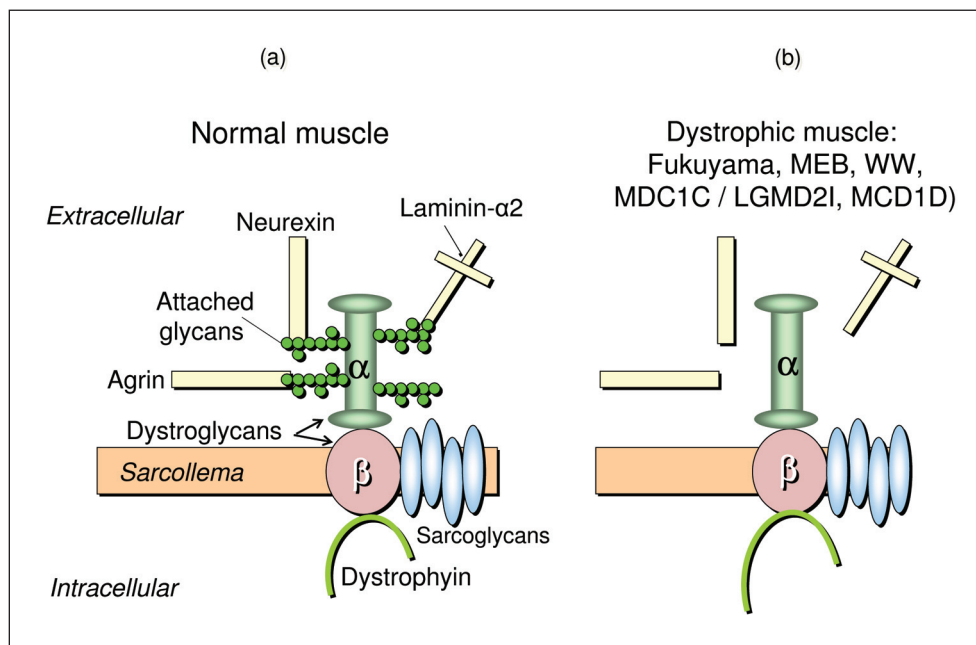


Fig 2. (A) Glycosylation of alpha-DG in normal muscle: glycans attached to alpha-DG link the dystrophin-glycoproteins complex to the extracellular matrix (agrin, neurexin, and laminin-alpha 2). (B) Loss of glycosylation of alpha-DG in the dystrophic alpha-dystroglycanopathies impedes the link with the extracellular matrix. Reproduced from Muntoni et al.³⁶.

yl groups of alpha-DG on either serine or threonine residues, following successive steps¹⁹. In this way, alpha-DG is sequentially modified by glycosyltransferases during the transportation from the rough endoplasmic reticulum to the *trans*-Golgi network²⁰. Protein O-mannosyl transferase 1 (POMT1) and protein O-mannosyl transferase 2 (POMT2) act in endoplasmic reticulum during the first step of the O-mannosyl glycan synthesis²¹, and POMGnT1 acts during the second step in the Golgi apparatus¹⁶. POMT1 transfers a mannosyl residue from dolichyl phosphate mannose to a serine or threonine residue in alpha-DG, and POMGnT1 adds an N-acetylglucosamine residue¹⁶. Co-expression of POMT1 and POMT2 is necessary for O-mannosyltransferase activity within the ER of mammalian cells²¹. However, it is still unclear whether these glycans are directly involved in ligand binding or play some other role. Fukutin and FKRPs are putative glycosyltransferases and their exact function and localization are controversial. Initially, it seemed that fukutin acted in the *cis*-Golgi compartment, while FKRPs probably acted in the rough endoplasmic reticulum. Matsumoto et al.²² analysed the precise localization of fukutin and FKRPs in muscle cells and suggested that FKRPs localizes in the rough endoplasmic reticulum and with POMT1 may play a role in the first O-mannosylation step of alpha-DG. Others reported that FKRPs and fukutin are localized subcellularly in the medial-Golgi apparatus^{23,24} but that the mutations in the FKRPs gene would lead to endoplasmic reticulum retention and consequent diminution in the Golgi apparatus²⁴. According to Yamamoto et al.²⁵, although the functions of fukutin continue unclear, it seems to interact with alpha-DG during glycosylation but binding to the core area of alpha-DG instead of its sugar chain. Also concerning FKRPs, it has recently been supposed that in normal and mutant mice it is detected in the sarcolemma and coexists with DG in the native dystrophin-glycoprotein complex, perhaps having its localization mediated by DG²⁶. It has not yet been demonstrated that LARGE is a glycosyltransferase itself but the Kanagawa et al.²⁷ found that alpha-DG interacts with LARGE at two different domains, one involved with the glycosylation process itself and the other at intracellular level (N-terminal domain of alpha-DG) for defining a recognition motif necessary to initiate functional glycosylation. Therefore, in order to stimulate alpha-DG hyperglycosylation LARGE needs to both physically interact with alpha-DG intracellularly and function as a glycosyltransferase²⁸. The two interactions are essential for the occurrence of the link between alpha-DG and laminin as well as other extracellular matrix ligands. LARGE also interacts with a homologous glycosyltransferase (glycosyltransferase-like 1B-GYLTL1B) named LARGE2 that is highly similar and also localizes to the Golgi apparatus but has lesser tissue expression and has not been detected in mus-

cle and brain²⁹. It is supposed that also LARGE2 is involved in alpha-DG glycosylation and that both show functional integration²⁹. Although it has not yet been demonstrated that LARGE and LARGE 2 are glycosyltransferases themselves, their overexpression in cultured cells induce alpha-DG hyperglycosylation and laminin-binding; therefore, this biological activity *in vivo* suggests that both act as glycosyltransferases³⁰. *Indeed*, LARGE2 was found to support the maturation of alpha-dystroglycan more effectively than LARGE³¹.

The structure, biosynthesis, and pathology of O-mannosyl glycans have been described in details by Endo^{13,15,32,33}, Barresi and Campbell³⁴ and Martin³⁵. It is still under analyses whether these glycans are directly involved in ligand binding or play some other role³⁰.

Molecular changes in genes that codified glycosyltransferases affecting this step of the DAG chain of links are responsible for the pathogenesis of five CMDs named alpha-dystroglycanopathies or CMDs by defects of the O-mannosyl-glycosylation of alpha-DG^{1-7,15,30,33,36-50}: Fukuyama CMD (FCMD), "muscle-eye-brain" (MEB) disease, Walker-Warburg syndrome (WWS), MDC1C and MCD1D.

After being glycosylated alpha-DG links to the following component of the DAG chain, laminin alpha-2, a glycoprotein that together with collagen IV is a major constituent of the basement membrane of the extracellular matrix⁵¹ (Fig. 1). Its linkage to alpha-DG was firstly demonstrated by Ibragimov-Beskrovnya⁵¹. All the components of the laminin family are essential for the assembly and architectural integrity of the mammals' basement membranes⁵². The molecule of laminin is a heterotrimer composed of three chains, alpha, beta e gamma, whose spatial arrangement has the form of a cross. The long arm of the cross corresponds to the long arms of the three chains closely assembled and each one of the three short arms of the cross comes from a different chain. At the moment 16 laminin isoforms are known, each one formed by a specific combination of alpha, beta, and gamma chain that allows a broad capacity of interaction with cellular receptors such as integrins and other extracellular ligands. Different laminins have their own spatial and temporal expression. In a recent review on the role of laminins in physiological and pathogenic conditions, Schéele et al.⁵³ reported that one of the specific basement membrane functions is "to govern cell fate by inducing intracellular signalling cascades". In fact the broad spatial distribution of laminin genes products offers tissue specificity as each tissue has its proper ligands. The temporal expression of laminins influences the processes of proliferation, differentiation, adhesion, and migration in different stages of normal life or in pathologic states⁵². The role of laminin in the nervous system is less well defined than in the muscle, but its adhesive molecule is supposed to have an important role

in peripheral nerve regeneration, influencing neurite outgrowth, neural differentiation, and synapse formation⁵⁴.

Laminin-2 or laminin alpha-2, also named merosin, is a heterotrimer composed of alpha-2, beta-1, and gamma-1 chains, that is specifically found in the basement membranes of striated muscle, Schwann cells and placental trophoblasts⁵⁵. Vuolteenaho et al.⁵⁶ described the complete primary structure of the human laminin M chain (merosin), assigned it to 6q22-23 and reported its tecdial distribution, including in human fetal tissues. The term merosin was changed to muscle laminin alpha-2 by Burgeson et al.⁵⁷ in 1994. In muscle, laminin alpha-2 acts as the most important ligand for the surface receptors of the muscular fiber, and is essential for controlling the transmission of force from the interior of the cytoskeleton. During the myogenesis, laminin alpha-2 is also involved in the stability and survival of the myotubes that develop normally in the first stages but collapsed and degenerated when the mature, contractile function of the muscular cell begins⁵⁸. The biological functions of the rest of the chains of laminin complex (alpha-1, alpha-4 and alpha-5 chains) include the formation of basement membranes during muscle myogenesis and development, and probably a role in signal transmission events during muscle formation and muscle regeneration⁵⁹.

Molecular changes in the gene responsible by codifying laminin alpha-2 are the source of the most common form of CMD, termed MDC1A or merosin-deficient CMD⁶⁰.

In addition to the connection with alpha-DG, laminin alpha-2 also connects with the sarcolemma through the glycoprotein named integrin, and to the rest of the extracellular matrix network mainly through collagen IV unit (Fig 1).

Integrins are heterodimeric cell adhesion receptors organized in a transmembrane arrangement and extracellular domains that organize the cytoskeleton and links to the extracellular matrix. They constitute the major cell surface receptors allowing cell-extracellular matrix adhesion⁶¹. In humans at least 18 alpha and eight beta subunits have been identified that compose 24 heterodimers with a great diversity of functions and tecdial specificity^{61,62}. Playing as adhesion receptors, integrins act in the transduction of signals to the cell interior and receive intracellular signals that control their own ligand-binding affinity. Similarly to laminin, through these signaling pathways, integrins participate in proliferation, differentiation, apoptosis, and cell migration at different developmental stages^{61,62}.

The specific integrin for the muscular fibre is integrin alpha-7/beta-1 that represents another link between the muscle fiber and the extracellular matrix that is independent of DG. Mutations in the integrin alpha7 gene are responsible by a very rare form of CMD⁶³.

The link with laminin is the last step of the DAG chain

but in the basement membrane, laminin alpha 2 binds to other laminins, nidogen (that binds to collagen IV and perlecan), agrin, fibronectin and different macromolecules⁶⁴. In addition to laminin, type IV collagen is the most abundant glycoprotein in the basement membrane and all together the components of the basement membrane form a thin pericellular matrix that is involved in many cellular functions (gene expression, adhesion, migration, differentiation, and apoptosis)^{64,65}. Through its carboxyl-terminal globular domain collagen IV interacts with the amino-terminal domain of the collagen VI alpha-1 chain⁶⁵ and this connection is essential for anchoring the basement membrane of the muscular fiber to the other components of the extracellular matrix and maintaining the structural muscle integrity⁶⁵⁻⁶⁸. Collagen VI provides a microfilamentous network in the extracellular matrix of the muscular tissue, as well as in other organs⁶⁶⁻⁶⁸. Lampe and Bushby⁶⁹ revised the literature concerning the main roles of collagen VI: it also interacts with several extracellular matrix components such as other fibrillar collagens, perlecan, glycoproteins associated to elastin, proteoglycans and fibronectin. In addition to these mechanical functions it also acts in cycle signaling, homeostasis control, tissue development and architecture, as well as in repair processes such as wound healing⁶⁹.

Collagen VI is formed by three chains: alpha-1, alpha-2, and alpha-3, each one encoded by a different gene. The structure and macromolecular organization of type VI collagen was described by Engel et al.⁶⁶, and later by Baldock et al.⁷⁰ and Ball et al.⁷¹ reported in details the biochemical basis of the tetrameric and microfibrillar organization. The three chains contribute to the formation of a triple helical monomer: first they associate through the C-terminal globular domain and then the triple helix folds from the C to N terminus to form the monomer that is the basic collagen VI structure⁶⁶. The globular amino and carboxi-terminal regions of each monomer are assembled in an antiparallel manner by disulfide bonds into dimers that are aligned laterally to form tetramers. Each tetramer is therefore made by eight smaller subunits corresponding to the globular amino and carboxi-terminal regions of the monomeric components of the microfibril. The tetramers are then secreted and associated end-to-end to form a characteristic microfibrils repeat that constructs a network of microfibrils (Fig 3). The formation of monomers, dimers and tetramers occurs intracellularly, while the microfibril assembly occurs in the extracellular space^{66,69-71}.

Mutations in the genes that codify the three collagen VI subunits lead to the second most common form of CMD, Ullrich atonic-sclerotic CMD⁷²⁻⁷⁵ and to Bethlem myopathy^{76,77} that now is included into CMD classification⁸.

After this brief explanation about the normal structure of the DAG/extracellular matrix complex we present

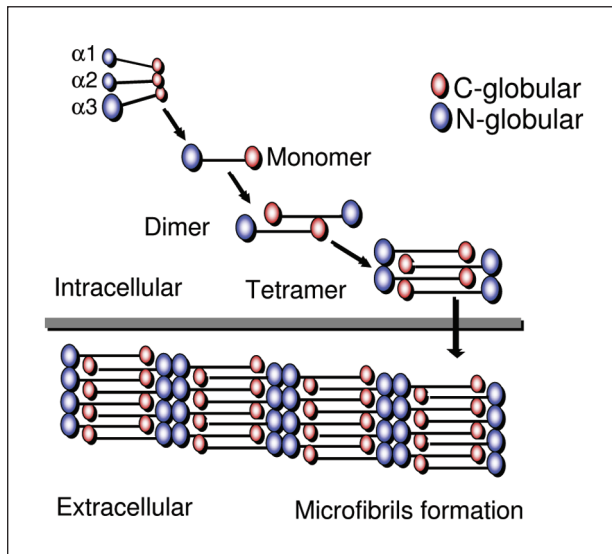


Fig 3. Partial reproduction of Fig 1 from Baldock et al.¹¹⁴: "a diagram showing the assembly of collagen VI microfibrils from the three alpha chains. The formation of monomers, dimers and tetramers occurs intracellularly, while microfibril assembly occurs in the extracellular space"⁷⁰.

the main pathogenic aspects and therapeutic perspectives of the most common forms of CMD that are caused by changes in this complex: CMD with merosin (laminin alpha-2) deficiency (MDC1A); collagen type VI related CMDs (Ullrich CMD and Bethlem myopathy); CMDs with abnormal glycosylation of alpha-DG (Fukuyama CMD, MEB disease, WWS, MDC1C, MDC1D).

Finally, we present the main pathogenic data and therapeutic researches about the much rare rigid spine CMD caused by a defect of an endoplasmic reticulum protein, selenoprotein N.

MEROSIN-DEFICIENT CONGENITAL MUSCULAR DYSTROPHY: MDC1A

Pathogenesis

MDC1A is caused by mutations in the laminin alpha-2 gene (LAMA2) and was first described by Tomé et al. in 1994⁶⁰. In the same year, Hillaire et al.⁷⁸ demonstrated that this specific CMD is linked to 6q2, and soon after Heibling-Leclerc et al.⁷⁹ found the first homozygous mutations in LAMA2 gene in two patients who had been reported by Tomé et al.⁶⁰. The disease has congenital onset and progressive course and patients manifest severe muscular weakness and atrophy, diffuse contractures, inability to walk and facial dysmorphism^{61,2,60}. Diffuse low-density areas in the cerebral white matter are the hallmark of this condition^{1,2,60}. In a few patients who have partial laminin alpha-2 deficiency, a later onset and a milder course can be observed^{1,2}. On muscle biopsies the dystrophic pattern is marked with fiber size variation, huge interstitial

proliferation and fatty infiltration^{1,2,60}. Necrosis and regeneration features are less prominent. Taniguchi et al.⁸⁰ showed that regenerative features are less prominent in MDC1A and FCMD than in Duchenne muscular dystrophy. FCMD and MDC1A muscular samples of patients at different stages of life showed increased expression of extracellular matrix genes and downregulation of genes that codify structural components of mature muscle. This active interstitial fibrosis with less active regeneration of muscle cell components suggested that FCMD and MDC1A could be primary fibrotic diseases⁸⁰.

Due to the fact that the alpha-2 subunit of laminin is also expressed in the basal lamina of Schwann cell-axon unit, a peripheral demyelinating neuropathy affecting predominantly motor fibers⁸¹, but also the sensitive ones⁸², is a feature of laminin alpha-2-deficient CMD. In the *dy* mouse model laminin alpha-2 is expressed in the endoneurium surrounding the Schwann cell/myelin sheath and interacts with dystroglycan and/or alpha 6 beta 4 integrin located at the outer membrane of Schwann cell/myelin sheath. Its expression influences the peripheral myelinogenesis⁸³. However, peripheral motor nerves involvement has not been found in Brazilian children with MDC1A^{84,85} and this fact might be due to the type and location of the mutation⁸⁴.

The characteristic pattern of white matter abnormality associated to MDC1A has been extensively analysed. Opposite to the peripheral nerve, in which laminin alpha 2 is associated only with myelinated and not with unmyelinated nerve fibers and is involved in the myelin stability⁸⁶, a role for laminin alpha-2 in central myelination has not been confirmed. Villanova et al.⁸⁷ found that laminin alpha-2 chain is localized to the basal lamina of all cerebral blood vessels and supposed that it may be important for the selective filtration capability of the blood-brain barrier. In patients with MDC1A the lack of laminin alpha-2 may lead to an abnormality of the blood-brain barrier causing impaired selective filtration. Caro et al.⁸⁸ postulated that disruption of the blood-brain barrier associated with laminin alpha-2 leads to increased water content, resulting in abnormal white matter signal intensity. Using magnetic resonance spectroscopy and apparent diffusion coefficient mapping, Leite et al.⁸⁹ detected abnormally high free-water concentrations in the white matter of our Brazilian patients with MDC1A and more prominent changes in the parietal, frontal, and temporal white matter. They also found no correlation between the extent of white matter abnormality on MRI and the clinical status as well as the degree of laminin alpha-2 deficiency (partial or total). Sijens et al.⁹⁰ in a patient with MDC1A observed that the concentrations of all metabolites detected by brain magnetic resonance spectroscopy were decreased only in the white matter. This observation

combined with fractional anisotropy decrease and apparent diffusion coefficient increase in the white matter indicated a presence of vasogenic edema in the white matter. The same was concluded by Brockmann et al.⁹¹ that observed a metabolite profile suggesting white matter edema which supports the hypothesis that laminin alpha2 deficiency results in leakage of fluids across the blood-brain barrier. Anyway, the pathophysiology of the white matter changes is not completely elucidated.

LAMA 2 mutations are markedly variable spanning all protein domains⁹²⁻⁹⁴ and, in general, the molecular diagnosis is not considered a priority in children with MDC1A, due to the homogeneous phenotype, the easy immunohistochemical analysis of laminin alpha-2 chain in muscle^{1,2,60} and skin⁹⁵, and the characteristic white matter abnormalities on neuroimaging. However, Siala et al.^{96,97} have emphasized the utility of mRNA analysis in cases of MDC 1A to understand the mechanism of the mutation and the genotype-phenotype correlation. Recently, also Oliveira et al.⁹⁸ emphasized the utility of analyzing genotype/phenotype correlations in MDC1A. In 26 patients they reported 96% of mutation detection rate using full genomic sequencing and complementary DNA analysis. They detected new polymorphisms and novel nonsense and truncating mutations, as well as the first fully characterized gross deletion. Interestingly, in the two patients with the milder clinical presentation and a partial reduction in muscle laminin-alpha2 one of the alleles showed a missense mutation that was not observed in any of the severe cases⁹⁸.

Therapeutic perspectives

From 1994, knock-out mouse or spontaneous mutant mouse strains have been identified as animal models for MDC1A with total and partial deficiency⁹⁹⁻¹⁰⁵, and experimental therapeutic strategies have been attempted^{102,103,106-116}. In mice, Kuang et al.^{102,103} were successful in obtaining the expression of a human laminin alpha 2 chain transgene under the regulation of a muscle-specific creatine kinase promoter. They detected the synthesis of laminin alpha-2 in muscle and a marked improvement of the clinical and histopathological changes; however, there was no improvement of the peripheral neuropathy and they concluded that potential new therapies have to be also directed to restore laminin alpha-2 in other tissue as CNS and peripheral nerves. Vilkin et al.¹⁰⁶ studying both human and mouse myogenic cells verified that pure muscle cells are able to synthesize laminin alpha-2 chain. Using primary muscle cell culture transplantation, they found that laminin alpha-2 is secreted and expressed in both non-dystrophic muscles of an immunodeficient mouse and dystrophic muscles of a CMD animal model. However, not all myoblasts or muscle fibers that they form are capable of secretion and deposition in vivo as cell spreading and dif-

fusion of the protein remain restricted to the injection site. In addition, the success of myoblast transplantation depends on future advances for controlling the immune response of the receptor and his biochemical adequacy with the donor. In dy^W/dy^W mice a "replacement therapy" using a mini-agrin (miniaturized form of agrin) resulted in a lesser muscle degeneration and in a decrease of mortality that is mediated through the linking of muscle basement membrane to the DGC, and not to integrin¹⁰⁷. The first attempt of somatic gene therapy to treat mice with laminin alpha-2 deficiency was performed by Qiao et al.¹⁰⁸. They used adeno-associated viral vector for delivering mini-agrin gene into dy^W/dy^W mice and obtained an improvement of the dystrophic pattern. Mini-agrin not only ameliorates the phenotype in those mice but also decelerates disease progression when applied at late stages. Meinen et al.¹⁰⁹ demonstrated that full-length agrin is also capable of promoting this improvement. This result indicates the role of DG not only in the pathogenesis of CMDs caused by hypoglycosylation of alpha-DG, but also in MDC1A. The improvement of the phenotype may be due to the link between 'non-muscle' laminins and DG through miniagrin, therefore maintaining the sarcolemma stability. These authors¹⁰⁹ also supposed that since the genetic manipulation in dy^W/dy^W mice can prevent apoptosis, it might be possible that antiapoptotic agents act synergistically with mini-agrin. Therefore, a future therapeutic attempt for patients with MDC1A could be the combination of antiapoptotic drugs with the expression of mini-agrin in muscle, and/or the up-regulation of endogenous agrin. Gawlik et al.¹¹⁰⁻¹¹² generated laminin alpha-2 deficient mice expressing laminin alpha-1 in peripheral nerves and muscles. Dy^{3K}/dy^{3K} mice harboring the laminin alpha-1 transgene in these tissues presented reduced muscular dystrophy and show peripheral myelination. The finding that transgenically expressed laminin alpha-1 compensates the lack of laminin alpha-2 in muscles and peripheral nerves indicates that laminin alpha-1 can be an ideal candidate for replacing laminin alpha-2 chain in MDC1A¹¹². In addition, the authors considered that laminin alpha-1 overexpression induced by gene therapy would be paralogous, therefore minimizing adverse immunological responses. They also generated dy^{3K}/dy^{3K} laminin-a2 deficient mice in which the expression of alpha-DG in muscle is increased¹¹². Using transgenic expression of laminin-a1, they obtained a restoration of the basement membrane and a normalization of alpha-DG hyperexpression, therefore demonstrating a probable interaction between laminin-a1 chain and alpha-DG¹¹². Laminin-a1 also restored the link with the integrin at the sarcolemma, what represents an additional way for increasing the muscle cell stability. Hagiwara et al.¹¹³ tested bone marrow transplantation in laminin-alpha2-deficient (dy) mice and reported an in-

crease in lifespan, growth rate, muscle strength, and respiratory function. Xu et al.¹¹⁴ demonstrated that embryonic or postnatal overexpression of cytotoxic T cell GalNAc transferase (Galgt2) (the latter using adeno-associated virus) is effective in inhibiting the development of the muscular dystrophy or altering the disease progression in skeletal muscles of *dy^(w)* by increasing the expression of agrin. Fukada et al.¹¹⁵ reported another possible therapeutic strategy for MDC1A. In normal animals, laminin alpha-2 is produced in CD90(+) cell fractions (from mesenchymal cells) not depending on its fusion with myogenic cells. They demonstrated that the number of C90+ cells increases during muscle regeneration process *in vivo* and as those cells can be transplanted to the animal model of MDC 1A, perhaps they represent a new source of cellular therapy. Allamand et al.¹¹⁶ using drugs that force reading through premature termination codons (PTCs) demonstrated that the mutant mRNAs were strongly stabilized in myotubes derived from MDC1 A after administration of negamycin but were not able to allow re-expression of laminin alpha-2, perhaps consequently to translational or post-translational troubles. They concluded that this novel form of therapy requires more studies to define the nucleotide context of PTCs, the mechanism of mRNA stability and its translation into a functional protein. Finally, the future treatment of lamininopathies may include stem-cell approaches as well as gene therapy⁵³.

COLLAGEN VI RELATED MUSCLE DISORDERS

Pathogenesis

Mutations in each one of the three collagen VI genes, COL6A1 (21 q22.3), COL6A2 (21 q22.3), and COL6A3 (2 q37) that encode respectively the alpha-1, alpha-2 and alpha-3 chains of collagen VI, cause two types of muscle disorders: Bethlem myopathy, with mild or moderate phenotype, and Ullrich CMD, with severe phenotype^{1-4,6,7,69,117}. Bethlem myopathy is a slowly progressive disorder with variable age of onset, congenital or within the first or second decade of life, and marked flexion contractures of several joints^{7,69,76,77,117-119}. Ullrich CMD is an early onset condition with severe weakness and progressive course that manifests proximal joint contractures and marked distal hyperlaxity. Protruding calcanei, follicular hyperkeratosis, and abnormal scarring are other frequent clinical signs^{1-4,6,7,69,72,73,118,119}.

Until few years ago, Bethlem myopathy and Ullrich CMD were separate entities with distinct modes of inheritance; after the recognition of heterozygous in-frame deletions acting in a dominantly-negative way in patients with Ullrich phenotype the boundaries between Bethlem myopathy and Ullrich CMD became narrow⁶⁹. According to Lampe and Bushby⁶⁹ it is becoming clear that BM and UCMD represent opposite end points of a clinical con-

tinuum in which individuals presenting with intermediate phenotypes could be considered to have either "mild UCMD" or "severe BM". The concept of a spectrum of collagen VI-related disorders with marked clinical and genetic heterogeneity has emerged from the recent advances on the molecular mechanism of both diseases⁶⁹. The complex genotype/phenotype correlations that have been broadly analysed in these two conditions clearly indicate that in both collagen VI-related disorders the main pathomechanism is due to the disruption of collagen VI anchorage to the basal lamina of the muscular fibers^{74,75,120-138}.

Presently more than 60% of patients with collagen VI related disorders have autosomal dominant or recessive mutations identified¹²¹; however, the highly polymorphic nature of the three genes suggests the need of specific methods of mutations analysis for performing an adequate genetic counseling¹²¹. Since the triple helical deletions are readily detectable by RT-PCR analysis, Lampe et al.¹²² recommend that mutation analysis checks these regions first by RT-PCR prior to screen all the 107 coding exons.

The data from molecular analysis in Bethlem myopathy revealed that COL6A1 is the most involved gene and a splice site mutation seems to be the most common, not only in COL6A1 as also in COL6A2 and COL6A3 genes^{123,124}. The α 1 exon 14 skipping mutation is the most commonly reported in Bethlem patients^{75,121,123-125}, leading to reduced amounts of collagen VI protein, some of which is structurally abnormal with an impaired ability to form microfibrils⁶⁹. As different kinds of mutations have been found, the pathogenic mechanism of Bethlem myopathy and the degree of clinical severity depend on the effect of the mutation on the structure, biosynthesis, secretion and assembly of collagen VI. In patients with different types of mutations, many have attempted to interpret the way by which the mutation alters the collagen VI organization and defines genotype/phenotype correlations. For example, a heterozygous nonsense mutation causing an alpha-1 premature stop codon and a decrease of available alpha-1 chains consequently reduces production and secretion of collagen VI¹²⁶. Otherwise, a heterozygous missense mutation interrupting the repetitive amino acid sequence (glycine mutation) that forms the characteristic collagen triple helix either in the alpha-1 or alpha-2 chains does not affect collagen VI intracellular monomer, dimer, and tetramer assembly or secretion, but the mutant tetramers are anomalous (kinked) and the extracellular microfibrils are functionally deficient¹²⁷. Patients reported with a heterozygous in-frame deletion in the triple-helical domain resulting in exon skipping in the COL6A1 gene have either a typical clinical picture with finger contractures¹²⁵ or a course more severe than that commonly reported¹²⁸. Some data indicate that large deletions and mutations inside the triple-helical collagen VI monomer he-

lix formed by alpha-1, 2 and 3 polypeptides are associated with a phenotype more severe than those mutations occurring in the amino-terminal globular region¹¹⁸. Pepe et al.¹²⁹ found two large and highly similar heterozygous COL6A1 genomic deletions, spanning from intron 8 to exon 13 or intron 13, in two patients, one with Bethlem myopathy and the other with moderate Ullrich CMD. They noted that as a similar deletion had been previously reported in the severe Brazilian case with Ullrich CMD, the presence of a deletion-prone region involving the minisatellite in intron 8 of COL6A1 could be suggested¹²⁹.

Lampe and Bushby⁶⁹ summarized the most frequent pathogenic mechanisms in Bethlem myopathy: single amino acid substitutions disrupting the Gly-Xaa-Yaa motif of the triple helical domain in COL6A1, COL6A2, or COL6A3^{77,121,127,130}, and splice site mutations^{75,121,125,128} which cause skipping of COL6A1 exon 14 during pre-mRNA splicing and consequently in-frame deletion of 18 amino acids from the triple helical domain of the $\alpha 1$ chain. In the first case, mutations towards the N terminal region of the triple helix may cause kinking of the tetramers in the normally straight supercoiled triple helical region, thus reducing their ability to form microfibrils¹²⁷ and exerting a dominant negative effect. Baker et al.¹²⁴ confirmed that the majority of the Bethlem myopathy mutations occur toward the N-terminal end of the triple helix, that is a critical region of the collagen VI monomer ($\alpha 1:\alpha 2:\alpha 3$) is critical for efficient dimer, tetramer, and microfibril assembly. However, in the second case, the interpretation of the influence of missplicing mutations on collagen VI organization is difficult and needs caution⁶⁹.

Recently, in one patient with Bethlem myopathy it was described a novel mutation that apparently did not affect the assembly, and it was suggested that its effect could be influencing collagen VI interactions in the extracellular matrix¹²⁴.

In relation to Ullrich phenotype, the initial molecular studies in COL6A2 and COL6A3 genes pointed only to recessive mutations^{73,74,118}. Lampe and Bushby⁶⁹ revised the literature about the type of mutations in Ullrich CMD and reported a large number of the mutations that introduce premature termination codons with consequent nonsense mediated mRNA decay and loss of the mutated chain; another common type of mutation is by splicing that leads to in-frame exonic deletions as well as in-frame genomic deletions. Different missense changes can also occur within the triple helical and C terminal domains of COL6A2 and the N-terminal domains of COL6A3 their interpretation is difficult.

In 2003, the first heterozygous in-frame deletions acting in a dominantly-negative way was found in the COL6A1 gene⁷⁵ in one Brazilian patient with severe Ullrich phenotype¹³¹; soon, more patients with a dominantly act-

ing mutation in the COL6A1 were reported¹³²⁻¹³⁴, and finally this same type of mutation has also been found in Ullrich patients with mutations in COL6A2 and COL6A3 genes¹³². Heterozygously occurring N-terminal triple helical in-frame deletions allow mutant monomers to form dimers but secreted tetramers are abnormal with a consequent dominant negative effect on microfibrillar assembly¹³². In general, the complete absence of collagen VI in the extracellular matrix depends on mutations that exert a strong dominant-negative effect and compromise intracellular assembly of dimers, tetramers, and extracellular microfibrils¹³². However, the pathogenic effect of the in-frame heterozygous deletions is still poorly understood and the genetic counselling of patients and their families is a difficult task, as it has been also demonstrated by Peat et al.¹³⁵.

Pace et al.¹³⁶ recently considered that in spite of the great number of different types of mutations that have been already identified detailed analyses of the effects of the mutations on assembly of the protein have been conducted only in a small number of patients^{75,124,125,127,132}. Pace et al.¹³⁶ investigated in eight patients the mechanism by which different dominant glycine mutations in the collagen VI triple helix produce a spectrum of clinical phenotypes from mild Bethlem myopathy to severe UCM, therefore pointing to the fact that the collagen VI related disorders form a continuum of clinical severity. Their study¹³⁶ identified that in both, Bethlem and Ullrich CMD, heterozygous glycine mutations toward the N-terminal end of the triple helix impair the collagen VI assembly in two different ways, therefore producing different phenotypes: in the first that correspond to less compromised patients, collagen VI dimers accumulated in the cell but microfibril formation in the medium is moderately reduced, with no significant collagen VI decrease in the extracellular matrix; in the second, that corresponds to severely affected Ullrich patients, the assembly defects are more pronounced, some secreted collagen VI tetramers are not disulfide bonded, microfibril formation in the medium is altered, and collagen VI is decreased in the extracellular matrix¹³⁶. Pace et al.¹³⁶ supposed that the relative positions of the mutations contribute to understand why some glycine mutations in this region have no detectable effect on disulfide bonding whereas others result in secretion of nondisulfide bonded tetramers. In conclusion, they demonstrated that in patients with different dominant glycine mutations a mild phenotype is associated to a mild assembly defect whereas severe assembly defects result in moderate-to-severe phenotype.

Okada et al.¹³⁸ found that in Japanese population mutations in collagen VI genes can lead to a collagen VI deficiency in the sarcollema (where normally it is strongly delineated) but with apparently normal amount in the interstitium. They sequenced the three collagen VI genes in

26 Japanese patients with primary collagen VI deficiency that in Japan accounts for 7.2% of CMD cases. By immunohistochemical analysis they found that most patients had sarcolemma-specific collagen VI deficiency and five had complete collagen VI deficiency, i.e. sarcolemma plus interstitium. In the former group all mutations were sporadic dominant; however, in spite of the occurrence of this apparently specific localization of mutated collagen VI, they could not define any genotype/phenotype correlation. Kawahara et al.^{139,140} also found that in Ullrich CMD due to heterozygous mutations in COL6 genes collagen VI is preserved in the interstitium but lost in the sarcolemma. As in normal conditions collagen VI is particularly abundant close to the cells and in intimate contact with basement membranes surrounding myocytes, its lack caused by heterozygous mutations in COL6 genes diminish the anchorage of collagen VI microfibrils to the extracellular matrix.

Finally, Lampe et al.¹²² recently concluded that in collagen VI related disorders the genotype-phenotype correlation does exist and that the type and location of exon skipping mutation is predictive for severity and inheritance. They compared the molecular data of patients with Ullrich CMD with de novo dominant negative heterozygous splice mutations in COL6A1, COL6A2, and COL6A3, Ullrich CMD with recessively acting splice mutations, and Bethlem myopathy with heterozygous splice mutations. For the three types of mutations, i.e. dominant and recessive Ullrich CMD as well as dominant Bethlem myopathy, they discussed the different mechanisms by which the location of the skipped exon in relation to the molecular structure of the collagen chain and to the degree of interference on the collagen VI microfibrils assembly, strongly correlated with the clinical phenotype. Therefore, they add new evidence to the knowledge that in last instance the clinical severity depends on the ability of mutant chains to be incorporated in the final multimeric structure of collagen VI microfibrillar network¹²².

Although rarely, Ullrich phenotype can be detected even in patients who do not harbor mutations in the collagen VI genes and show a normal amount of collagen VI in the interstitium. Ishikawa et al.¹⁴¹ considered that these patients could have a primary abnormality of other not yet identified protein interacting with collagen VI in the sarcolemma causing a failure of collagen VI to anchor the basal lamina to the interstitium. However, according to Okada et al.¹³⁸ the possibility of mutations affecting the promoter regions or introns, or of overlooked mutations must be considered in such cases.

In conclusion, it is clear that the complex genotype-phenotype correlations of collagen VI-related disorders are next to be elucidated. Recently¹³⁷, three novel collagen VI chains, alpha-4, alpha-5, and alpha-6 were found

in mice in or close to basement membrane. These chains structurally resemble the collagen VI alpha-3 chain, apparently having the ability of substitute it in the constitution of the heterotrimers. This identification could implicate in new findings related to the molecular biology of Bethlem myopathy and Ullrich CMD.

Therapeutic perspectives

As for other types of muscular dystrophies, many researches focusing on possible effective therapies have occurred. An animal model of human Bethlem myopathy has already been described and the details about the composition and the role of collagen VI have been widely discussed^{117,142}.

For at least some types of mutations, Kawahara et al.¹³⁹ suggested a therapeutic strategy based on a replacement therapy: in fibroblasts culture from patients with Ullrich CMD and COL6A1 glycine mutation, they verified that fibroblasts adhesion was markedly reduced but could be recovered by the addition of a medium with normal collagen VI¹³⁹. Recently, Merlini et al.¹⁴³ reported the results of an open pilot trial with oral cyclosporin A in five patients with collagen VI myopathies. After a month of treatment a new muscular biopsy showed a decrease of the mitochondrial and apoptotic changes in the myofibers as well as an increase of regenerative signs¹⁴³. According to Angelin et al.^{144,145}, an anomalous mitochondrial depolarization has been demonstrated in myoblasts from patients with Ullrich CMD, and cyclosporin A as well as intracellular Ca (2+) chelators, probably, prevent such anomalous mitochondrial depolarization and a consequent ATP depletion. Also Zou et al.¹⁴⁶ recently reported another possible implication for future therapies. By means of immunofluorescence staining and Western blot analysis *in vitro* they found that the secretion and deposition of collagen VI in the extracellular matrix depends on interstitial fibroblasts but not on myogenic cells¹⁴⁶. Studies on animal models for collagen VI related disorders may further offer good perspectives for therapeutic researches^{147,148}.

DISORDERS OF GLYCOSYLATION OF ALPHA-DG (ALPHA-DYSTROGLYCANOPATHIES)

Pathogenesis

The alpha-dystroglycanopathies include different forms of dystrophies whose clinical severity varies from WWS that is associated with ocular abnormalities and CNS malformations and is not compatible with survival beyond the first years of life, to forms of LGMD with later onset later and pure muscular involvement. Five forms of CMDs are alpha-dystroglycanopathies¹⁻⁷: FCMD, MEB disease, WWS, MDC1C and MDC1D (Table 2). FCMD, MEB disease and WWS form a spectrum of increasing clinical severity regarding muscle, eyes and brain malformation

Table 2. Genotype/Phenotype correlations in CMDs caused by alpha-dystroglycanopathy (the most common phenotype associated to each gene is underlined>, and the bibliography refers to the first molecular identification).

FKRP: fukutin related protein	FKTN: fukutin	POMGnT1	POMT1: protein O-mannosyl transferase 1	POMT2: protein O-mannosyl transferase 2	glycosyltransferase "like": LARGE
LGMD2I ¹⁹⁷	FCMD ¹⁹⁹	MEB ¹⁶	WWS ²⁰⁵	WWS ¹⁵⁶	two patients with severe CNS involvement: MDC1D ²⁰⁸ and WW ²⁰⁹
MDC 1C ¹⁵²	WWS ²⁰⁰	WWS ⁵⁰	MDC with mental retardation, microcephaly, structural brain changes, muscle hypertrophy and eventual myopia ²⁰⁶	MEB ¹⁷¹	
WWS ¹⁹⁸	MEB ⁵⁰	LGMD2? with severe myopia and normal intelligence ^{50,203}	MEB ⁵⁰	MDC with microcephaly, severe mental deficiency, possible ocular changes ^{171,172}	
MEB ¹⁹⁸	Myocardiopathy, minimal or absent muscle weakness, normal intelligence ²⁰¹	MDC with brain malformations without ocular abnormalities ²⁰⁴		MDC and cerebellar involvement ⁵⁰	
MDC with cerebellar cysts ¹⁵¹	LGMD2M ²⁰²			LGMD2? mild, with normal intelligence and inflammatory changes on muscle biopsy ²⁰⁷	
MDC with variable cortical, cerebellar and pontine dysplastic changes ¹⁴⁹				LGMD2? with mental retardation ⁵⁰	

FCMD: Fukuyama congenital muscular dystrophy; LGMD: limb girdle muscular dystrophy; MDC: muscular dystrophy, congenital; MEB: muscle-eye-brain; POMGnT1: protein O-mannose beta1,2-N-acetylglucosaminyltransferase; WWS: Walker-Warburg syndrome.

and MCD1D, with only one report, is also associated to severe muscular and CNS involvement. MDC1C can manifest pure, although severe, muscular involvement or presents associated clinical and radiological CNS involvement, suggested by mental retardation, cerebellar cysts and white matter abnormalities on neuroimaging¹⁴⁹⁻¹⁵¹. The lack of CNS involvement in some patients can suggest that skeletal muscle is more sensitive to perturbation of dystroglycan³⁰. The clinical and diagnostic particularities of each dystroglycanopathy were discussed in Part I of this review. The genetic and clinical heterogeneity of dystroglycanopathies are remarkable and, except for FCMD is impossible to correlate a particular gene with a specific phenotype (Table 2).

The pathogenic mechanism that explains the severe forms of CMDs associated with CNS manifestations emerged in 2001 from different works^{16, 152-154}. Brockington et al.¹⁵² identified a gene that codifies a protein of the fukutin family termed fukutin related protein (FKRP) assigning it to 19q13.3. Mutations in the FKRP gene were found in children with a severe form of CMD who manifested inability to walk and muscle hypertrophy but no CNS changes and whose muscle samples showed a secondary deficiency of laminin alpha-2 immunostaining and a decreased expression of alpha-dystroglycan (DG). They

named this new genotype/phenotype association as MD-C1C and suggested that the mutations in the FKRP gene causing a defective glycosilation (hypoglycosilation) of alpha-DG would explain the basic pathologic mechanism in MDC1C¹⁵². Hayashi et al.¹⁵³ found that the highly glycosylated epitope of alpha-DG was markedly deficient in skeletal and cardiac muscle and reduced in brain tissue obtained from patients with FCMD. These findings suggested that also fukutin could be involved in the glycosylation of alpha-DG that, when altered, could induce a disruption in the extracellular surface membrane of the muscle fiber, also influencing CNS development¹⁵³. Yoshida et al.¹⁶ found that the MEB gene codifies a glycosyltransferase named POMGnT1 that is involved in O-mannosyl glycosylation and, therefore, suggested that an altered glycosylation could be a new pathomechanism for CMDs with neuronal migration disorder¹⁶. Kano et al.¹⁵⁴ reported a selective deficiency of alpha-DG in MEB patients; therefore, they suggested that POMGnT1 acts on alpha-DG and that altered glycosylation of alpha-DG is involved in the pathogenesis of MEB.

At last, in 2002 two works from Campbells' research group at the Iowa University^{17,18} demonstrated that patients with MEB and FCMD, who carry mutant POMGnT1 and fukutin genes, respectively, had an abnormal hypo-

glycosylation of alpha-DG that abolished its binding with the ligands, laminin alpha-2, agrin, and neuexin of the extracellular matrix. The same occurred in the myodystrophy (*myd*) mouse that carried a LARGE mutation and in the knock-out for brain-selective alpha-DG mouse. In the knock-out mouse they found aberrant cerebral and cerebellar migration probably derived from a discontinuous pial surface basal lamina (glia limitans); they considered that the aberrant migration, similar to that seen in patients with MEB and FCMD, was due to the lack of DG preventing its high-affinity binding to the extracellular matrix laminin. These results identify alpha-DG as having an essential role in both muscle and brain development and function and suggested that POMGnT1, fukutin, and LARGE may have a role in transferring sugars to alpha-DG, i.e. act as glycosyltransferases. The recognition of the involvement of other two glycosyltransferases, POMT1 and POMT2, in the pathogenesis of CMDs came soon after¹⁵⁵⁻¹⁵⁶.

During the last seven years, many have discussed and elucidated the pathogenesis of the defective glycosylation of alpha-DG, and the particular cortical involvement that is observed in many of them^{5,711,13,15,17,18,30,33,34,36-47,157-161}. It is supposed that other subtypes of CMD will prove to depend on mutations in genes as yet unidentified but also involved in alpha-DG glycosylation^{39,45,46,48}. In addition, from the first reports on congenital disorders of glycosylation, it seemed unlikely that the glycosyltransferases could have a role only in glycosylation of alpha-DG and the importance of looking for other protein targets has been emphasized^{37,38}.

The role of alpha-DG in developing brain and in neuronal migration has been studied in animals models^{11,18,158,159,162,163}. Zaccaria et al.¹⁶² found that in adult mice alpha-DG is expressed in neurons of the cerebral cortex, hippocampus, olfactory bulb, basal ganglia, thalamus, hypothalamus, brainstem and cerebellum; in addition the astrocytes and their endfeet around blood vessels and the endothelial cells at the blood-brain barrier, also expressed DG. During CNS development, alpha-DG is expressed in the ventricular zone and in basement membranes, therefore participating in neuronal proliferation, in the constitution of the meningeal layer and in migration process as the radial glia is attached to the pial basement membrane¹⁵⁸. In DG-null mice, then abnormal glycosylation of alpha-DG leads to different defects of neuronal migration that at the maximum of rupture of the pial-glial limitans, closely resemble the cobblestone aspect of cortex in human lissencephaly type II¹⁸.

The number of glycosyltransferases involved in the glycosylation of alpha-DG and their different possibilities of regional and developmental expression explain the spectrum of malformations variability and clinical severity that are observed in the different CMDs caused by alpha-dys-

troglycanopathies. Yamamoto et al.¹⁶⁴ analysed the expression and localization of fukutin, POMGnT1, and POMT1 in the CNS and discussed the mechanism of lesions linked to these three glycosyltransferase whose changes are represented by migration disorders in FCMD, MEB, and WWS. They observed that fukutin, POMGnT1, and POMT1 are expressed especially in astrocytes that compose the astrocytic glia limitans and therefore is involved in the formation of the basement membrane. As the latter was found disrupted in fetal cases of FCMD, MEB, and WWS the defect in any of the three glycosyltransferases can be related to the pathogenesis of CNS lesions. These are also expressed in immature neurons what suggests their probable involvement in neuronal migration itself. In mature neurons POMGnT1 and POMT1 are expressed but fukutin is rarely positive indicating that at late stages of development POMGnT1 and POMT1 have a more crucial role than fukutin¹⁶³. In addition, fukutin is expressed in pancreas, kidneys, bronchi, salivary gland, alimentary tract and skin in both fetal and adult mice, and in autopsied FCMD cases it was found decreased in kidneys, lung, skin and intestine¹⁶³. These findings demonstrate the role of alpha-DG glycosylation also in non-muscle systems both during development and in the adult; studies with the aim of clarify why muscle and brain symptoms predominate in FCMD would be useful for better understanding the complex pathogenic mechanism of alpha-dystroglycanopathies¹⁶³.

The wide phenotypical variation of the alpha-dystroglycanopathies, in particular the FKR-related myopathies, has not a clear pathogenic basis. A study analysed patients with FKR mutations who had also CNS alterations and concluded that the distribution of *FKRP* gene mutations did not allow any particular genotype/phenotype correlation concerning the amount of CNS malformations¹⁴⁹.

Keramaris et al.²⁴ considered that the type of missense point mutation could in part explain the phenotypical variation of FKR gene mutations that alter the normal subcellular localization of FKR protein in the Golgi apparatus: the protein is kept in the endoplasmic reticulum and diminishes in the Golgi apparatus. Therefore, each mutation could act in two ways, directly altering the presumed glycosyltransferase activity and further altering the protein function by the mislocalization²⁴. In patients with FKR mutations, the residual expression of alpha-DG detected by immunolabeling and Western blot can be correlated with the clinical severity and with the type of mutation¹⁶⁸. Patients with MDC 1C who exhibit a severe phenotype are compound heterozygotes (one missense and one nonsense mutation) or have homozygous missense mutations; they present absent or strongly decreased alpha-DG expression. Patients with LGMD2I at the severe end of the clinical spectrum (Duchenne-like) tend to present a marked reduction in the immunolabeling of alpha-DG and

are compound heterozygotes (a missense mutation and either another missense or a nonsense mutation) while patients at the milder end (later onset) generally present variable immunohistochemical pattern, sometimes only minimally changed, and in general are homozygous for the common C826A/Leu279Ile FKR mutation¹⁶⁸.

POMT1 and POMT2 mutations are not associated to the same clinical heterogeneity that is observed in the FKR and in general cause classical WW or MEB phenotypes. However, out of these classical phenotypes, different types and degrees of cortical and posterior fossa malformations have been described^{156,169-174}, with or without eye involvement; mental retardation, and calf as well as thigh enlargement has also been related^{170,171}. Yanagisawa et al¹⁷² hypothesized that patients with POMT1 and POMT2 mutations could share the same phenotype because, according to Many et al.²¹, both glycosyltransferases form a heterodimeric complex that is responsible for the catalysis of the first step in O-mannosyl glycan synthesis. Messina et al.¹⁷⁵ emphasized that POMT1 and POMT2 mutations have a wider clinical spectrum than initially thought and that, excluding MEB and WWS phenotypes, mutations causing frameshifts and stop codons were associated to the most severe phenotypes with predominant cerebellar hypoplasia commonly found. Godfrey et al.⁵⁰ analysed eight patients with POMT1 mutations and nine with POMT2 mutations and suggested that *POMT1* and *POMT2* mutations do not seem to manifest a hierarchical involvement of muscle and brain, being associated with significant central nervous system involvement even in patients with relatively mild weakness and who remain ambulant (LGMD2K). In this large revision about alpha-dystroglycanopathies not related to FKR mutations Godfrey et al.⁵⁰ found a broad correlation between the amount of depleted glycosylated epitope and phenotypic severity.

Recently another study¹⁶⁵ demonstrated a good correlation between the clinical phenotype and the amount of glycosylated alpha-DG with the most severe cases being associated to a lack or marked reduction of glycosylated alpha-DG. However, this correlation was strong in patients with POMT1, POMT2 and POMGnT1 mutations, but not consistent in patients whose mutations had occurred in fukutin and FKR genes.

The amount of dystroglycan hypoglycosylation is detected utilizing two monoclonal antibodies (IIH6 and VIA4) that recognize only the functional glycosylated epitope of alpha-DG³⁰; further immunohistochemical studies will be useful for identifying all the glycan structures induced by glycosyltransferases, in particular LARGE activity³⁰.

Considering all the alpha-dystroglycanopathies that have already been supposed by means of hypoglycosylation on the immunohistochemical analysis of muscle biopsies, Muntoni et al.⁵ pointed to a rate of confirmed mo-

lecular diagnosis around 65% of the cases, suggesting that more genes have yet to be identified. The same is clearly demonstrated by the work from Godfrey et al.⁵⁰ that described many patients who had a phenotype suggesting alpha-dystroglycanopathy but no mutations in any of the six glycosyltransferases already identified. Therefore, they concluded that undefined genes are likely to be involved in the pathogenesis of the dystroglycanopathies and that the identification of these genes may provide additional information on the pathway of glycosylation of alpha-DG.

Other great evidence pointing to the probability of the existence of more genes codifying glycosyltransferases not yet identified comes from the molecular studies from patients with WWS. In spite of its worldwide distribution and therefore the good availability of patients' DNA for genomic analysis, only one third of the cases has been associated to mutations in one of the six genes involved in the O-manosylation pathways^{50,166,167}. It is supposed then that additional loci are going to be identified soon^{30,167}.

Finally, Moore and Hewitt³⁰ recently hypothesized that genetic variation between patients for other genes that might have compensatory functions could contribute to this clinical heterogeneity.

Therapeutic perspectives

Glycobiology of O-mannosyl glycans is a broad and open field of research that can favor the understanding of the pathogenesis and consequently the advent of a possible therapeutic strategy for the alpha-dystroglycanopathies. The researches about the long-term potential of glycotherapies for CMD have begun with the first reports about the defective alpha-DG glycosylation as a new pathogenic mechanism for some subtypes of^{7,11,13,15,32-34,36-48,157,176,177}. The central point of these researches is that the overexpression of LARGE promotes the attachment of glycans to alpha-DG, therefore restoring its ligand-binding function¹⁷⁸.

Barresi et al.¹⁷⁸ demonstrated that in Large(myd)mice overexpression of LARGE leads to a recovery of alpha-DG function as a receptor and ameliorates the dystrophic phenotype; in normal mice induces the synthesis of glycan-enriched alpha-DG with high affinity for extracellular ligands. They also demonstrated that in cell lines derived from patients with WWS and MEB (POMT1, POMGnT1 or fukutin mutations) as well as in transfected cell cultures from all cell lines, gene transfer of LARGE restores the laminin-binding activity of alpha-DG and results in hyperglycosylation of alpha-DG; these data indicate that, at least in this experimental over-expression system, LARGE may act in a different glycosylation pathway¹⁷⁸. Therefore, up regulation of this gene might represent a therapeutic approach for the muscular dystrophies caused by defects

of alpha-DG glycosylation¹⁷⁸. The Large myd mouse is an excellent and complete model for glycosylation-deficient CMDs as displays skeletal, cardiac and tongue muscle pathology, defective retinal transmission, and neuronal migration defects¹⁷⁹.

The LARGE homologue, glycosyltransferase-like 1B (GYLTL1B), named LARGE2, shows similar sequence and genomic organization and as LARGE-1 is localized in the Golgi apparatus, what suggests its function in glycosylation²⁹. Since transient overexpression of LARGE2 in mouse myoblast cell line increased alpha-DG glycosylation and its affinity to bind laminin, Grewal et al.²⁹ hypothesized that partial functional redundancy might occur between LARGE 1 and LARGE 2; therefore, the latter should be considered an additional potential therapeutic target. The details about the enzymatic activity of LARGE and LARGE 2 and the identification of the glycan structures that both induce remain undefined³⁰. In addition, it is probable that LARGE 2 overexpression could compensate not only LARGE deficiency as also that of other glycosyltransferases¹⁷⁷. Indeed, it is expected that LARGE 2 could be a candidate gene for an eventual non CMD-like genetic disease, as LARGE 2 has very low expression in muscle and brain³⁰.

However, the CMDs caused by alpha-dystroglycanopathies constitute a therapeutic challenge because any intervention might be early, even prenatally and would need to reach multiple tissues. In addition to the potentially therapeutic overexpression of LARGE 1 and 2, other possible strategies have been discussed^{37,38,40,178,180,181}: to obtain synthetic donors of sugars that could serve as glycan analogues; to carry on enzyme replacement via gene therapy, and to provide ectopic expression or upregulation of other glycosyltransferases that might compensate the loss of function of that altered by the mutation. A similar mechanism has been tested in a mouse model of Duchenne muscular dystrophy in which the dystrophic process was inhibited by the overexpression of Galgt2, another glycosyltransferase not implicated in CMD^{48,176,182}. This promoted the synthesis of a form of alpha-DG that was followed by the recruitment of utrophin at the sarcolemma, therefore restoring its stability. Recent works pointed to the fact that potential therapeutic strategies firstly depend on the complete elucidation of the molecular mechanism that is involved in the formation of DG structure, ligands and function, not only in muscles but also in non-neuromuscular tissues^{48,176}.

The glycosylation itself is poorly understood and many points have to be considered. According to Scianra et al.¹⁷⁶, there might be that glycosyltransferases target other, unidentified, molecules that, hypoglycosylated, could also contribute to the pathogenesis of the alpha-dystroglycanopathies. They also stressed the importance

of better analyzing the interaction between both, alpha and beta subunits, for DG function¹⁷⁶.

Brancaccio¹⁵⁷ discussed the possibility of the existence of a primary dystroglycanopathy, and hypoglycosylated, or even hyperglycosylated alpha-DG molecules also involved either in functional or pathological status; as the other authors above reported, he also emphasized that a possible therapeutic option could come from the compensatory glycosylation function of paralogue glycosyltransferases¹⁵⁷.

Besides the therapeutic perspectives, in the topic correspondent to each subtype of CMD, we have already mentioned the genetic animal models, in general mice that have been studied in the researches on laminin alpha-2 deficient CMD, collagen VI related disorders, and many different types of alpha-dystroglycanopathies. Other, unusual, animal models have been utilized. In zebrafish, all the six identified or putative glycosyltransferases implicated in the pathogenic mechanism of the human muscular dystrophies were found to have similar patterns and expression than in patients, inclusively throughout early development. Therefore the zebrafish can be a useful model system for future researches^{183,184}. Another experimental model for muscular dystrophies associated to disorders of alpha-DG glycosylation has also been found in mutant *Drosophila*¹⁸⁵. The experimental studies in animal models provide new perspectives for clarifying the pathogenic mechanism of the CMDs and for testing potential therapeutic strategies. Unfortunately, until the moment the possibility of transferring some of the good results obtained in animal experiments to humans, in vivo, has to be considered with moderate optimism.

RIGID SPINE CMD

Pathogenesis

Rigid spine syndrome CMD is characterized by marked limitation in the flexion of the spine, gradual development of scoliosis leading to reduced respiratory vital capacity and respiratory failure as well as joints contractures. In 2001, Moghadaszadeh et al.⁹ identified the SEPNI gene and several mutations resulting in rigid spine CMD, suggesting that selenoprotein N can be involved in redox reactions into the cell, protecting it from oxidant damage. Petit et al.¹⁸⁶ demonstrated that SEPNI is a glycoprotein-localized within the endoplasmic reticulum that is more abundant in human fetal tissues than in adult ones, including skeletal muscle. Although it is not a muscle specific protein and it is ubiquitously expressed, its expression is particularly marked in cultured myoblasts and decreases in differentiating myotubes, suggesting a role in early development and in cell proliferation and regeneration¹⁸⁶. In zebrafish embryos deficient for SEPNI, muscle organization and myofibrils architecture are altered during early development¹⁸⁷.

In 2002, Ferreira et al.¹⁸⁸ concluded that rigid spine CMD and the most severe form of classic multimincore disease are different phenotypes associated with mutations in SEPNI gene. Later, they¹⁸⁹ also demonstrated homozygous SEPNI deletion in patients with CMD with Mallory body-like inclusions. Finally Clarke et al.¹⁹⁰ described SEPNI mutations in two sisters with a diagnosis of typical congenital fiber-type disproportion without minicore lesions or dystrophic changes. Therefore the spectrum of selenoprotein-related disorders includes rigid spine CMD, multimincore myopathy, desmin-related myopathy with Mallory body-like inclusions and congenital fiber-type disproportion. Rederstorff et al.¹⁹¹ confirmed the importance of selenium-containing proteins in muscle formation, maintenance and repair and attested that in patients with selenoprotein-related disorders most of mutations are nonsense mutations and deletions, likely to induce a loss of function.

As multimincore myopathy is also caused by mutations in RyR1 gene that codifies the major component of the ryanodine receptor intracellular calcium release channel^{192,193}, the relationships between SEPNI and RyR1 could explain this overlapping spectrum of congenital myopathies¹⁹⁴. In the zebrafish embryo, Juryneć et al.¹⁹⁴ demonstrated that the two proteins participate of the same cellular differentiation events and of the regulation of calcium fluxes. SEPNI acts as a modifier of the RyR channel. In the absence of SEPNI, RyR channels lose their normal sensitivity to redox conditions. This finding explains because mutations affecting either factor lead to similar diseases¹⁹⁴. Lescure et al.¹⁹⁵ recently stressed the importance of also studying the correlation between SEPNI gene and protein and other genes involved in similar phenotypes, like desmin, alpha-actin and different selenoproteins acting in muscles and myocardium. In addition, the molecular function of SEPNI and the role of the selenocystein (Sec) insertion that not only integrates the protein itself but also controls its mRNA quality needs to be further analysed¹⁹⁵.

Therapeutic perspectives

Recently Rederstorff et al.¹⁹⁶ reported a patient with a homozygous point mutation at the selenocystein (Sec) codon (c.G1385A) that converted the normal codon UGA to UAA, therefore preventing synthesis of a full-length active SePN protein. Then, they¹⁹⁵ "engineered a mutant tRNA^{Sec} gene carrying a point mutation in the anticodon, thereby restoring the base-pair complementarity with the SEPNI mutated codon; in patient-derived primary fibroblasts the corrector tRNA^{Sec} gene allowed read-through of the UAA stop codon, thus enabling synthesis of the full-length SePN protein"¹⁹⁶. This is the first attempt of a therapeutic strategy for rigid spine CMD and other SEPNI related

disorders, using a mutation-specific approach. Considering the highly specific molecular context of SEPNI mutations, the authors¹⁹⁵ discuss in details and emphasize the efficiency and technical advantages of this alternative strategy in relation to conventional gene-therapy strategies.

In conclusion, the advances in the field of molecular genetics, cell biology and biochemistry have been the source of a better knowledge on the pathogenic mechanisms involved in the CMDs. However, the development of effective therapies is still at a preclinical stage, except for an attempt of treating patients with Ullrich phenotype with cyclosporin A¹⁴³.

Unfortunately, the induction of a functional protein by gene modification, cell therapy or pharmacological agents has been a long and difficult task. The effective therapy is coming but employing it in large-scale clinical trials constitutes a challenge that is much greater in undeveloped countries.

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