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The role of defective glycosylation in congenital muscular dystrophy

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The dystrophin glycoprotein complex (DGC) is an assembly of proteins spanning the sarcolemma of skeletal muscle cells. Defects in the DGC appear to play critical roles in several muscular dystrophies due to disruption of basement membrane organization. *O*-mannosyl oligosaccharides on α-dystroglycan, a major extracellular component of the DGC, are essential for normal binding of α-dystroglycan to ligands (such as laminin) in the extracellular matrix and subsequent signal transmission to actin in the cytoskeleton of the muscle cell. Muscle-Eye-Brain disease (MEB) and Walker-Warburg Syndrome (WWS) have mutations in genes encoding glycosyltransferases needed for *O*-mannosyl oligosaccharide synthesis. Myodystrophic *myd* mice and humans with Fukuyama Congenital Muscular Dystrophy (FCMD), congenital muscular dystrophy due to defective fukutin-related protein (FKRP) and MDC1D have mutations in putative glycosyltransferases. These human congenital muscular dystrophies and the *myd* mouse are associated with defective glycosylation of α-dystroglycan. It is expected other congenital muscular dystrophies will prove to have mutations in genes involved in glycosylation.
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***Abbreviations:* CMD: congenital muscular dystrophy; DGC: dystrophin glycoprotein complex; EST: expressed sequence tag; FCMD: Fukuyama Congenital Muscular Dystrophy; FKRP: Fukutin-related protein; LGMD: limb-girdle muscular dystrophy; MEB: Muscle-Eye-Brain disease; PCR: polymerase chain reaction; POMGnT1: Protein *O*-Mannosyl β1,2-*N*-acetylglucosaminyltransferase 1; POMT1: Protein *O*-Mannosyltransferase 1; RT: reverse transcription; WWS: Walker-Warburg Syndrome.**

Introduction

Glycosylated proteins are found in archaebacteria, eubacteria, and eukaryotes [1]. In an excellent recent review, Spiro [1] lists over 40 different sugar-amino acid linkages involving 8 amino acids and 13 monosaccharides, and representing the products of *N*- and *O*-glycosylation, C-mannosylation, phosphoglycation, and glypiation (formation of the glycophosphatidylinositol anchor). These sugar-protein bonds result in a huge diversity of protein-bound glycans. Although the biological functions of these glycans have been the subject of much debate and experimentation, specific roles for specific glycans have been described in only a relatively small number of cases. It has become clear in recent years that many congenital human diseases are due to mutations that cause defective glycosylation [2–8] and that such mutations can also cause abnormal development in mice and flies [9]. The abnormal phenotypes resulting from these mutations have shown that a large number of proteins and protein-bound glycans must function as essential signaling molecules in cell-cell interactions. This review will focus on five human diseases and a mouse model in which defective glycosylation results in congenital muscular dystrophy (CMD).

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Dystroglycan and the dystrophin glycoprotein complex

The dystrophin glycoprotein complex (DGC) is an assembly of proteins spanning the sarcolemma of skeletal muscle cells (dystrobrevin, dystroglycan, dystrophin, sarcoglycans, sarcospan, syntrophins) [10–12]. Defects in the DGC appear to play critical roles in several muscular dystrophies due to disruption

of basement membrane organization [13,14]. Dystroglycan (dystrophin-associated glycoprotein), a major component of the DGC, was first cloned by Kevin Campbell and colleagues in 1992 [15]. Dystroglycan is expressed in many cell types and composed of α- and β-subunits encoded by a single mRNA [16]. The protein is synthesized as a precursor propeptide that is post-translationally cleaved and differentially glycosylated to yield α- and β-dystroglycans.

Protein *O*-mannosylation is rare in mammals and is observed in a limited number of glycoproteins of brain, peripheral nerve, and skeletal muscle [17]. The tetrasaccharide NeuAcα2,3Galβ1,4GlcNAcβ1,2Manα1-*O*-Ser/Thr is the major sialylated O-glycosidically-linked oligosaccharide in α-dystroglycan from bovine peripheral nerve [17,18], rabbit skeletal muscle [19], and sheep brain [20]. The NeuAcα2,3Galβ1,4GlcNAc moiety of this sugar chain can block the binding of laminin to α-dystroglycan [17,18,21] suggesting that the sugar chain is involved in binding.

Figure 1 shows the organization of dystroglycan within the sarcolemma. Dystroglycan acts as a link between the extracellular matrix and intracellular actin. In skeletal muscle, the extracellular 156 kDa heavily glycosylated α-dystroglycan binds both β-dystroglycan, a transmembrane protein, and extracellular ligands such as the globular G domain of merosin, whereas dystrophin binds both β-dystroglycan and the intracellular contractile protein actin [15,21–25]. The merosin M-chain (or a2-laminin) is one of three subunits of laminin-2 which is highly expressed in striated muscle and peripheral nerve.

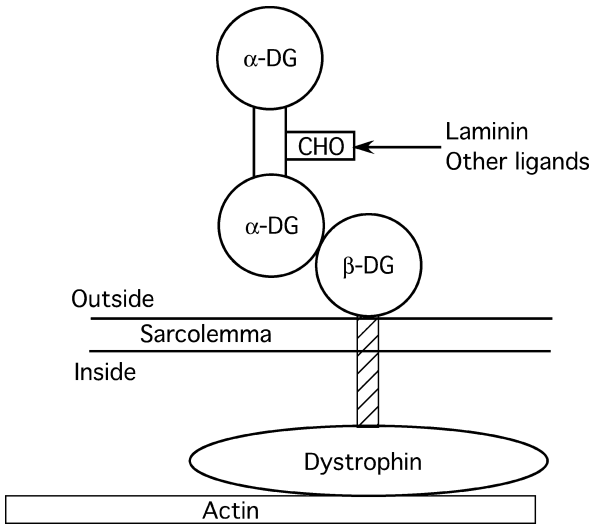

Figure 1. A diagram of the dystrophin glycoprotein complex (DGC). α-DG, α-dystroglycan, a dumb-bell shaped molecule at the external surface of the skeletal muscle cell sarcolemma. β-DG, β-dystroglycan, a transmembrane glycoprotein spanning the sarcolemma. The hatched section of β-dystroglycan is the transmembrane segment which interacts on the cytoplasmic side with dystrophin. CHO, carbohydrate moieties linked by *O*-mannosyl residues to Ser/Thr residues of the "mucin" segment of α-dystroglycan. α2-Laminin (and probably other proteins) of the extracellular matrix interact with α-dystroglycan via these carbohydrate moieties.

Although the exact functions of α- and β-dystroglycan and the other components of the DGC are not known, they appear to provide a link between the extracellular matrix and the interior cytoskeleton of the muscle cell [18,25–27]. The DGC may play a role in cell-cell signaling and adhesion, and may regulate intracellular calcium concentrations and provide structural support to the sarcolemma. Several cell surface and transmembrane molecules (laminins, integrins, lectins) have been proposed as ligands for dystroglycans [12,27–29]. The disruption of the binding of α- and β-dystroglycan and other components of the DGC to their respective ligands may lead to weakened anchorage of muscle fibers to the extracellular matrix with very early (*i.e.*, embryonic) and rapid muscle dysfunction and necrosis [14]. This process occurs in many congenital muscular dystrophies (Table 1 and below).

Congenital muscular dystrophy

Mutations in the dystrophin gene that result in absent or abnormal dystrophin with resulting disruption of the interactions between dystrophin and either β-dystroglycan or actin (Figure 1) lead to Duchenne or Becker muscular dystrophy respectively [12,30]. Mutations interfering with the interaction of extracellular ligands and α-dystroglycan (Figure 1) are responsible for an etiologically heterogeneous group of autosomal recessive congenital muscular dystrophies (CMD, Table 1) which can have associated brain and eye abnormalities [30–38]. The diagnostic criteria for CMD include dystrophic changes of biopsied skeletal muscle and onset of muscle weakness and contractures at birth or in the first few months of life. The incidence of CMD is 4.65 in 100,000 indicating it is among the most frequent autosomal recessive neuromuscular diseases [39]. The most common CMD (about 40% of the cases) is MDC1A (Table 1) due to mutations in the gene (LAMA2) encoding merosin (the α2-chain of laminin-2). Secondary deficiencies of merosin occur in other CMDs (MDC1B, MDC1C, MEB, FCMD; Table 1).

FCMD, MEB, and WWS (Table 1) are complex muscular dystrophies with a wide spectrum of brain and eye malformations [40–44]. The brain findings include neuronal migrational abnormalities resulting in coarse gyri with an abnormally nodular surface ("cobblestone cortex", previously called type II lissencephaly), hypoplasia of pons and cerebellum, absence or hypoplasia of corpus callosum, simplified or absent brain convolutions, dilatation of ventricles, cystic degeneration of white matter, and hydrocephalus [33,34,36,38,45]. The eye abnormalities may include microphthalmia, buphthalmos, anterior chamber defects, congenital cataracts, optic nerve atrophy, retinal dysplasia, myopia, and coloboma. In contrast, MDC1C (Table 1) presents only with muscular dystrophy [46–49]. The spectrum of a combination of brain, eye, and muscle abnormalities leads to differing degrees of motor developmental delay, physical disability, muscle pathology, and elevation of serum creatine kinase, along with variable presentation of

Table 1. Congenital muscular dystrophies and LGMD2I

Type	*Gene locus*	*Location*	*Protein*	*OMIM**
Merosin-deficient	MDC1A, LAMA2	6q22–23	α2-laminin	156225
Secondary merosin deficiency 1	MDC1B	1q42	Unknown	604801
Secondary merosin deficiency 2	MDC1C	19q13.3	Fukutin-related protein (FKRP)	606612 606596
MDC1D	MDC1D	22q12.3–q13.1	LARGE	603590
Limb Girdle type 2I	LGMD2I	19q13.3	FKRP	607155
Fukuyama	FCMD	9q31	Fukutin	253800
Muscle-Eye-Brain	MEB	1p33–34	POMGnT1	253280
Walker-Warburg Syndrome	WWS	9q34.1	POMT1	236670
Rigid Spine Syndrome	RSMD1 (SEPN1)	1p35–36	Selenoprotein N	602771
Myopathy	COL6A1	21q22.3	α1-Collagen VI	120220
Ullrich	COL6A2, UCMD	21q22.3	α2-Collagen VI	120240
Ullrich	COL6A3, UCMD	2q37	α3-Collagen VI	120250
α7-Integrin-deficient	ITGA7	12q13	α7-Integrin	600536

*OMIM, Online Mendelian Inheritance in Man (http://www.ncbi.nlm.nih.gov/).

mental retardation and structural brain and eye defects. The most severe and often lethal phenotype is generally associated with WWS followed in decreasing severity by MEB, FCMD, and MDC1C.

The role of glycosylation in congenital muscular dystrophy

Four of the CMDs (FCMD [50,51], MEB [45,51], WWS [52], and MDC1C [48]) are associated with deficient immunostaining of α-dystroglycan in the basal lamina of skeletal muscles as demonstrated by immunohistochemical techniques using antibodies against α-dystroglycan such as VIA4-1 [40–44]. However, α-dystroglycan staining can be normal in muscle from MEB and FCMD patients using a polyclonal antibody which can detect hypoglycosylated full-length α-dystroglycan [51]. The data indicate that VIA4-1 is directed against the glycans on α-dystroglycan and that therefore α-dystroglycan is underglycosylated in muscle from MEB and FCMD patients [53]. This was further confirmed by ligand overlay assays which showed that α-dystroglycan is hypoglycosylated.

A recent study [54] described a 17 year old girl presenting with congenital muscular dystrophy, profound mental retardation, white matter changes, and subtle structural abnormalities on brain MRI. Her skeletal muscle biopsy showed reduced immunolabelling of α-dystroglycan. Immunoblotting with an antibody to a glycosylated epitope demonstrated a reduced molecular weight form of α-dystroglycan that retained some laminin binding activity. This patient was found to have a missense mutation (G1525A, Glu509Lys) and and a 1bp insertion (1999insT) in the human homologue of the mouse LARGE gene. This gene encodes a putative glycosyltransferase (see below). This is the first description of mutations in the human LARGE gene and the authors named this new disorder MDC1D.

Fukuyama Congenital Muscular Dystrophy (FCMD)

FCMD is an autosomal recessive congenital muscular dystrophy, first described in Japan in 1960 [55]. By 1981, more than 200 cases had been recognized clinically in Japan [56]. Patients manifest generalized muscle weakness and hypotonia from early infancy and most are unable to walk without support. All are mentally retarded and some have seizures, abnormal electroencephalograms, and abnormal CT scans. Histologic changes in skeletal muscle are characteristic of muscular dystrophy [57]. The brain malformations in FCMD include cerebral and cerebellar micropolygyria associated with defective neuronal migration, fibroglial proliferation of the leptomeninges, hydrocephalus, focal interhemispheric fusion, and hypoplasia of the corticospinal tracts [56].

Toda *et al.* [58] performed homozygosity mapping and genetic linkage analysis with polymorphic microsatellite markers in 21 FCMD families and assigned the FCMD mutation to 3 loci on 9q31–q33. Further mapping narrowed the gene location to 9q31 [59].

Kobayashi *et al.* [60] described a haplotype shared by 87% of FCMD chromosomes indicating most chromosomes bearing the FCMD mutation are probably derived from a single founder. A 3 kb retrotransposal insertion of tandemly repeated sequences exists within this candidate gene in all FCMD chromosomes carrying the founder haplotype [61]. The predicted protein was called fukutin [61]. The function of fukutin is unknown but it has an amino acid sequence suggestive of a glycosyltransferase [62,63].

Kondo-Iida *et al.* [64] undertook a systematic analysis of the FCMD gene in 107 unrelated patients and identified 4 novel nonfounder mutations in 5 patients. The frequency of severe phenotypes was significantly higher among probands who were compound heterozygotes carrying a point mutation on one allele and a founder mutation on the other than among probands

who were homozygous for the 3 kb retrotransposon [64,65]. No FCMD patients were detected with homozygous nonfounder point mutations. This finding indicates that such homozygotes are embryonically lethal and may explain why few FCMD cases have been reported in non-Japanese populations.

Fukutin mRNA transcripts are expressed in control fetal and adult brain but are greatly reduced in FCMD brains [66]. Tissue *in situ* hybridization studies of FCMD brain showed that fukutin transcripts are expressed in neurons in regions with no dysplasia but that transcripts were nearly undetectable in the overmigrated dysplastic region. The authors hypothesized that fukutin may influence neuronal migration.

A marked deficiency of highly glycosylated α-dystroglycan in skeletal and cardiac muscle and reduced amounts in brain tissue were found in samples from FCMD patients using immunohistochemistry and immunoblotting [50]. A deficiency of fukutin and normal levels of β-dystroglycan were found in all tissues examined [50]. These findings support the suggestion that fukutin deficiency affects the glycosylation of α-dystroglycan.

Although α-dystroglycan is expressed in both MEB and FCMD muscle samples, the protein is in a hypoglycosylated form which cannot bind to the ligands laminin, neurexin, and agrin [51].

Muscle-Eye-Brain Disease (MEB)

MEB (Table 1) was first described in 1978 [67]. It is an autosomal recessive congenital muscular dystrophy with severe early-onset muscle weakness and high serum creatine kinase. The disease causes associated abnormalities of the brain (cobblestone type brain malformation of intermediate severity, mental retardation, hydrocephalus, abnormal EEG) and eye (severe congenital myopia, congenital glaucoma, pallor of the optic discs, retinal hypoplasia) [36–38,68]. The MEB gene was mapped to 1p32–p34 by linkage analysis and homozygosity mapping in 8 families with 12 affected individuals [38,69]. This distinguishes MEB from WWS which has phenotypic similarities but maps to 9q34.1 (Table 1).

Two groups [45,70,71] recently reported the cloning and expression of a gene encoding a β1,2-*N*-acetylglucosaminyltransferase with significant homology to the gene encoding UDP-GlcNAc:α-3-D-mannoside β1,2-*N*-acetylglucosaminyltransferase I, an enzyme essential for the synthesis of hybrid and complex N-glycans [72]. The recombinant enzyme incorporated GlcNAc from UDP-GlcNAc in β1,2 linkage to the *O*-linked Man of the *O*-mannosyl glycopeptides CYA(Manα1-O-T)AV [70,71] and *N*-acetyl-AAP(Man-T)PVAAP-NH_2 [45]. The enzyme has been named Protein *O*-Mannosyl β1,2-*N*-acetylglucosaminyltransferase 1 (POMGnT1) and is probably required for the synthesis of the NeuAcα2,3Galβ1,4GlcNAcβ1,2Manα1-O-Ser/Thr tetrasaccharide on proteins such as α-dystroglycan. The gene contains 22 exons and has a 1980 bp open reading frame encoding a protein with 660 amino acids and a domain structure typical of Golgi-associated type II transmembrane proteins [45,70,71]. Northern blot analysis detected a $\sim$3 kb mRNA transcript in 23 normal tissues tested indicating human POMGnT1 is constitutively expressed. An additional weaker 3.4 kb mRNA band was observed in spinal cord, lymph node and trachea, suggesting the presence of 2 transcriptional initiation sites or alternative splicing in these tissues. The human POMGnT1 gene maps to 1p33–p34.

Independent loss-of-function mutations of the POMGnT1 gene were reported in six patients with MEB [45]. Several distinct single base substitutions (4) and deletions (2) were found; five of the patients were homozygotes and one was a compound heterozygote [45]. More recently, seven more novel disease-causing mutations in the POMGnT1 gene were found in six patients with suspected MEB, severe FCMD or WWS, bringing the total number of POMGnT1 mutations to thirteen [73]. None of the thirteen mutant POMGnT1 genes expressed enzymatically active proteins indicating that MEB is inherited as a loss-of-function of POMGnT1 [74].

MEB has a worldwide distribution and a clinical spectrum broader than recognized previously [73]. POMGnT1 mutations must therefore be considered in WWS and other "muscle-brain" congenital muscular dystrophy patients. POMGnT1 enzyme activity can be measured in muscle biopsies from MEB patients providing a rapid and relatively simple diagnostic test for this disease [75]. POMGnT1 assays of muscle biopsies should probably be used as a screening procedure for MEB in all CMD patients associated with brain malformations.

A selective deficiency [76] and hypoglycosylation [51] of α-dystroglycan has been reported in MEB skeletal muscle by immunohistochemical techniques using anti-α-dystroglycan antibodies directed against the carbohydrate moiety. The data indicate that α-dystroglycan is a potential target of POMGnT1 and that altered glycosylation of α-dystroglycan plays a critical role in the etiology of MEB by interfering with the binding of α-dystroglycan to ligands such as laminin, neurexin, and agrin.

Walker-Warburg syndrome (WWS)

WWS (Table 1) is an autosomal recessive congenital muscular dystrophy (CMD) associated with characteristic brain and eye malformations [33]. The name is based on the publications of Walker [77] and Warburg [78–80]. In a study on WWS by Dobyns *et al.* in 1989 [33], four abnormalities were present in all patients checked for these anomalies: type II lissencephaly (21/21), cerebellar malformation (20/20), retinal malformation (18/18), and CMD (14/14). Two other frequently observed abnormalities were dilatation of the cerebral ventricles with or without hydrocephalus (20/21) and anterior chamber malformation (16/21). Other abnormalities occurred less frequently, *e.g.*, congenital macrocephaly with hydrocephalus (11/19), congenital microcephaly (3/19), Dandy-Walker malformation (10/19), microphthalmia (8/21), ocular colobomas (3/15), congenital cataracts (7/20), and genital anomalies in males (5/8). Median survival in this series of patients was 9 months [33]. FCMD

(Table 1), a related autosomal recessive CMD, consists of similar but less frequent and less severe cerebellar and retinal abnormalities. Skeletal muscle merosin (α2-laminin) is completely absent in merosin-deficient CMD (Table 1) and is significantly reduced in FCMD, but was consistently preserved in five WWS patients [34]. However, two WWS patients with severely deficient skeletal muscle merosin expression have been reported [81]; this effect was considered to be secondary. Ultrasonography has been used for prenatal diagnosis.

A genome-wide linkage analysis in 10 consanguineous families with WWS indicated the existence of at least three WWS loci [52]. Candidate genes for WWS were selected based on the findings (see above) that MEB and FCMD were due to abnormalities in α-dystroglycan glycosylation. Since POMGnT1 encodes an *O*-mannoside *N*-acetylglucosaminyltransferase, the possible involvement of *O*-mannosyl glycan synthesis was considered in WWS [52].

A human gene homologous to the yeast protein *O*-mannosyltransferases (which attach mannose in O-glycosidic linkage to the Ser/Thr residues of proteins) has been cloned, and the protein has been named Protein *O*-Mannosyltransferase 1 (POMT1) [82]. The POMT1 mRNA is expressed as a 3.1 kb transcript in all tissues tested with highest levels in testis and fetal brain. Alternative splicing of several exons in all tissues predicts the generation of several protein isoforms. The most common mRNA variant encodes a 725 amino acid protein with 54% similarity to the yeast enzymes. Computer prediction suggests that POMT1 is an integral membrane protein of the endoplasmic reticulum. The POMT1 locus has been assigned to human chromosome 9q34.1 by somatic cell hybrids, radiation hybrids, and linkage analysis [82].

The candidate gene approach (analysis of the locus for POMT1) in 15 consanguineous families with WWS [52] revealed POMT1 mutations in 6 of 30 unrelated patients [52]. Of five distinct mutations identified, two were nonsense mutations, two were frameshift mutations and one was a missense mutation. Immunohistochemical analysis of muscle from patients with POMT1 mutations indicated the absence of α-dystroglycan glycosylation.

The strong conservation of protein motifs between human POMT1 and the yeast *O*-mannosyltransferases suggests that POMT1 has a similar enzymatic function. Until recently, it has not been possible to measure any enzyme activity for recombinant human POMT1. However, Manya *et al.* [83] were able to demonstrate that the human *POMGnT1* gene encoded an active protein *O*-mannosyltransferase activity *in vitro* using a GST fusion protein of α-dystroglycan as acceptor, dolichol-phospho-mannose as donor, and *n*-octyl-β-D-thioglucoside-solubilized mammalian cell membranes as an enzyme source. They then showed that co-transfection of mammalian cells with both *POMT1* and *POMT2* (another human gene homologous to yeast protein O-mannosyltransferases) was necessary for enzyme activity but expression of either *POMT1* or *POMT2* alone was insufficient. It was suggested that a heterophilic interaction between POMT1 and POMT2 is involved in the formation of the active enzyme.

Fukutin-related protein (FKRP), MDC1C and LGMD2I

Database screening using the mouse fukutin sequence as a probe resulted in the cloning of a new gene highly homologous to fukutin. The encoded protein was named fukutin-related protein (FKRP) [48]. The human FKRP sequence was determined by a combination of EST assembly, RT-PCR and RACE. The cDNA had a 1485 bp open reading frame encoding a 495 amino acid protein with a domain structure typical of Golgi-associated type II transmembrane proteins. The amino acid sequence suggests that FKRP could be a glycosyltransferase. Northern blot analysis showed a 4.0 kb transcript expressed predominantly in skeletal muscle, placenta, and heart, and relatively weakly in other tissues. The FKPR gene (12 kb) has three noncoding exons and a single large exon of 3.8 kb that contains part of the 5′-untranslated region and the entire open reading frame and 3′-untranslated region. Radiation hybrid mapping localized the FKRP gene to chromosome 19q13.3 [48]. Mutations in the FKRP gene lead to either severe CMD (MDC1C) [48,49], or to a later onset and milder allelic form of CMD called limb-girdle muscular dystrophy 2I (LGMD2I) [47].

FKRP mutations were identified in 7 families with MDC1C [48]. Onset of the disease occurs in the first weeks of life with a severe phenotype, *i.e.*, inability to walk, muscle hypertrophy, and marked elevation of serum creatine kinase. However, most of the patients have normal brain structure and function. Nine of the mutations were missense mutations and 2 were nonsense mutations. In 4 families, the affected individuals were compound heterozygotes; in the other 3, the patients were homozygous for the particular mutation. The 11 mutations were all different. MDC1C patients have a secondary deficiency of α2-laminin and a profound reduction in α-dystroglycan immunostaining. In addition, the molecular weight of α-dystroglycan is reduced in muscle. Together, these findings suggest that this protein is abnormally glycosylated in MDC1C and is central to the pathogenesis of the disorder.

Autosomal recessive limb-girdle muscular dystrophies (LGMD) represent a genetically heterogeneous group of diseases characterized by a progressive involvement of skeletal muscles [47,84]. They show a wide spectrum of clinical courses, varying from very mild to severe. The onset of symptoms in CMD is within the first few months of life whereas in LGMD they can occur in late childhood, adolescence or adult life. Several loci and genes responsible for autosomal recessive LGMDs have been mapped and identified.

Driss *et al.* [84] reported the clinical data, muscle biopsy findings, and results of genetic linkage analysis in a large consanguineous Tunisian family with 13 individuals suffering from autosomal recessive LGMD. Clinical features included variable age of onset, proximal limb muscle weakness and wasting, predominantly affecting the pelvic girdle. Creatine kinase levels

were usually high in younger patients. Muscle biopsy showed dystrophic changes with normal expression of dystrophin but abnormal expression of other proteins of the dystrophin glycoprotein complex (DGC). Genetic linkage analysis mapped this new form of autosomal recessive LGMD (LGMD2I) to chromosome 19q13.3 and excluded the known LGMD loci as well as ten additional candidate genes.

Mutation analysis of FKRP in 25 potential LGMD2I families, including some with a severe and early onset phenotype, identified mutations in individuals from 17 families [47]. Age at onset of symptoms ranged from less than 6 months to 40 years. Affected individuals from 15 families had an identical C826A (Leu276Ileu) mutation, including five that were homozygous for this change. Patients with the C826A change had the clinically less severe LGMD2I phenotype, suggesting this is a less disruptive FKRP mutation than those found in MDC1C. The spectrum of LGMD2I phenotypes ranged from infants with an early presentation and subsequent Duchenne-like disease course including cardiomyopathy, to milder phenotypes compatible with a favourable long-term outcome. A variable reduction of α-dystroglycan expression was observed in the skeletal muscle biopsy of all individuals studied. Several cases showed a deficiency of α2-laminin either by immunocytochemistry or Western blotting. Linkage analysis identified at least 2 possible haplotypes in linkage disequilibrium with this mutation.

The LARGE Gene and the *myd* Mouse

In 1999, Peyrard *et al.* [85] reported the cloning of a human gene with homology to the *N*-acetylglucosaminyltransferase gene family. The gene (LARGE) occupies over 664 kb and is one of the largest human genes. The gene contains 16 exons and encodes a 4.3 kb cDNA and a 756 amino acid protein with two putative glycosyltransferase sequences [86]. The mouse Large ortholog was also cloned and encodes a protein with over 97% identity to the human counterpart. Both genes are widely expressed. The mouse and human genes map to chromosomes 8C1 and 22q12.3–q13.1 respectively.

The mouse myodystrophy (*myd*) mutation was first described in 1976 [87]. It is an autosomal recessive mutation which causes a diffuse and progressive myopathy. Muscle from the *myd* homozygotes appears dystrophic with degenerating and regenerating fibers, inflammatory infiltrates, central nuclei, and variation in fiber size [88]. Homozygotes have an abnormal gait, show abnormal posturing when suspended by the tail, and are smaller than littermate controls. Serum creatine kinase is elevated. The *myd* mutation maps to mouse chromosome 8 [87,89–91].

Grewal *et al.* [86,92] have shown that the gene mutated in the *myd* mouse encodes LARGE. An intragenic deletion of exons 4–7 in *myd* causes a frameshift and a premature termination codon before the first of two putative glycosyltransferase domains. Immunoblots with the VIA4-1 monoclonal antibody to α-dystroglycan show reduced binding in the *myd* mouse suggesting hypoglycosylation of this protein. Michele *et al.* [51] have demonstrated that *myd* mice show abnormal neuronal migration in cerebral cortex, cerebellum, and hippocampus, and disruption of the basal lamina of the basement membrane. The data suggest that dystroglycan targets proteins to functional sites in brain through its interactions with extracellular matrix proteins.

The muscular dystrophy phenotype of the *myd* mouse is not confined to skeletal muscle, but is also present in the heart and tongue [93]. Immunohistochemistry indicates disruption of the DGC in skeletal and cardiac muscle. Quantitative Western blotting shows a general increase in the expression of DGC proteins in mutant skeletal muscle and a decrease in mutant cardiac muscle. Overlay assays show loss of laminin binding by α-dystroglycan in *myd* mouse skeletal and cardiac muscle, and in brain. The phenotype of the *myd* mouse is not restricted to muscular dystrophy but also includes ophthalmic and central nervous system defects (*e.g.*, evidence of defective neuronal migration). The phenotype is therefore similar to the human "muscle-eye-brain" types of congenital muscular dystrophy discussed above and can serve as an animal model for studying the function of glycosylation in these diseases.

Concluding remarks

The demonstration of mutations in genes encoding established or putative glycosyltransferases in five different types of inherited human muscular dystrophy and in a mouse model suggests that post-translational processing of proteins may play an important role in their pathogenesis. Abnormal glycosylation of α-dystroglycan is a major feature in these pathologies and probably results in an impaired ability of α-dystroglycan to interact with its normal extracellular matrix partners giving rise to progressive muscle degeneration and abnormal neuronal migration. Brain-selective deletion of dystroglycan in mice is sufficient to cause CMD-like brain malformations suggesting that defects in dystroglycan are central to the pathogenesis of structural and functional brain abnormalities seen in CMD [94]. Since the glycosylation-deficient congenital muscular dystrophies show accompanying brain abnormalities, it is highly probable that abnormal dystroglycan ligand interactions underlie the pathogenic mechanism of "muscle-brain" diseases.

The abnormal proteins in two of the CMDs discussed above (MEB and WWS) are glycosyltransferases almost certainly required for the synthesis of the NeuAcα2,3Galβ1,4GlcNAc β1,2Manα1-O-Ser/Thr tetrasaccharide on α-dystroglycan. Abnormal α-dystroglycan glycosylation is also a key element of FCMD, MDC1C, MDC1D and the *myd* mouse. Since none of the genes mutated in the above CMDs is homologous either to known sialyltransferases [95] or galactosyltransferases [96], it is unlikely they are involved in the synthesis of the NeuAcα2,3Galβ1,4- moiety of the *O*-mannosyl tetrasaccharide. Other epitopes have been found on *O*-mannosyl oligosaccharides, *e.g.*, the Lewis X antigen which has an α1,3-linked fucose [20] and the GlcNAcβ1,6(GlcNAcβ1,2)Manα1-O-Ser/Thr moiety [97–100]. Furthermore, 30% of the O-linked

glycans in brain are O-linked via mannose [97] indicating that other as yet unknown *O*-mannosyl glycans exist. It is clearly essential to have a great deal of effort devoted to the elucidation of the structures of the glycans bound to α-dystroglycan and other proteins of the DGC in health and disease. Comparison of glycan structures in the muscle and brain of normal controls and of dystrophic patients or animal models will inevitably lead to the identification of many more CMD genes.

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