



Review

The congenital muscular dystrophies in 2004: a century of exciting progress

Francesco Muntoni^{a,*}, Thomas Voit^b

^aDepartment of Paediatrics and Neonatal, Dubowitz Neuromuscular Unit, Imperial College School of Medicine, Hammersmith Hospital Campus, Du Cane Road, London W12 0NN, UK

^bDepartment of Pediatrics and Pediatric Neurology, University Hospital Essen, Hufelandstrasse 55, D-45122 Essen, Germany

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Abstract

The congenital muscular dystrophies are a heterogeneous group of inherited disorders. The clinical features range from severe and often early fatal disorders to relatively mild conditions compatible with survival into adult life. The recent advances in the genetic basis of congenital muscular dystrophies have allowed to significantly improve our understanding of their pathogenesis and clinical diversity. These advances have also allowed to classify these forms according to a combination of clinical features and primary biochemical defects. In this review we present how the congenital muscular dystrophies field has evolved over the last decade from a clinical and genetic point of view. © 2004 Elsevier B.V. All rights reserved.

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1. Introduction

Congenital muscular dystrophy (CMD) is a common and clinically heterogeneous condition. Firstly described by Frederick Eustace Batten in 1903 [1], the condition has been increasingly recognised and approximately 1500 articles on CMD can now be found on Pubmed. A detailed historical review can be found in Voit and Tomé, 2004 [2]. In the last decade a significant input to the field came from the activity of the European Neuromuscular Centre CMD Consortium, which convened eight workshops of which the proceedings have all been published in *Neuromuscular Disorders* [3–9].

There are important regional variations regarding the occurrence of different CMD variants; in addition, the clinical features and spectrum of severity are only now becoming more clear. However, the only detailed epidemiological figures available regards the North East of Italy, with incidence and prevalence figures of 4.65×10^{-5} and

8×10^{-6} , respectively [10]. This suggests that CMD is one of the most common neuromuscular disorders. This is also the clinical experience of both authors.

2. Congenital muscular dystrophies: classification

Until recently patients with different CMD variants were assigned to a specific category on the basis of the main clinical features and country of origin. More recently, however the molecular genetic data have indicated that this approach, while still valid in many respects, has its limitation when applied to genetic counselling. It is now well recognized, for example, that virtually all patients with ‘Fukuyama type congenital muscular dystrophy’ (FCMD) of non-Japanese descent do not have mutations in the *fukutingene* and therefore have different conditions. Similarly, allelic mutations of a number of genes give rise to different ‘clinical’ conditions. The classification of CMD therefore has to rely on the clinical features of affected individuals together with the identification of the genetic and biochemical defects. Ten genes causing specific forms of CMD have so far been identified; a number of conditions have also been described which are clinically and

* Corresponding author. Tel.: +44-208-383-3295; fax: +44-208-740-8281.

E-mail address: fmuntoni@ic.ac.uk (F. Muntoni).

genetically different from these 10 diseases. This suggests that the number of CMD variants will be in excess of those currently recognized, and it is the opinion of the authors that at least 20 genes responsible for different forms of CMD will eventually be identified.

3. CMD diseases: towards a biochemical classification

Taking into account the primary genetic defects, one can recognize the following disease categories:

1. Genes encoding for structural proteins of the basal membrane or extracellular matrix of the skeletal muscle fibres. This includes *collagen 6 genes*; *laminin $\alpha 2$ chain* and *integrin $\alpha 7$* .
2. Genes encoding for putative or demonstrated glycosyltransferases, that in turn affect the glycosylation of dystroglycan, an external membrane protein of the basal membrane. Genes belonging to this category include *POMT1*; *POMGnT1*; *fukutin*; *fukutin-related protein (FKRP)*; *Large*.
3. *Selenoprotein 1*, which encodes an ER protein of unknown function.

Table 1 summarises the genes involved in the known forms of CMD together with their main clinical features,

while Fig. 1 shows the schematic location of number of these proteins in the extracellular matrix.

3.1. Genes encoding for structural proteins of the basal membrane or extracellular matrix of the skeletal muscle fibres

Two of the three variants belonging to this group, namely CMD with laminin $\alpha 2$ deficiency and the variant with collagen VI deficiency are amongst the most common forms of CMD.

3.1.1. Congenital muscular dystrophy with laminin $\alpha 2$ deficiency (also known as merosin-deficient CMD; or MDC1A)

Primary deficiency of laminin $\alpha 2$ accounts for ~30–40% of all patients with CMD although regional variations do occur. Initially identified by Tomé et al. [11], this variant was called the classical, occidental type CMD, or merosin-deficient CMD. The latter name indicated the deficiency of the trimer formed by the combined expression of laminin $\alpha 2$, laminin $\beta 1$ and laminin $\gamma 1$. Subsequent studies localized the disorder to the region of the *LAMA2* gene on chromosome 6q2 [12], and mutations in the corresponding gene were identified shortly after [13].

Table 1
Genetically recognized forms of CMD

| Protein category | Disease | Abbreviation | Gene symbol | Gene location | Protein | Serum CK |
|---|--|--------------|-------------|---------------|--|----------|
| Extracellular matrix proteins | Merosin deficient CMD | MDC1A | LAMA2 | 6q2 | laminin $\alpha 2$ | ↑↑↑ |
| | Ullrich syndrome (1,2,3) | UCMD1 | COL 6A1 | 21q2 | Collagen VI | = ↑ |
| | | UCMD2 | COL 6A2 | 21q2 | collagen VI | |
| | | UCMD3 | COL 6A3 | 2q3 | collagen VI | |
| | Integrin $\alpha 7$ deficiency | | ITGA7 | 12q | Integrin $\alpha 7$ | = |
| Glycosyltransferases (variants with abnormal glycosylation of α -dystroglycan) | Walker Warburg syndrome | WWS | POMT1 | 9q34 | Protein- <i>O</i> -mannosyltransferase | ↑↑↑ |
| | Muscle-eye-brain | MEB | POMGnT1 | 1p3 | <i>O</i> -linked mannose beta 1,2- <i>N</i> -acetylglucosaminyltransferase | ↑↑↑ |
| | Fukuyama CMD | FCMD | FCMD | 9q3 | Fukutin | ↑↑↑ |
| | CMD+secondary merosin deficiency 1 | MCD1B | ? | 1q4 | ? | ↑↑ |
| | CMD+secondary merosin deficiency 2 | MCD1C | FKRP | 19 | Fukutin related protein | ↑↑↑ |
| | CMD with mental retardation and pachygyria | MDC1D | LARGE | 22q | Large | ↑↑ |
| Proteins of the endoplasmic reticulum | Rigid spine syndrome | RSMD1 | SEPN1 | 1p3 | Selenoprotein N, 1 | = ↑ |

=, normal; ↑, mildly elevated; ↑↑, moderately elevated; ↑↑↑, severely elevated.

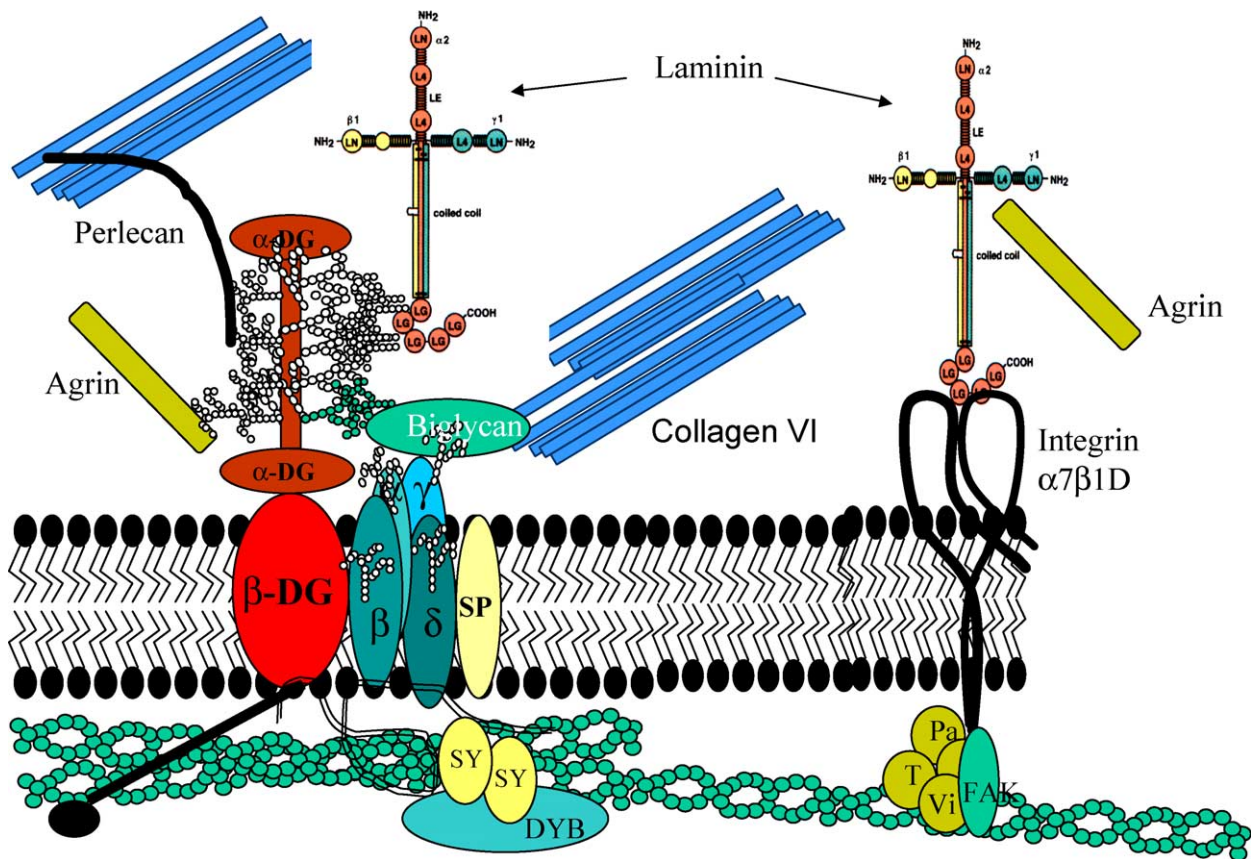


Fig. 1. This figure summarises the location of a number of proteins described in this article. In particular α -dystroglycan, laminin $\alpha 2$, the integrin complex and collagen VI can be visualised.

Laminin is an abundant protein in the extracellular matrix and takes the form of a cross-shaped heterotrimer through the association of an α , β and γ chain, each of which are encoded by separate genes [14]. To date five α ($\alpha 1$ – $\alpha 5$) chains, three β ($\beta 1$ – $\beta 3$) chains and three γ ($\gamma 1$ – $\gamma 3$) chains have been identified [15]. The predominant forms expressed in skeletal muscle are laminin-2 ($\alpha 2$ – $\beta 1$ – $\gamma 1$, also known as merosin) and laminin-4 ($\alpha 2$ – $\beta 2$ – $\gamma 1$). Despite the original name of ‘merosin deficient CMD’, both laminin-2 and 4 are affected by mutations in the LAMA2 chain gene; this condition is now also known as MDC1A.

Laminins are secreted into the extracellular matrix and bind to a number of other macromolecules such as nidogen, agrin, and collagen IV in the extracellular matrix, and to the two main transmembrane laminin receptors, dystroglycan and integrins. The main action of laminins is the cell–cell recognition, differentiation, cell shape, movement, transmission of force, and tissue survival [16,17]. Laminin-2 is expressed in the striated muscle basement membrane as well as in the basal lamina of the cerebral blood vessels, in the developing white matter tracts and Schwann cells.

3.1.1.1. Clinical features of MDC1A. Children affected by MDC1A invariably present at birth or in the first few months of life with hypotonia and weakness; respiratory and feeding

problems can also be present although not so severe to require the need for ventilatory support at birth [18]. Contractures can occur, but severe arthrogyrosis is rare. Prominence of the calves can be observed in the early phases of the disorder, but the phenotype is more commonly an atrophic one. Weakness affects the limbs more proximally than distally, and axial muscles are severely affected as well. Limited ocular movements resulting in partial external ophthalmoplegia can be observed in the later stages [19].

The maximal motor ability is only sitting unsupported; often children can stand with some form of support but only rarely walk with support. In the personal series of the authors, only two of 46 cases with total laminin $\alpha 2$ chain deficiency achieved independent standing and limited ambulation that was eventually lost following the development of a progressive scoliosis. Muscle power does not change significantly in most cases; however, clear progression of weakness can be occasionally documented. Increased flexion deformity at the hips, knees, elbows and ankles, followed by rigidity and scoliosis of the spine occur almost invariably. In view of the severe phenotype, conservative management is usually preferred to orthopaedic procedures and spinal surgery is often not a realistic option for these children.

Frequent complications in MDC1A include respiratory failure, feeding problems and failure to thrive [20]. With regard to respiratory function, an invariable complication is severe restrictive respiratory syndrome. Nocturnal hypoventilation occurs at ages ranging from 5 to early teens. Treatment with night-time non-invasive positive pressure ventilation delivered by facemask resolves these symptoms and affects the long-term prognosis of this condition.

The failure to thrive, common in MDC1A, is accompanied by increased risk of aspiration pneumonias. Early speech and language and dietician input is indicated; gastrostomy should be considered in children who are failing to thrive or have uncoordinated/unsafe swallow [20]. Cardiac failure is rare in MDC1A [21], but a proportion of cases have a mild to moderate left ventricular hypokinesia [22].

3.1.1.2. Central and peripheral nervous systems involvement. In the human brain, laminin $\alpha 2$ is expressed in the basement membrane of blood vessels including the capillaries that form the blood–brain barrier [23]; on the brain surface, laminin $\alpha 2$ is expressed in the glia limitans suggesting a role in the guidance of neuronal migration; in addition, laminin $\alpha 2$ expression has been observed along developing axon tracts, where it interacts with $\beta 1$ integrin and might have a role in myelin membrane formation in oligodendrocytes [24]. Brain magnetic resonance imaging (MRI) studies invariably show white matter changes in patients with MDC1A after the age of 6 months. These changes can be demonstrated as increased signal intensity of white matter on T₂-weighted MRI [25] (Fig. 2A) and are diffuse, although they spare the internal capsule, corpus callosum, basal ganglia, thalami, and cerebellum. Using fast-spin echo MRI sequence, these changes can be demonstrated already at birth. To date, no patient with mutation-proven complete laminin $\alpha 2$ deficiency and normal white matter after age of 6 months has been reported. Brain MRI represents therefore a powerful tool in the study of patients with this form of CMD. In addition to the white matter abnormalities, structural brain changes have been reported in some patients with complete or mutation-proven partial laminin $\alpha 2$ deficiency. These included occipital polymicrogyria/agyria, and hypoplasia of pons and/or cerebellum [26,27]. Whenever present (~5% of cases), occipital agyria is associated with mental retardation (cognitive function is otherwise normal in MDC1A) and epilepsy. This latter is a frequent complication of MDC1A and in the experience of the authors it can affect up to 30% of cases [28].

Visual function is normal. Electrophysiological studies have however shown that visual and somatosensory evoked responses are usually abnormal in MDC1A [29].

Children with MDC1A have a motor demyelinating neuropathy; sensory nerve function is unaffected in young children [30], but involvement of these nerves can be demonstrated in older patients.

3.1.1.3. The LAMA2 gene: genotype–phenotype correlations. The LAMA2 gene is composed of 64 exons. The resulting protein is structurally organized into six domains: the N-terminal domain VI participates in polymerization and is important for integrin binding [31]; domains V, IIIb and IIIa contain cystein-rich EGF-like repeats resulting in rigid, rod-like structure; domain III is important for entactin/nidogen binding; domains Vb and IVa are predicted to form globular structures while the laminin long arm binds to agrin [32]. The coiled-coil forming domains II and I are important for the assembly of the heterotrimer, while the C-terminal end is formed by the G-domain, composed of five globular LG-modules which are important for binding cell-surface receptors. In particular, the LG-domains 1–3 and 4–5 bind to α -dystroglycan, and this binding is also important for the induction of AChR clustering. In addition, the LG 4–5 modules are required for basement membrane assembly [33] (Fig. 1).

A wide spectrum of mutations including stop, missense, non-sense, splice and deletion mutations of the LAMA2 gene spread over the entire length of the gene and leading to complete or partial laminin $\alpha 2$ deficiency has been reported (reviewed in Ref. [37]). Mutations precluding the synthesis of domains I and II, and/or of the G-domain typically result in severe phenotype. The correlation between phenotype and genotype is however complex for mutations that occur outside these areas and in some instances lead to a severe phenotype even in patients with mutations that allow the expression of relatively high level of protein. Prenatal diagnosis is available following molecular genetic studies and the immunostaining of the trophoblast, a tissue which also expresses laminin $\alpha 2$ chain (reviewed in Refs. [38,39]).

3.1.1.4. Pathological diagnosis and differential diagnosis of MDC1A. Absence of laminin $\alpha 2$ from skeletal muscle gives rise to a typical dystrophic picture with massive muscle fiber necrosis and regeneration combined with endo- and perimyseal fibrosis that can be detected already immediately after birth [11]. Prominent inflammatory infiltrate can lead to the erroneous diagnosis of congenital inflammatory myopathy [40]. From a diagnostic point of view, it is essential to use multiple antibodies directed against different portions of laminin $\alpha 2$ especially in cases in whom residual expression of the protein is found [41,42]. In patients with complete laminin $\alpha 2$ deficiency, a concomitant marked reduction of α -dystroglycan, and of laminin $\beta 2$ and integrin $\alpha 7$ is observed. The laminin heavy chains $\alpha 4$ and $\alpha 5$ are characteristically overexpressed [34,36], while the light chains $\beta 1$ and $\gamma 1$ as well as β -dystroglycan are expressed at normal levels [34,35].

Laminin $\alpha 2$ is also expressed at the junction of the dermis and epidermis in skin, and its expression in this tissue can therefore be used for diagnostic purposes [43].

In cases with partial deficiency of laminin $\alpha 2$ (see below), variable combinations of antibody staining can be observed [41,42] and this can help to assign one individual

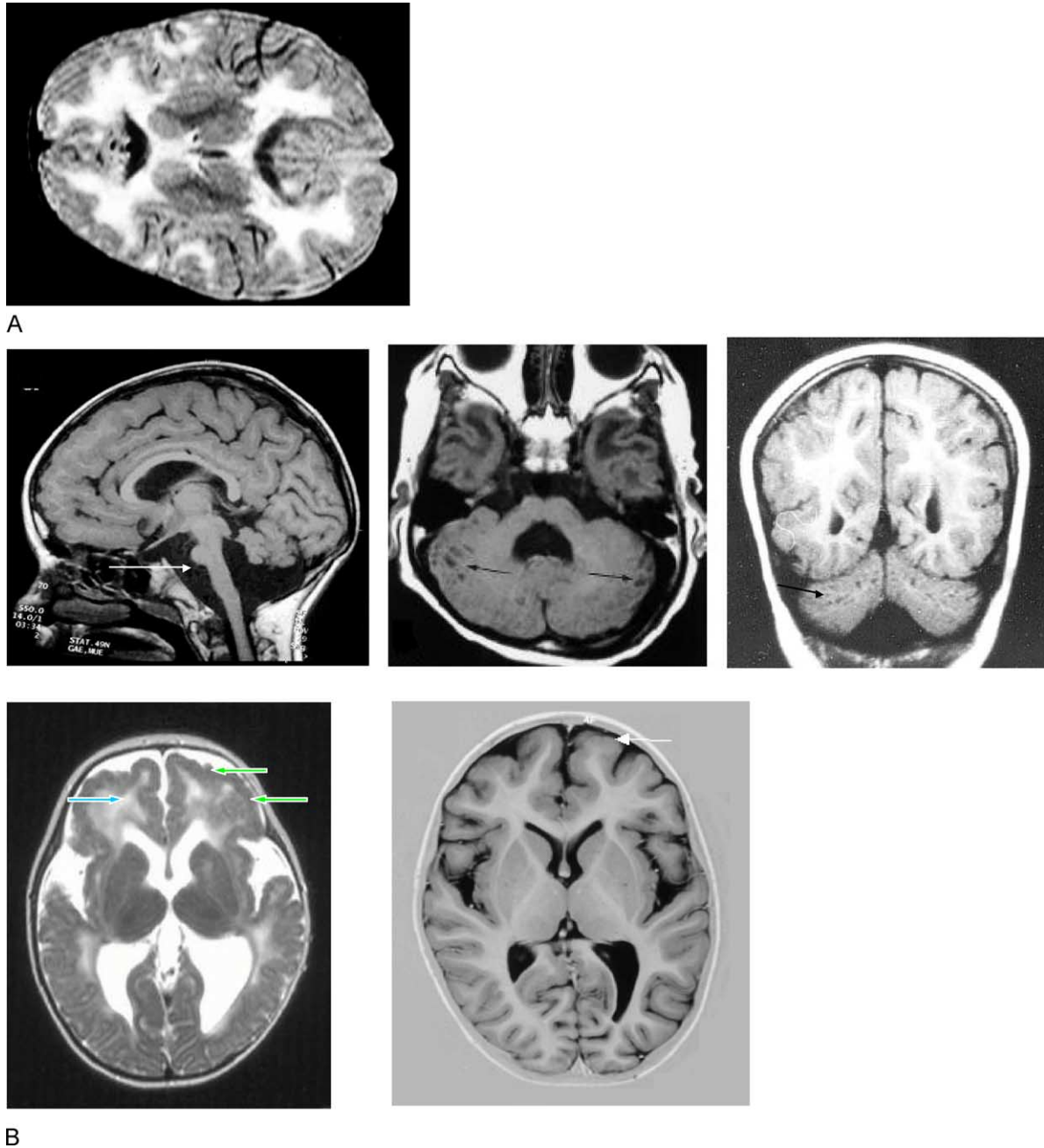


Fig. 2. (A) Typical white matter changes observed in MDC1A. The corpus callosum and the cerebellum are spared. (B) Typical 'cobblestone' complex, seen in FCMD; MEB; MDC1D and some patients with MDC1C. The brainstem is flattened (white arrow, top left panel), cerebellar cysts are present (black arrows, top panel central and right). The cortical folding in the frontal and parietal areas is abnormal because of thickened cortex (arrows, bottom panels).

patient to a primary laminin $\alpha 2$ deficiency. In some patients however it can be difficult to distinguish the pattern of a partial primary deficiency from the secondary reduction observed in conditions due to mutations in glycosyltransferases (see below). A combined study of dystroglycan expression using antibodies against the core protein and the glycosylated epitopes is essential in these cases, also followed by Western blot analysis of α -dystroglycan. It should also be noted that abnormal expression of laminin $\alpha 2$ on Western blots occurs also in patients with fukutin-related-protein gene defects who also show partial

immunocytochemical reduction of α -dystroglycan and laminin $\alpha 2$ [44,45]. Similar secondary changes in laminin $\alpha 2$ chain expression can be found also in MEB, Walker Warburg syndrome (WWS) and FCMD. It is therefore of critical importance for reaching a diagnosis in patients with partial laminin $\alpha 2$ reduction to integrate clinical (brain imaging; peripheral nerve electrophysiology) and molecular data into the diagnostic approach.

3.1.1.5. Clinical variant of MDC1A: partial (primary) laminin $\alpha 2$ deficiency. In rare patients allelic milder

mutations of the laminin $\alpha 2$ chain gene result in partially preserved expression of laminin $\alpha 2$ and in clinically milder phenotypes [28,46,47]. The spectrum varies from milder variants of CMD (with ability to acquire independent ambulation) to a limb girdle muscular dystrophy. The typical involvement of the central nervous system is a helpful clinical hint that allows to suspect this condition. As also observed in MDC1A, epilepsy is a common complication of patients even if mildly affected from a skeletal muscle point of view. In addition mildly reduced nerve conduction velocities can also be documented in these patients; CK levels are elevated (3–30 \times). A small proportion of patients with partial laminin $\alpha 2$ deficiency follows a severe course, indistinguishable from complete deficiency. The reason for this is likely related to the involvement of a crucial portion of the laminin molecule that results in the production of a non-functional protein.

3.1.2. Ullrich congenital muscular dystrophy

This is probably the second most common variant of CMD. Originally described in 1930, the classical features of Ullrich congenital muscular dystrophy (UCMD) are represented by the combination of congenital contractures of the proximal joints, torticollis, kyphoscoliosis associated with hyperelasticity of the distal joints [48]. Normal intelligence and respiratory failure are also integral features.

3.1.2.1. Clinical features of UCMD. Typical features of UCMD patient are presentation in the neonatal period with muscle hypotonia, kyphosis of the spine, frequently combined with proximal joint contractures, torticollis, and hip dislocation. At the same time the distal joints show striking hyperlaxity with extended talipes and protruding calcaneus; however, hyperlaxity may be absent in severe cases. The spinal kyphosis and the proximal contractures can be transient or at least improve under physiotherapy. Contractures tend however to recur and eventually affect also the previously lax ankles, wrists and fingers; particularly severe (as in Bethlem myopathy) are the long finger flexion contractures. Maximum motor function is very variable. Some patients never walk whereas others achieve ambulation in time or with delay up to the fourth year. Progressive functional difficulties mostly secondary to increased contractures leading to loss of ambulation after a period of independence are common. Many patients have a characteristic facial appearance with a rounded face with slight drooping of the lower lid and prominent ears. The skin typically shows follicular hyperkeratosis, a sign already noted by Ullrich. Additional more uncommon features are cheloid formation and a softer consistency of the skin in the palms and soles. Scoliosis that may require surgical correction is a common complication. Ventilatory insufficiency almost invariably develops in the first or second decade.

3.1.2.2. The collagen six genes in UCMD. Collagen VI is a ubiquitously expressed extracellular matrix protein

composed of three chains, $\alpha 1$, $\alpha 2$, and $\alpha 3$, that form a monomer made up of two globular domains connected by a triple helical structure. Prior to secretion into the extracellular space, the three chains assemble in the cytoplasm into antiparallel dimers which associate laterally into tetramers [49]. These associate end to end to form a microfibrillar network that interacts with the fibronectin network, biglycan and collagen 4 (Fig. 1).

The three chains are encoded by the genes *COL6A1* and *COL6A2* on chromosome 21q22.3 and *COL6A3* on chromosome 2q37. Mutations in the *COL6A2* gene leading to UCMD were first reported in 2001 [50].

The spectrum of the clinical phenotype in patients with recessive mutations in the *COL6A* genes is rapidly expanding. In addition to the typical phenotype including congenital kyphosis, torticollis, proximal contractures, and distal hyperlaxity, followed by delayed motor development and later respiratory compromise, milder patients who did not have neonatal contractures and showed normal motor milestones have now been reported. At the other end of the spectrum, clearly there are patients in whom the distal laxity is absent.

Collagen VI (ColVI) staining of the muscle biopsy is a useful diagnostic tool for UCMD; however, the changes in some patients with *COL6A* gene mutations can be very subtle, and minimal reduction of the protein can therefore be significant but difficult to appreciate. While there is no straightforward correlation between protein levels and phenotype, cases with completely absent protein are severely affected. On the other hand, cases with minimal reduction can be mildly or severely affected. When ColVI expression was variably reduced, skeletal muscle showed a most prominent loss in the basal lamina [51], whereas labelling of the connective tissue, basal lamina of capillaries and vessels was normal or almost normal. It is therefore possible that in some patients the changes in ColVI expression could be so subtle to escape detection. This obviously complicates the diagnostic process as the identification of mutations in the *COL6A* genes is not a trivial task (see below). ColVI is also expressed in skin where staining can be decreased in the papillary dermis and around skin hair follicles.

Fibroblast cultures have been used to study the molecular mechanisms of ColVI reduction. A number of mutations lead to non-sense-mediated mRNA decay of the affected α chain and thereby precluded correct assembly and secretion of ColVI tetramers, while others resulted in reduced secretion of abnormal monomers that could not properly assemble into dimers and tetramers and formed abnormal microfibrillar networks. Parents carrying a recessive mutation showed reduction of the corresponding RNA levels but in vitro long-term matrix deposition of ColVI was normal [49]. Recent data suggest that 'severe' dominant mutations can result in dimer formation and secretion of abnormal tetramers which exert a strong dominant negative effect on microfibrillar assembly. This lead to a loss of

normal localization of collagen VI in the basement membrane and eventually resulted in a severe phenotype [52]. This information is very important when providing genetic counseling to individuals with ‘UCMD’ phenotype, as clearly dominant and recessive mutations can both result in a UCMD phenotype.

Studies on muscle fibers from a *Col6a1* knock out mouse model revealed reduced contractile force and disturbed intracellular calcium homeostasis. More recently, *Col6a1*^{-/-} muscles were shown to have a loss of contractile strength associated with ultrastructural alterations of sarcoplasmic reticulum and mitochondria and spontaneous apoptosis. This was secondary to abnormal activation of the mitochondrial permeability transition pore which could be rescued following administration of cyclosporin A (CsA) [53]. These findings therefore link a defect of the extracellular matrix to a mitochondrial dysfunction followed by apoptosis which is preventable using cyclosporin. This observation can now be exploited for therapeutic intervention.

3.1.2.3. Muscle pathology in UCMD. Skeletal muscle pathology in UCMD ranges from mildly myopathic to overtly dystrophic with increased variation of fiber size, some necrotic fibers and prominent endo- and perimysial fibrosis and adipose tissue substitution. A reduced or absent Col6 labelling suggests a diagnosis of UCMD. However, this has recently been reported in patients with no mutations in the *COL6* genes, suggesting a secondary down-regulation of this protein in some other conditions [54]. Subtle deficiencies of collagen VI characterise some patients with UCMD; in particular the link between the collagen VI fibrils and the basal lamina can be lost [54]. Perlecan, collagen IV and laminin $\alpha 2$ expression is normal.

3.1.2.4. Differential diagnosis of UCMD. While the recognition of the typical phenotype in a young child with all the associated clinical features is straightforward, older patients who are severely weak and ventilated might share features with patients with rigid spine syndrome. Muscle MRI can help in the differential diagnosis [55].

The clinical overlap with Bethlem myopathy has been already highlighted; another condition that shares some clinical features with UCMD is central core disease, in which very significant distal laxity can be present. The lack of diaphragmatic weakness and skin involvement helps to suspect CCD. Finally, we and others have identified a number of families with evocative features of UCMD in whom however the collagen VI expression was normal. Mild mental retardation complicated the clinical picture in some of these patients and linkage analysis excluded the involvement of the collagen VI gene in the informative families [8]. This likely represents an entirely different and novel disorder.

3.1.3. Integrin $\alpha 7$ deficiency

Integrins are heterodimeric transmembrane glycoproteins consisting of an α and a β chain. Integrin $\alpha 7\beta 1$ is a major laminin $\alpha 2$ receptor in skeletal myotubes and mature myofibers. Integrin $\alpha 7\beta 1$ expression and localization is laminin $\alpha 2$ -dependent [56] (Fig. 1). Primary deficiency of integrin $\alpha 7$ appears to be an exceptionally rare form of CMD. So far only three patients with normal laminin $\alpha 2$ but absent integrin $\alpha 7$ were found to carry causative mutation in the integrin $\alpha 7$ gene [57,58]. Clinically, these patients rather suffered from a mild congenital myopathy with delayed motor milestones, and muscle biopsies only showed mild variation of fibre size. The direct diagnosis of integrin $\alpha 7$ deficiency from immunostaining is hampered by the developmental regulation and interindividual variation seen especially in the first two years of life where integrin $\alpha 7$ expression as detected with the available antibodies is frequently low. From these studies it can be concluded that integrin $\alpha 7$ remains a candidate for causing a probably very rare form of CMD or congenital myopathy but the nosological place of this disorder remains to be further characterised.

3.2. Disorders of O-glycosylation/ α -dystroglycanopathies

Five conditions belonging to this group have been identified so far; they are characterised by mutations in proven or putative glycosyltransferases and all share an abnormally glycosylated dystroglycan. This finding is likely to be central to the muscle pathology that characterises these variants. There is good evidence to suggest that a similar pathological process is also responsible for the structural brain and eye involvement seen in these forms. α and β -dystroglycan are encoded by a single gene *DAG1*, which undergoes post-translational cleavage to give rise to two glycoproteins which are tightly associated via non-covalent interactions [59]. The primary sequence of α -dystroglycan predicts a molecular mass of 72 kDa. However, the mass of α -dystroglycan in skeletal muscle is 156 kDa, while in brain and peripheral nerve it is 120 kDa. This is the result of tissue specific patterns of post-translational modification which this protein undergoes. Whilst dystroglycan contains four potential N-linked glycosylation sites (three of which are in β -dystroglycan and one in α -dystroglycan), it is the O-linked glycosylation that makes the major contribution to the observed molecular weight [60]. Multiple O-linked glycosylation sites are located in the serine–threonine-rich ‘mucin’ domain of α -dystroglycan. β -Dystroglycan interacts with the C terminal region of α -dystroglycan at the membrane periphery and dystrophin, utrophin, caveolin, actin and Grb2 in the cytoplasm, thereby linking the extracellular matrix with cytoplasmic and signalling components of the muscle fibre [61] (Fig. 1).

Dystroglycan is essential for basement membrane formation and its complete disruption in mice is embryonically lethal as a result of the failure to form the basement

membrane (Reichert's membrane) that separates the embryo from the maternal circulation. Conditional mutagenesis resulting in striated muscle-specific disruption leads to loss of the dystrophin–glycoprotein complex and muscular dystrophy in mouse [62]. Similar brain-specific disruption perturbs the glia limitans with consequent overmigration of the neurons into the subarachnoid space resulting in loss of cortical layering like in human type II (or cobblestone) lissencephaly [63]. An example of the cobblestone complex can be found in Fig. 2B. The targeted mutations of dystroglycan therefore recapitulate to some extent the pathological events observed in the CMD variants which belong to this category, namely WWS, muscle-eye-brain disease (MEB), FCMD, congenital muscular dystrophy 1C (MDC1C) and 1D (MDC1D). All these conditions are collectively referred to as α -dystroglycanopathies.

In addition, a growing number of CMD syndromes also appear to be characterised by loss of glycosylated α -dystroglycan. While it is very likely that also these CMD variants are secondary to proteins involved in the glycosylation of α -dystroglycan, this is at the moment speculative. For this reason we have grouped these forms in Section 4, as forms 'in search of a genetic defect'.

In the sections below we will briefly describe the main clinical features of the five different diseases; at the same time we will also highlight how these five conditions are essentially linked by an identical pathogenetic process (i.e. the hypoglycosylation of α -dystroglycan) and that allelic mutations of several of these genes can result in different syndromes, as a result of the severity of the mutation and the resulting hypoglycosylation of α -dystroglycan [64].

3.2.1. *Fukuyama congenital muscular dystrophy*

FCMD was first described by Yukio Fukuyama from Japan in 1960 [65]. The disorder is particularly frequent in Japan where it represents the second most common form of muscular dystrophy after Duchenne dystrophy. The molecular basis for the high frequency of FCMD in Japan is secondary to a founder mutation that will be described below.

3.2.1.1. Clinical features of FCMD. The classical picture of a child with FCMD is the combination of generalized muscle weakness, severe brain involvement with mental retardation, frequent occurrence of seizures and abnormal eye function. First symptoms may occur in utero with poor fetal movements or at birth where asphyxia is not uncommon. Severe arthrogryposis is however unusual. Functional improvement is frequently observed and most patients achieve standing with support and occasionally are able to take a few steps with support between the age of 2 and 8 years. Enlargement of the calves, quadriceps muscles and tongue is common. Progressive weakness then develops and respiratory failure in the middle–late teens is an invariable complication.

The life expectancy averages about 15 years but survival into the mid 1920s is becoming increasingly possible [66].

Progressive contractures including hips, ankles and knees are an early feature and scoliosis commonly follows the loss of independent sitting after 9 years of age.

Rare more severely affected individuals might only sit with support and cannot control their head. Severe weakness is usually combined with profound mental retardation, and these patients typically do not speak meaningful words, whereas the majority learn to speak short sentences and may even become able to read and write a few characters. Most patients develop seizures before 3 years of age.

Cardiac involvement (dilated cardiomyopathy) is almost invariable and typically develops in the second decade of life.

About 50% of the classical FCMD cases show signs of ocular involvement ranging from abnormal eye movements, poor visual pursuit, and strabismus to severe myopia, hyperopia, or cataracts. At the more severe end of the spectrum however there can be retinal detachment and microphthalmos.

The brain changes in FCMD are broadly speaking similar to those in other forms of α -dystroglycanopathy. These are collectively recognised as part of the type II lissencephaly spectrum which encompasses the 'cobblestone' polymicrogyria–pachygyria on one end and the complete agyria on the other. The regular layering of the cerebral cortex is perturbed or lost, and overmigration of neurons beyond the glia limitans into the leptomeninges develops during early fetal life. Hemispheric fusion can be observed; obstructive hydrocephalus is rare and only few patients require shunting. The cerebellum shows cystic lesions under the cerebellar cortex containing granular cells and mesenchymal tissue. In addition brain MRI typically shows a transient delay of myelination that tends to gradually diminish with age.

3.2.1.2. The *fukutin* gene. FCMD is caused by mutations of the *fukutin* gene on chromosome 9q31 [67]. Its protein product, fukutin, has sequence homologies with bacterial glycosyltransferase, but its precise function is unknown.

A retrotransposal insertion into the 3' UTR of fukutin mRNA accounts for 87% of FCMD chromosomes and is considered to be a relatively mild mutation as it only partially reduces the stability of the full length mRNA. In keeping with this interpretation, combined heterozygotes between this mutation and deletions or non-sense mutations have a more severe phenotype than individuals homozygous for the retrotransposon. While targeted inactivating mutations of both alleles in the mouse are not compatible with life, recently two patients with functional null mutations in a homozygous state were identified. Interestingly, they both had a more severe WWS-like phenotype indicating that complete loss of fukutin function is compatible with life in the human [68,69].

3.2.1.3. Pathological studies. Various abnormalities of proteins of the plasma membrane and extracellular matrix had been described over the years in FCMD. In particular laminin $\alpha 2$ and several proteins of the dystrophin-associated glycoprotein complex were found to be abnormal in FCMD muscle. Following the suggestion that fukutin was a glycosyltransferase, Hayashi et al. demonstrated a complete loss of glycosylated α -dystroglycan from FCMD muscle, identifying the involvement of this molecule as a possible substrate for the deficiency of a putative glycosyltransferase [70].

In addition abnormally glycosylated α -dystroglycan in FCMD has lost most of its laminin $\alpha 2$, neurexin and agrin binding abilities [63]. Electron microscopy confirms a disruption of the muscle fiber basal lamina. The brains of FCMD fetuses characteristically show breaches in the glia limitans–basal lamina. The glia limitans is formed by the endfeet of astrocytes, and in situ hybridisation and immunohistochemical analyses suggest that fukutin is normally expressed in both fetal and adult glial cells (some of which are astrocytes) in addition to neurons [71]. A similar pattern of abnormal neuronal migration was observed in mice with a brain-specific disruption of α -dystroglycan [72] suggesting that impaired dystroglycan function plays a significant role in the central nervous system manifestation of FCMD. One recent pathological study in FCMD additionally reported an abnormal migration of neurones intermingled in the pontomedullary region and ventrolateral pontine surface in the brainstem, suggesting a disruption of both radial and tangential neuronal migration [73].

3.2.2. Muscle-eye-brain disease

MEB is a form of CMD of similar severity to FCMD and is characterised by eye involvement (congenital myopia and glaucoma, retinal hypoplasia), mental retardation and structural brain involvement (pachygyria, flat brainstem and cerebellar hypoplasia). MEB was first described as a separate entity by Pirkko Santavuori and colleagues in Finland in 1977 [74]. The recent identification of the primary genetic defect allowed to identify also MEB cases outside Finland and to broaden the clinical spectrum of the condition.

3.2.2.1. Clinical features. The severity of MEB varies and can be broadly correlated with the molecular genetic defect. Typically, MEB patients present in the neonatal period with profound muscle hypotonia, and poor visual alertness. Patients at the severe end of the spectrum remain bedridden, never achieve sitting, head control, visual contact. These patients may die during the first years of life. Moderately affected patients usually show high myopia, but have some preserved vision enabling them to establish contact. Their maximum motor ability is to sit unsupported, and occasionally speak a few words. Muscle enlargement can be present. Patients at the milder end of the spectrum may

acquire ambulation for a number of years. Often their functional abilities are more impaired by the coexistence of spasticity and ataxia than muscle weakness. Vision is preserved in these patients and limited verbal communication skills possible. Epilepsy is a common complication of MEB. Long-term survival is similar to FCMD, and 85% of the Finnish patients reach adulthood.

The eye involvement is more severe than in FCMD. Typical features are a high myopia, retinal dysplasia, persistent hyperplastic primary vitreous, glaucoma and cataracts. Later on, progressive high myopia may lead to retinal detachment. Giant amplitudes ($> 50 \mu\text{V}$) on visual evoked potentials are typically present in MEB patients but this is not an invariable feature.

The severity of the central nervous system involvement varies and more recently has been correlated with the genotype (see below). Patients at the severe end of the spectrum have the pachygyria/polymicrogyria/agyria complex and show a nodular, ‘cobblestone’ surface at anatomical inspection [75]. Other features are partial absence of the corpus callosum, hypoplasia of the pyramidal tracts and obstructive hydrocephalus requiring a shunt. On the other hand, patients at the milder end of the spectrum may only show flattening of the brainstem and cerebellar changes including vermis hypoplasia and cerebellar cysts. These changes of the posterior fossa are typically associated with transient dysmyelination; the frontal cortex might show pachygyric changes.

3.2.2.2. The *POMGnT1* gene and protein. The gene responsible for MEB is the glycosyltransferase *O*-mannose β -1,2-*N*-acetylglucosaminyltransferase (*POMGnT1*) which catalyzes the transfer of *N*-acetylglucosamine to *O*-mannose of glycoproteins, including dystroglycan [76]. Recently, the group of Toda reported a number of novel *POMGnT1* mutations in patients of both Japanese and Korean origin, suggesting that MEB has a wider demographical prevalence than originally appreciated [77]. Mutations identified included a combination of missense, non-sense and frameshifting mutations and a more severe phenotype of patients carrying mutations towards the 5' of the gene compared to those located towards the 3'. This has led to a degree of diagnostic confusion with WWS in a few of the most severe cases. Patients with the most common mutation were found to have reduced *POMGnT1* activity [76], this has since been confirmed by the group of Endo who analysed an additional 13 *POMGnT1* mutations [78]. A comparable reduction in skeletal muscle *POMGnT1* enzymatic activity has also been reported in four patients with MEB who carried either missense or other mutations resulting in loss of the open reading frame [79]. These authors have therefore proposed that *POMGnT1* activity of muscle biopsies be used as a screening procedure for MEB.

Protein studies on MEB muscle from these patients documented dramatic loss of muscle α -dystroglycan expression indicating that *POMGnT1* plays an essential

role in α -dystroglycan glycosylation [80]. In addition the loss of glycosylated α -dystroglycan, but preserved expression of core α -dystroglycan together with a loss of laminin α -2 binding capacity was demonstrated in MEB muscle [63].

3.2.3. Walker-Warburg syndrome

WWS is the most severe α -dystroglycanopathy and associated with a life expectancy of less than 3 years. It is a recessive disorder described for the first time in 1942 [81] although the full delineation of the syndrome was completed later on [82]. Characteristic features are CMD in combination with type II lissencephaly and retinal malformation. While originally considered to be a genetically homogeneous condition, recent genetic data indicate a surprisingly high degree of genetic heterogeneity. So far three genes (the *POMT1*; *fukutin* and *FKRP* gene) have been implicated in WWS, but they account for only a minority of cases. This finding, and the observation that abnormal α -dystroglycan also characterises the cases still in search of a primary genetic defect, suggests that the WWS phenotype may represent the severe phenotypic spectrum of mutations affecting genes that are involved in the process of α -dystroglycan glycosylation.

3.2.3.1. Clinical features of WWS. WWS is an extremely severe condition. Characteristic features are encephaloceles and severe hydrocephalus often already detected prenatally. The brain shows complete type II lissencephaly/agyria, combined with pontocerebellar hypoplasia [83]. Obstructive hydrocephalus complicates the clinical picture in a number of cases. In addition to the marked weakness, immobility in WWS patients is compounded by virtual absence of the pyramidal tracts. Severe feeding difficulties are invariable and tube or gastrostomy feeding is required. Additional features are blindness which results from both anterior and posterior chamber eye malformations, and genital anomalies in males. Muscle bulk is usually very reduced, and contractures may already be present at birth or develop rapidly thereafter. Histopathological features of muscular dystrophy may already be present at birth but in a few cases these changes were subtle and only became evident after a few months of life.

Unilateral or bilateral microphthalmia is common and the optic nerves are often hypoplastic or absent. Ocular colobomas usually involving the retina can be present and other retinal changes include retinal detachment. Anterior chamber malformations include cataracts, iris malformation or hypoplasia, and congenital or infantile glaucoma secondary to an abnormal anterior chamber angle.

On MRI the brain shows complete or near-complete absence of gyration and widespread, confluent white matter changes. The corpus callosum is usually absent and partial fusion of cerebral hemispheres is common. The cerebral cortical mantle is thin, with or without associated widening of the lateral ventricles. Severe atrophy of the cerebellar

vermis and hemispheres and a flattened aspect of the pons and brainstem are invariable. Arachnoid cysts are common, particularly also in the posterior fossa, where meningo- or encephaloceles can be found.

Pathological studies confirm the cobblestone type of lissencephaly, complete loss of cortical layering accompanied by a markedly abnormal vascular architecture both on the surface of the brain and in the cortex.

3.2.3.2. The *POMT1* gene and protein. Mutations in the *O*-mannosyltransferase 1 (*POMT1*) were recently identified in a relatively small proportion (6/30) of a well-characterized cohort of patients with WWS suggesting genetic heterogeneity [84]. *POMT1* catalyses the first step in *O*-mannosyl glycan synthesis [85]. A second putative *O*-mannosyltransferase, *POMT2*, shows an expression pattern in adults that overlaps with *POMT1*. While no mutations in the *POMT2* gene have as yet been identified in WWS or any other disorder [9,84,85], recent data indicate that both *POMT1* and *POMT2* form a complex which confers the enzymatic *O*-mannosyltransferase activity [86].

α -Dystroglycan immunolabelling is severely reduced in patients with *POMT1*-linked WWS [84] and can also be reduced in peripheral nerve [9]. Among other genes responsible for WWS, there are both the *fukutin* and *FKRP* gene, but they only account for a fraction of WWS cases. Abnormal α -dystroglycan expression has been documented in patients with WWS who did not carry mutations in the *POMT1* gene or either *FKRP* or *fukutin*, suggesting that defects in other as yet unidentified glycosyltransferases may underlie WWS [87].

3.2.4. Congenital muscular dystrophy 1C

There are two surprising features of *FKRP*-related myopathies: the first one is that they are very common especially among Caucasians; the second one relates to the spectrum of severity which gives rise to the largest phenotypic spectrum of muscular dystrophies so far connected to mutations of a single gene. This ranges from in utero onset, WWS phenotype to mild LGMD variants with onset in adulthood.

3.2.4.1. *MDC1C*: clinical features. The 'typical' form of *MDC1C* was recognised a few years ago by Muntoni as a combination of weakness and functional achievements similar to those observed in *MDC1A* [88]. This form was characterised by secondary laminin α 2 chain deficiency, but brain imaging and intelligence were normal. The main features of these patients are weakness and hypotonia from birth or the first few months of life (but no arthrogryposis), followed by a marked delay of motor milestones. The maximum motor capacity was to sit or to take a few steps with support in the first decade of life. Progressive respiratory muscle weakness leading to ventilatory insufficiency was a constant feature in the first or second decade of life.

Other characteristic features were marked enlargement of the leg muscles, sometimes followed by striking tongue hypertrophy. Wasting and weakness of the shoulder muscles and facial weakness were common. CK levels were always very elevated (20–75×) and cardiac involvement also present in the form of a dilated cardiomyopathy.

3.2.4.2. The severe end of the spectrum: MDC1C with structural brain involvement. This is the most recent addition to the FKRP-related group of conditions and well illustrates the hierarchy of central nervous system involvement in patients with FKRP mutations. At the ‘milder’ end of the ‘CNS-involvement spectrum’, there are patients with mild mental retardation and structural changes of the cerebellum with cerebellar cysts but normal brain stem and eye examination [89]. A number of similar cases have now also been described from Tunisia and Algeria [90].

More recently, we identified a patient with CMD, severe mental retardation ponto-cerebellar hypoplasia, cerebellar cysts and supratentorial changes with thickening of the frontal cortex which were indistinguishable from those observed in MEB. In addition this patient also had retinal changes with abnormal pigmentation and progressive myopia resulting in bilateral retinal detachment and blindness. This patient only achieved sitting with support and died at age 7 [91]. Another patient had a WWS phenotype including agyria, ponto-cerebellar hypoplasia and microphthalmia and blindness who also carried *FKRP* mutations. This patient died at the age of 3 [91].

3.2.4.3. LGMD2I: the mildest end of the spectrum. Patients with LGMD2I may present in childhood, adolescent or adult life and can often maintain the ability to walk for life. Intelligence and brain structure as judged by MRI is normal. Patients with LGMD2I may be divided into a Duchenne-like group, who have early onset and loss of independent ambulation in the teens, and a milder group with later onset and preserved ambulation after the second decade [92]. In a recent study, more than half of LGMD2I patients had evidence of dilated cardiomyopathy, which was not consistently associated with an early or more severe presentation [93]. Respiratory failure affected a third of the patients with a Duchenne-like phenotype after the age of 16 years.

3.2.4.4. The FKRP gene and protein. The *FKRP* gene consists of four exons encoding for a protein which, like fukutin, is targeted to the medial-Golgi apparatus. Sequence homology suggests this to be a member of the phosphosugar transferase family [94]. Mutation analysis in MDC1C revealed either two missense mutations or a missense mutation combined with a null mutation. However, the most common *FKRP* mutation (C826A leading to Leu276Ile), very commonly associated with LGMD2I, has not been observed in MDC1C so far [93].

This ‘LGMD2I’ mutation has been calculated to occur at a heterozygous frequency of 1:400 in the UK [93]. The second allelic mutation in patients with the Leu276Ile change determines the severity of the LGMD2I phenotype although the degree of intra-familial clinical variability suggests that additional factors may also play a significant role [92].

So far no patient was reported with two null *FKRP* alleles and perhaps this would not be compatible with life.

From a biochemical point of view, α -dystroglycan is abnormal in all patients with *FKRP* mutations. There typically is a clear correlation between the residual expression of α -dystroglycan and the phenotype. Patients with MDC1C displayed a profound depletion of α -dystroglycan, while patients with LGMDI with a Duchenne-like severity typically had a moderate reduction in α -dystroglycan. Individuals with the milder form of LGMD2I showed a variable but subtle alteration in α -dystroglycan immunolabelling [95].

3.2.5. MDC1D and the large^{myd} mouse

The myodystrophy (*myd*; now renamed Large^{myd}) mouse carries a loss of function mutation in the *LARGE* gene encoding for a putative bifunctional glycosyltransferase. Homozygous Large^{myd} mice display a severe, progressive muscular dystrophy and a mild cardiomyopathy in addition to retinal, peripheral and central nervous system involvement. Abnormalities in neuronal migration are observed in the brain particularly the cortex and cerebellum which is similar to that seen in fukutin-deficient mice. A profound loss of muscle α -dystroglycan has also been observed.

The substrate of *LARGE* is at present unclear. More recently, one patient with a G1525A (Glu509Lys) missense mutation and a 1 bp insertion, 1999insT has been reported and this disorder named MDC1D. This 17-year-old girl presented with CMD, profound mental retardation, white matter changes and subtle structural abnormalities on brain MRI. Her skeletal muscle biopsy showed reduced immunolabelling of α -dystroglycan and immunoblotting demonstrated a reduced molecular weight form of α -dystroglycan that did however, retain some laminin binding activity [96].

3.3. Rigid spine with muscular dystrophy Type 1: deficiency of selenoprotein N, an endoplasmic reticulum protein of unknown function

The rigid spine syndrome’ clinical phenotype was first recognized by Victor Dubowitz [97]. The study of informative families in the recent years succeeded to assign the locus for CMD with spinal rigidity (RSMD1) to chromosome 1p35–36. Further studies allowed to identify the selenoprotein N gene, *SEPN1*, as the cause of RSMD1 [98]. Elegant subcellular studies revealed that selenoprotein N is an endoplasmic reticulum glycoprotein with a main isoform corresponding to a 70 kDa protein containing a single selenocysteine residue [99].

3.3.1. Clinical features of RSMD1

The most common presentation of RSMD1 is that of axial hypotonia and weakness often noticed already in the first year of life, but usually in a child with otherwise normal motor milestones and no significant contractures. Motor difficulties because of a combination of mild/moderate proximal muscle weakness, mild Achilles tendon tightness and rigidity of the spine are also quite common, but only exceptionally affected patients may not achieve independent ambulation. Ambulation is usually maintained into adulthood unless a severe progressive scoliosis that cannot be treated surgically develops. The overall muscle bulk is reduced, especially the medial aspects of the thighs. The most prominent clinical feature is spinal rigidity and scoliosis due to contractures of the spine extensor muscles which may develop between 3 and 12 years. In typical cases this will be in form of a lumbar lordoscoliosis with a pelvic tilt and with cervical spine stiffness. If present, stabilization by thoraco-lumbar orthosis and early surgical fixation of the spine may be helpful. Rare patients do not develop prominent spinal rigidity, although axial muscle weakness is present. Contractures usually are mild and affect the ankles, and only occasionally the temporo-mandibular joint, with limitation of mouth opening, or also the finger extensors. An additional feature of these patients is the nasal speech secondary to palatal weakness.

Vital capacity due to stiffness of the rib cage is low and decreasing over time, and this is almost invariably aggravated by diaphragmatic weakness leading to respiratory failure. It is important to be aware that there often is a significant discrepancy between the overall functional abilities of affected individuals who are able to walk with no problems, and the compromise of lung function. Many patients require ventilatory assistance already in the first decade, some as early as at 2 years of age. On the other hand, an active life with good quality and reasonable muscle function can be maintained over many years, possibly decades, if non-invasive ventilation is instituted early enough.

CK levels are normal or mildly elevated ($n-2\times$). A specific pattern of skeletal muscle involvement on muscle imaging (CT or MRI) has been described: this can help to suspect the condition.

3.3.2. Muscle pathology in RSMD1

Muscle specimens show evidence of myopathy with increased variation of fiber size and some increase of endomyseal fibrosis. Early on there is usually no or very little necrosis and also little regeneration. Many specimens will show unevenness of oxidative enzyme staining with overt core-like areas in some. Predominance and relative hypotrophy of type I fibers is frequently observed. Biopsies of severely affected patients or from the paraspinal muscles may show overt dystrophic changes with a strong increase of endo- and perimyseal fibrosis and some increase of internal nuclei but still frank necrosis and regeneration will

not be prominent features [100]. Staining for laminin $\alpha 2$ and collagen 6 is normal.

3.3.3. Differential diagnosis of RSMD1

A proportion of patients with multicore–minicore disease (MMD) share a very similar clinical picture with RSMD1 patients. This is not surprising considering that recently Ferreiro et al. found SEPNI mutations in a number of MMD patients [100]. More recently, mutations in SEPNI have been reported in individuals with a congenital myopathy characterised by Mallory-body deposits, further expanding the pathological spectrum of SEPNI-related myopathies [101]. There can be a partial clinical overlap with patients with UCMD, especially in the late stages of the disorder; however severe joint laxity, and follicular hyperkeratosis are not features of RSMD1. A few patients have an almost identical clinical phenotype as RSMD1 but do not have SEPNI mutations. In a few of these cases, there can be associated mild mental retardation.

4. Other forms of CMD in search of a genetic defect

There are a number of CMD conditions in search of a genetic defect. Some of them have simply been identified because the isolation of a gene has shown that the condition, previously thought to be homogeneous, is in actual fact heterogeneous. To this category belong WWS cases with no mutations in any of the known glycosyltransferases; RSS cases with no SEPNI mutation; cases with a LGMD with α -dystroglycan depletion but no mutation in any of the glycosyltransferases identified so far. One of these variants (MDC1B) has been mapped to chromosome 1q42, but the responsible gene is still elusive.

In the second category there are conditions in which the clinical features are sufficiently distinct to suggest that they represent specific entities. Among these there is a variant with cerebellar hypoplasia and megacysterna magna that appears very common in Italy; a variant with peripheral neuropathy; and a variant of UCMD with mental retardation (not linked to the collagen VI genes). These forms are reported in Table 2.

5. Concluding remarks

One year after the centennial anniversary of the first clear description of CMD, clinicians, pathologists, molecular and cell biologists are involved in further understanding pathogenesis and diversity of this group of disorders. Once considered the cinderellas of neuromuscular diseases, new insight on fundamental aspects related to muscle formation and survival, and to neuronal migration disorders have mostly been generated in the last few years from research into CMD. Further elucidation of the significant clinical and genetic heterogeneity, together with the best

Table 2
CMD variants without identified genetic defects

| Name | Main features | Laminin2 | α -Dystroglycan |
|---|--|----------|------------------------|
| Rigid spine syndrome unlinked to 1p | Marked RSS. CK normal or \uparrow | Normal | Normal |
| UCMD/mental retardation unlinked to COL6A genes | Distal laxity, proximal contractures normal and scoliosis. CK normal or \uparrow | Normal | |
| CMD/mental retardation + cerebellar hypoplasia | Mild-moderate mental retardation | | |
| CMD/mental retardation + cataracts | Isolated cerebellar hypoplasia (no cysts) CK normal or \uparrow | Normal | |
| CMD/mental retardation + adducted thumbs | Cataracts in the first year of life CK normal, \uparrow or $\uparrow\uparrow$ | Normal | Normal |
| CMD/muscle hypertrophy normal intellect 'Italian MEB' | Mental retardation ophthalmoplegia; CK \uparrow or $\uparrow\uparrow$ | Normal | Normal |
| CMD-LGMD/microcephaly | Normal MRI; CK $\uparrow\uparrow$ | Reduced | Reduced |
| CMD/mental retardation + peripheral neuropathy | Cerebellar hypoplasia; megacisterna magna; CK: $\uparrow\uparrow$ | Reduced | Reduced |
| | Mild mental retardation normal brain MRI; CK $\uparrow\uparrow$ | Normal | Reduced |
| | Microcephaly, pachigyria, occipital pachigyria; CK $\uparrow\uparrow$ | Reduced | Reduced |

clinical care for these patients, often severely disabled by their conditions, remains our challenge for the future.

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References

- Batten F. Three cases of myopathy, infantile type. *Brain* 1903;26: 147–8.
- Voit T, Tome F. The congenital muscular dystrophy. *Myolog*, Engel A Ed 2004;.
- Dubowitz V. 22nd ENMC sponsored workshop on congenital muscular dystrophy held in Baarn, The Netherlands, 14–16 May 1993. *Neuromuscul Disord* 1994;4:75–81.
- Dubowitz V. 41st ENMC international workshop on congenital muscular dystrophy 8–10 March 1996, Naarden, The Netherlands. *Neuromuscul Disord* 1996;6:295–306.
- Dubowitz V. 50th ENMC international workshop: congenital muscular dystrophy, 28 February 1997 to 2 March 1997, Naarden, The Netherlands. *Neuromuscul Disord* 1997;7:539–47.
- Dubowitz V. 68th ENMC international workshop (5th international workshop): on congenital muscular dystrophy, 9–11 April, Naarden, The Netherlands. *Neuromuscul Disord* 1999;9:446–54.
- Muntoni F, Bertini E, Bonnemann C, et al. 98th ENMC international workshop on congenital muscular dystrophy (CMD), 7th workshop of the international consortium on CMD, 2nd workshop of the MYO-CLUSTER project GENRE. 26–28th October, 2001, Naarden, The Netherlands. *Neuromuscul Disord* 2002;12:889–96.
- Muntoni F, Guicheney P. 85th ENMC international workshop on congenital muscular dystrophy. 6th international CMD workshop. 1st workshop of the Myo-Cluster Project 'GENRE'. 27–28th October 2000, Naarden, The Netherlands. *Neuromuscul Disord* 2002;12:69–78.
- Muntoni F, Valero de Bernabe B, Bittner R, et al. 114th ENMC international workshop on congenital muscular dystrophy (CMD) 17–19 January, Naarden, The Netherlands: (8th workshop of the international consortium on CMD; 3rd workshop of the MYO-CLUSTER project GENRE). *Neuromuscul Disord* 2003;13: 579–88.
- Mostacciolo ML, Miorin M, Martinello F, Angelini C, Perini P, Trevisan CP. Genetic epidemiology of congenital muscular dystrophy in a sample from north-east Italy. *Hum Genet* 1996;97: 277–9.
- Tome FM, Evangelista T, Leclerc A, et al. Congenital muscular dystrophy with merosin deficiency. *C R Acad Sci III* 1994;317: 351–7.
- Hillaire D, Leclerc A, Faure S, et al. Localization of merosin-negative congenital muscular dystrophy to chromosome 6q2 by homozygosity mapping. *Hum Mol Genet* 1994;3:1657–61.
- 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.
- Engvall E. Laminin variants: why, where and when? *Kidney Int* 1993;43:2–6.
- Gullberg D, Tiger CF, Velling T. Laminins during muscle development and in muscular dystrophies. *Cell Mol Life Sci* 1999; 56:442–60.
- Vachon PHLF, Xu H, et al. Merosin and laminin in myogenesis; specific requirement for merosin in myotube stability and survival. *J Cell Biol* 1996;134:1483–97.
- Colognato H, Yurchenco PD. The laminin alpha2 expressed by dystrophic dy(2J) mice is defective in its ability to form polymers. *Curr Biol* 1999;9:1327–30.
- Philpot J, Sewry C, Pennock J, Dubowitz V. Clinical phenotype in congenital muscular dystrophy: correlation with expression of merosin in skeletal muscle. *Neuromuscul Disord* 1995;5:301–5.
- Philpot J, Muntoni F. Limitation of eye movement in merosin-deficient congenital muscular dystrophy. *Lancet* 1999;353:297–8.
- Philpot J, Bagnall A, King C, Dubowitz V, Muntoni F. Feeding problems in merosin deficient congenital muscular dystrophy. *Arch Dis Child* 1999;80:542–7.
- Gilhuis HJ, ten Donkelaar HJ, Tanke RB, et al. Nonmuscular involvement in merosin-negative congenital muscular dystrophy. *Pediatr Neurol* 2002;26:30–6.
- Spyrou N, Philpot J, Foale R, Camici PG, Muntoni F. Evidence of left ventricular dysfunction in children with merosin-deficient congenital muscular dystrophy. *Am Heart J* 1998;136:474–6.
- Villanova M, Malandrini A, Sabatelli P, et al. Localization of laminin alpha 2 chain in normal human central nervous system: an immunofluorescence and ultrastructural study. *Acta Neuropathol (Berl)* 1997;94:567–71.

- [24] Buttery PC, Ffrench-Constant C. Laminin-2/integrin interactions enhance myelin membrane formation by oligodendrocytes. *Mol Cell Neurosci* 1999;14:199–212.
- [25] 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.
- [26] Sunada Y, Edgar TS, Lotz BP, Rust RS, Campbell KP. Merosin-negative congenital muscular dystrophy associated with extensive brain abnormalities. *Neurology* 1995;45:2084–9.
- [27] Philpot J, Cowan F, Pennock J, et al. Merosin-deficient congenital muscular dystrophy: the spectrum of brain involvement on magnetic resonance imaging. *Neuromuscul Disord* 1999;9:81–5.
- [28] Herrmann R, Straub V, Meyer K, Kahn T, Wagner M, Voit T. Congenital muscular dystrophy with laminin alpha 2 chain deficiency: identification of a new intermediate phenotype and correlation of clinical findings to muscle immunohistochemistry. *Eur J Pediatr* 1996;155:968–76.
- [29] Mercuri E, Muntoni F, Berardinelli A, et al. Somatosensory and visual evoked potentials in congenital muscular dystrophy: correlation with MRI changes and muscle merosin status. *Neuropediatrics* 1995;26:3–7.
- [30] 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.
- [31] Engvall E, Wewer UM. Domains of laminin. *J Cell Biochem* 1996;61:493–501.
- [32] Kammerer RA, Schultheiss T, Landwehr R, et al. Interaction of agrin with laminin requires a coiled-coil conformation of the agrin-binding site within the laminin gamma1 chain. *Eur Mol Biol Org J* 1999;18:6762–70.
- [33] Yurchenco PD, Cheng YS, Campbell K, Li S. Loss of basement membrane, receptor and cytoskeletal lattices in a laminin-deficient muscular dystrophy. *J Cell Sci* 2004;117:735–42.
- [34] Cohn RD, Herrmann R, Wewer UM, Voit T. Changes of laminin beta 2 chain expression in congenital muscular dystrophy. *Neuromuscul Disord* 1997;7:373–8.
- [35] Cohn RD, Mayer U, Saher G, et al. Secondary reduction of alpha7B integrin in laminin alpha2 deficient congenital muscular dystrophy supports an additional transmembrane link in skeletal muscle. *J Neurol Sci* 1999;163:140–52.
- [36] Sewry CA, Philpot J, Mahony D, Wilson LA, Muntoni F, Dubowitz V. Expression of laminin subunits in congenital muscular dystrophy. *Neuromuscul Disord* 1995;5:307–16.
- [37] Allamand V, Guicheney P. Merosin-deficient congenital muscular dystrophy, autosomal recessive (MDC1A, MIM#156225, LAMA2 gene coding for alpha2 chain of laminin). *Eur J Hum Genet* 2002;10:91–4.
- [38] Naom I, D'Alessandro M, Sewry C, et al. The role of immunocytochemistry and linkage analysis in the prenatal diagnosis of merosin-deficient congenital muscular dystrophy. *Hum Genet* 1997;99:535–40.
- [39] Guicheney P, Vignier N, Zhang X, et al. PCR based mutation screening of the laminin alpha2 chain gene (LAMA2): application to prenatal diagnosis and search for founder effects in congenital muscular dystrophy. *J Med Genet* 1998;35:211–7.
- [40] Pegoraro E, Mancias P, Swerdlow SH, et al. Congenital muscular dystrophy with primary laminin alpha2 (merosin) deficiency presenting as inflammatory myopathy. *Ann Neurol* 1996;40:782–91.
- [41] Sewry CA, Naom I, D'Alessandro M, et al. Variable clinical phenotype in merosin-deficient congenital muscular dystrophy associated with differential immunolabelling of two fragments of the laminin alpha 2 chain. *Neuromuscul Disord* 1997;7:169–75.
- [42] Cohn RD, Herrmann R, Sorokin L, Wewer UM, Voit T. Laminin alpha2 chain-deficient congenital muscular dystrophy: variable epitope expression in severe and mild cases. *Neurology* 1998;51:94–100.
- [43] Sewry CA, Philpot J, Sorokin LM, et al. Diagnosis of merosin (laminin-2) deficient congenital muscular dystrophy by skin biopsy. *Lancet* 1996;347:582–4.
- [44] Bushby K, Anderson LV, Pollit C, et al. : Abnormal merosin in adults. A new form of late onset muscular dystrophy not linked to chromosome 6q2. *Brain* 1998;121:581–8.
- [45] Brockington M, Yuva Y, Prandini P, et al. 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.
- [46] Tan E, Topaloglu H, Sewry C, et al. Late onset muscular dystrophy with cerebral white matter changes due to partial merosin deficiency. *Neuromuscul Disord* 1997;7:85–9.
- [47] 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.
- [48] Ullrich O. Kongenitale, atonisch-sklerotische Muskeldystrophie. *Monatsschr Kinderheilkd* 1930;47:502–10.
- [49] Zhang RZ, Sabatelli P, Pan TC, et al. Effects on collagen VI mRNA stability and microfibrillar assembly of three COL6A2 mutations in two families with Ullrich congenital muscular dystrophy. *J Biol Chem* 2002;277:43557–64.
- [50] Camacho Vanegas O, Bertini E, Zhang RZ, et al. Ullrich scleroatonic muscular dystrophy is caused by recessive mutations in collagen type VI. *Proc Natl Acad Sci USA* 2001;98:7516–21.
- [51] Ishikawa H, Sugie K, Murayama K, et al. Ullrich disease: collagen VI deficiency: EM suggests a new basis for muscular weakness. *Neurology* 2002;59:920–3.
- [52] Pan TC, Zhang RZ, Sudano DG, Marie SK, Bonnemann CG, Chu ML. 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.
- [53] 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.
- [54] Ishikawa H, Sugie K, Murayama K, et al. Ullrich disease due to deficiency of collagen VI in the sarcolemma. *Neurology* 2004;62:620–3.
- [55] Mercuri E, Cini C, Pichiecchio A, et al. Muscle magnetic resonance imaging in patients with congenital muscular dystrophy and Ullrich phenotype. *Neuromuscul Disord* 2003;13:554–8.
- [56] Vachon PH, Xu H, Liu L, et al. Integrins (alpha7beta1) in muscle function and survival. Disrupted expression in merosin-deficient congenital muscular dystrophy. *J Clin Invest* 1997;100:1870–81.
- [57] Hayashi YK, Chou FL, Engvall E, et al. Mutations in the integrin alpha7 gene cause congenital myopathy. *Nat Genet* 1998;19:94–7.
- [58] Pegoraro E, Cepollaro F, Prandini P, et al. Integrin alpha 7 beta 1 in muscular dystrophy/myopathy of unknown etiology. *Am J Pathol* 2002;160:2135–43.
- [59] Michele DE, Campbell KP. Dystrophin-glycoprotein complex: post-translational processing and dystroglycan function. *J Biol Chem* 2003;278:15457–60.
- [60] Endo T, Toda T. Glycosylation in congenital muscular dystrophies. *Biol Pharm Bull* 2003;26:1641–7.
- [61] Henry MD, Campbell KP. Dystroglycan: an extracellular matrix receptor linked to the cytoskeleton. *Curr Opin Cell Biol* 1996;8.
- [62] Cohn RD, Henry MD, Michele DE, et al. : Disruption of DAG1 in differentiated skeletal muscle reveals a role for dystroglycan in muscle regeneration. *Cell* 2002;110:639–48.
- [63] Michele DE, Barresi R, Kanagawa M, et al. Post-translational disruption of dystroglycan–ligand interactions in congenital muscular dystrophies. *Nature* 2002;418:417–22.
- [64] Muntoni F, Brockington M, Torelli S, Brown SC. Defective glycosylation on congenital muscular dystrophies. *Curr Opin Neurol* 2004;17(2):205–9.

- [65] Fukuyama Y, Kwazura M, Haruna H. A peculiar form of congenital muscular dystrophy. *Paediatr Univ Tokyo* 1960;4:5–8.
- [66] Osawa M, Sumida S, Suzuki N, et al. Fukuyama type congenital muscular dystrophy. In: Fukuyama Y, Osawa M, Saito K, editors. *Congenital muscular dystrophies*. Amsterdam: Elsevier; 1997. p. 31–68.
- [67] Kobayashi K, Nakahori Y, Miyake M, et al. An ancient retrotransposal insertion causes Fukuyama-type congenital muscular dystrophy. *Nature* 1998;394:388–92.
- [68] Silan F, Yoshioka M, Kobayashi K, et al. A new mutation of the fukutin gene in a non-Japanese patient. *Ann Neurol* 2003;53:392–6.
- [69] 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.
- [70] Hayashi YK, Ogawa M, Tagawa K, et al. Selective deficiency of alpha-dystroglycan in Fukuyama-type congenital muscular dystrophy. *Neurology* 2001;57:115–21.
- [71] Yamamoto T, Kato Y, Karita M, et al. Fukutin expression in glial cells and neurons: implication in the brain lesions of Fukuyama congenital muscular dystrophy. *Acta Neuropathol (Berl)* 2002;104:217–24.
- [72] Moore SA, Saito F, Chen J, et al. Deletion of brain dystroglycan recapitulates aspects of congenital muscular dystrophy. *Nature* 2002;418:422–5.
- [73] Saito Y, Kobayashi M, Itoh M, et al. Aberrant neuronal migration in the brainstem of fukuyama-type congenital muscular dystrophy. *J Neuropathol Exp Neurol* 2003;62:497–508.
- [74] Santavuori P, Leisti J, Kruus S. Muscle, eye and brain disease: a new syndrome. *Neuropädiatrie* 1977;8:550.
- [75] Haltia M, Leivo I, Somer H, et al. Muscle-eye-brain disease: a neuropathological study. *Ann Neurol* 1997;41:173–80.
- [76] Yoshida A, 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.
- [77] Taniguchi K, Kobayashi K, Saito K, et al. Worldwide distribution and broader clinical spectrum of muscle-eye-brain disease. *Hum Mol Genet* 2003;12:527–34.
- [78] Manya H, Sakai K, Kobayashi K, et al. Loss-of-function of an *N*-acetylglucosaminyltransferase, POMGnT1, in muscle-eye-brain disease. *Biochem Biophys Res Commun* 2003;306:93–7.
- [79] Zhang W, Vajsar J, Cao P, et al. Enzymatic diagnostic test for muscle-eye-brain type congenital muscular dystrophy using commercially available reagents. *Clin Biochem* 2003;36:339–44.
- [80] 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.
- [81] Walker AE. Lissencephaly. *Arch Neurol Psychol* 1942;48:13–29.
- [82] Warburg M. Hydrocephaly, congenital retinal nonattachment, and congenital falciform fold. *Am J Ophthalmol* 1978;85:88–94.
- [83] Dobyns WB, Pagon RA, Armstrong D, et al. Diagnostic criteria for Walker-Warburg syndrome. *Am J Med Genet* 1989;32:195–210.
- [84] Beltran-Valero de Bernabe D, Currier S, Steinbrecher A, et al. Mutations in the *O*-mannosyltransferase gene POMT1 give rise to the severe neuronal migration disorder Walker-Warburg syndrome. *Am J Hum Genet* 2002;71:1033–43.
- [85] Willer T, Valero MC, Tanner W, Cruces J, Strahl S. *O*-mannosyl glycans: from yeast to novel associations with human disease. *Curr Opin Struct Biol* 2003;13:621–30.
- [86] Manya H, Chiba A, Yoshida A, et al. Demonstration of mammalian protein *O*-mannosyltransferase activity: coexpression of POMT1 and POMT2 required for enzymatic activity. *Proc Natl Acad Sci USA* 2004;101:500–5.
- [87] Jimenez-Mallebrera C, Torelli S, Brown SC, et al. Profound skeletal muscle depletion of alpha-dystroglycan in Walker-Warburg syndrome. *Eur J Paediatr Neurol* 2003;7:129–37.
- [88] Mercuri E, Sewry CA, Brown SC, et al. Congenital muscular dystrophy with secondary merosin deficiency and normal brain MRI: a novel entity? *Neuropediatrics* 2000;31:186–9.
- [89] Topaloglu H, Brockington M, Yuva Y, et al. FKRP gene mutations cause congenital muscular dystrophy, mental retardation, and cerebellar cysts. *Neurology* 2003;60:988–92.
- [90] Driss A, Noguchi S, Amouri R, et al. Fukutin-related protein gene mutated in the original kindred limb-girdle MD 2I. *Neurology* 2003;60:1341–4.
- [91] Beltrán Valero de Bernabé 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;41:61–6.
- [92] 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.
- [93] Poppe M, Cree L, Bourke J, et al. The phenotype of limb-girdle muscular dystrophy type 2I. *Neurology* 2003;60:1246–51.
- [94] Brockington M, Blake DJ, Prandini P, et al. Mutations in the fukutin-related protein gene (FKRP) cause a form of congenital muscular dystrophy with secondary laminin alpha2 deficiency and abnormal glycosylation of alpha-dystroglycan. *Am J Hum Genet* 2001;69:1198–209.
- [95] 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.
- [96] Longman C, 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.
- [97] Dubowitz V. Rigid spine syndrome: a muscle syndrome in search of a name. *Proc R Soc Med* 1973;66:219–20.
- [98] Moghadasszadeh B, Petit N, Jaillard C, et al. Mutations in SEPN1 cause congenital muscular dystrophy with spinal rigidity and restrictive respiratory syndrome. *Nat Genet* 2001;29:17–18.
- [99] Petit N, Lescure A, Rederstorff M, et al. an endoplasmic reticulum glycoprotein with an early developmental expression pattern. *Hum Mol Genet* 2003;12:1045–53.
- [100] Ferreira A, Quijano-Roy S, Pichereau C, et al. Mutations of the selenoprotein N gene, which is implicated in rigid spine muscular dystrophy, cause the classical phenotype of multimincore disease: reassessing the nosology of early-onset myopathies. *Am J Hum Genet* 2002;71:739–49.
- [101] Ferreira A, Ceuterick-de Groote C, Marks JJ, et al. Desmin-related myopathy with mallory body-like inclusions is caused by mutations of the selenoprotein N gene. *Ann Neurol* 2004;55:676–86.