CMLS, Cell. Mol. Life Sci. 60 (2003) 251–258
1420-682X/03/020251-08

CMLS Cellular and Molecular Life Sciences

Glycosylation defects in inherited muscle disease

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Abstract. The gene mutated in the myodystrophy mouse, a model of muscular dystrophy, encodes a putative glycosyltransferase, Large. Mutations in genes encoding proteins thought to be involved in glycosylation have now been identified in six human forms of muscular dystrophy. Hereditary inclusion body myopathy and Nonaka myopathy result from defects in sialic acid production. Two forms of congenital muscular dystrophy, Fukuyama-type and MDC1C, result from mutations in members of the fukutin family. MDC1C and limb girdle muscular dystrophy type 2I are allelic, as they are both associated with mutations in the *FKRP* gene. Mutations in *POMGnT*, which encodes an enzyme involved in the synthesis of *O*-mannosyl glycans, result in muscle-eye-brain disease – another congenital form of muscular dystrophy. Abnormal α-dystroglycan has been reported in the myodystrophy mouse, and in the congenital and limb girdle muscular dystrophies. Recent data have shown that there is altered glycosylation of the protein and that this reduces its ability to bind to extracellular matrix ligands such as laminin and agrin.

Key words. Dystroglycan; mouse model; muscular dystrophy; O-glycosylation; glycosyltransferase; laminin.

Introduction

Inherited muscular dystrophies are genetically very heterogeneous; over 25 genes have now been implicated in one or more forms of the disease (reviewed in [1–3]). Identification of these genes represents a resounding success for genetic strategies; indeed, identification of the X-linked gene for Duchenne muscular dystrophy (DMD) was one of the first results of positional cloning in the 1980s. The DMD gene encodes a cytoskeletal protein, dystrophin, a member of the β-spectrin/α-actinin family located in the muscle sarcolemma. Subsequently, a large number of proteins have been identified that interact with dystrophin; together, these are known as the dystrophin-associated protein complex (DAP or DPC) [2] or the dystrophin-associated glycoprotein protein complex (DGC) [3]. A schematic representation of this complex is shown in figure 1. Although there is a signalling role for the DGC [4], its main function is in maintaining sarcolemmal integrity by linking cytoskeletal actin (via dystrophin) to components of the extracellular matrix (via α-dystroglycan). Known ligands for α-dystroglycan include the laminin-α1 and -α2 chains, agrin and perlecan [5]. Mutations in almost all of the genes encoding components of the DGC or proteins that interact with the complex have been implicated in inherited forms of human muscular dystrophy or in animal models (reviewed in [3, 6]), although genetic forms of muscular dystrophy are not exclusively associated with the DGC [1].

Recent findings have implicated abnormal glycosylation as a pathomechanism underlying some forms of muscular dystrophy. First the gene mutated in the myodystrophy mouse was shown to encode a putative glycosyltransferase [7]; this was rapidly followed by reports of mutations in glycosyltransferase genes in several forms of human muscular dystrophy [8–10]. Abnormal glycosylation of a key component of the DGC, α-dystroglycan, has been implicated in several of these dystrophies. In this review, we give an overview of those muscular dystrophies and myopathies where abnormal glycosylation appears to play a role in the development of the disease.

The myodystrophy mouse

The mouse myodystrophy (*myd*) mutation arose spontaneously at the Jackson Laboratory and has an autosomal recessive mode of inheritance [11]. Using a positional

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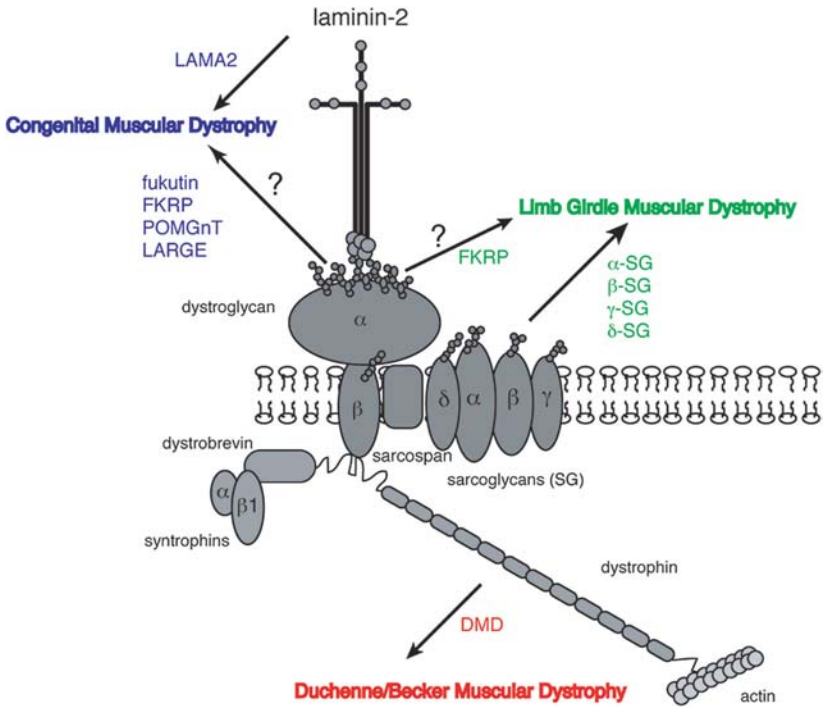

Figure 1. Schematic diagram of the skeletal muscle dystrophin-associated protein complex (not drawn to scale). The DGC is composed of dystrophin, the dystroglycan complex of α and β subunits, the sarcoglycans and sarcospan, α and β1 syntrophins and dystrobrevin. Of the DGC components identified to date, only dystroglycan and the sarcoglycans are known to be glycosylated (indicated by small circles). Mutations in genes encoding most of these components results in muscular dystrophy phenotypes in human and animal models (reviewed in [1, 3]). Laminin consists of heterotrimers of α, β and γ chains. α-Dystroglycan binds to the laminin α2 chain, the predominant α chain in skeletal muscle, linking the DGC to the extracellular matrix. This binding requires carbohydrate structures on α-dystroglycan. A large proportion of cases of congenital muscular dystrophy are due to mutations in the LAMA2 gene, encoding the laminin α2 chain. Null mutations in α-dystroglycan are embryonic lethal in mouse, and no mutations have been described in the gene in human muscular dystrophy. However, several studies have identified mutations in genes encoding known or putative glycosylation enzymes; fukutin, FKRP and POMGnT in human and LARGE in mouse and are discussed in the main text. All of these have been associated with abnormalities in α-dystroglycan, which are presumed to represent aberrant glycosylation of the protein.

cloning approach, we demonstrated that the causative mutation in *myd* is a deletion within a putative glycosyltransferase gene (*Large*) [7, 12]. This deletion of over 100 kb of genomic DNA removes exons 5–7 of *Large*, producing a 379-bp out-of-frame deletion within the coding region and a premature termination signal.

The human *LARGE* gene was so named because it covers over 660 kb of genomic DNA [13]. The messenger RNA (mRNA) is ubiquitously expressed, with highest levels in heart, brain and skeletal muscle. It is predicted to encode a glycosyltransferase, although this has yet to be confirmed experimentally. Large has an N-terminal transmembrane anchor, typical of glycosyltransferases. It also has a coiled-coil motif and two putative catalytic regions [7, 13]. These two catalytic domains are not closely related to each other, and each shows homology to a different family of glycosyltransferases. The proximal domain belongs to family 8 of glycosyltransferases [14] and has highest amino acid identity (~22%) to members of the bacterial WaaIJ family of putative α-glycosyltransferases involved in synthesis of lipopolysaccharides or lipo-oligosaccharides [15]. The distal domain, in contrast, is most closely related (31% amino acid identity) to i-β-1,3-*N*-acetylglucosaminyltransferase (iGnT) [16]. This enzyme is required for synthesis of the poly-*N*-acetyllactosamine backbone $(\mathrm{Gal}\beta1\rightarrow4\mathrm{GlcNAc}\beta1\rightarrow3)_n$, which is attached to *N*-glycans, *O*-glycans and glycolipids, and may be modified by structures such as sialyl Lewis^x. The enzyme activity of Large is currently under investigation.

Homozygous *myd* mice display a severe, progressive muscular dystrophy [11, 17]. The muscle basement membrane is thin and disrupted, and there are neuronal migration abnormalities in the brain, particularly the cortex and cerebellum [our unpublished data]. Because of the typical muscular dystrophy phenotype in *myd* mice, we investigated the glycosylation of DGC components, and our data suggested that glycosylation of skeletal muscle and brain dystroglycan is abnormal in the mutant mice [7]. Dystroglycan is produced as a precursor polypeptide that is posttranslationally modified and cleaved into the α and β subunits. α-Dystroglycan is a peripheral membrane

protein linking the DGC to the extracellular matrix, while the β subunit is a transmembrane protein interacting with both α-dystroglycan and dystrophin (fig. 1). By Western blotting, we found a marked reduction in the signal intensity of α-dystroglycan in skeletal muscle and brain using the monoclonal antibody VIA4$_1$, a widely used, commercially available monoclonal antibody raised against membrane preparations of rabbit skeletal muscle [18]. In contrast, an antibody to the β subunit showed no significant differences between *myd* and control tissues on Western blots, showing that synthesis and cleavage of the precursor dystroglycan protein is unaffected. The α-dystroglycan epitope recognised by VIA4$_1$ has not been defined, although the antibody does not recognise bacterially expressed protein, suggesting it may be a carbohydrate. In support of this, equivalent signals were seen in control and mutant tissues using a polyclonal antibody raised against an α-dystroglycan peptide [7, 19]. However, the size of α-dystroglycan on immunoblots using this antibody did not differ between control and affected animals, as might be expected if glycosylation of the protein is reduced in the mutant animals. One possibility is that the polyclonal antibody recognises only a subset of glycosylated isoforms that are not altered in *myd*.

The human *LARGE* gene maps to 22q [13]. No muscular dystrophy locus has been mapped to this region, and we have not yet identified mutations in *LARGE* in any muscular dystrophy patients. However, the gene remains a strong candidate for involvement in human muscular dystrophy. The coding region of *LARGE* is interrupted by very large introns that are rich in repeat sequences, properties that may predispose it to deletions and duplications, and we are currently developing screening strategies to test for such rearrangements within the gene.

Human muscular dystrophies

In human muscular dystrophy, many of the glycosyltransferase mutations described to date are associated with congenital forms of the disease. Congenital muscular dystrophy (CMD) is a clinically and genetically heterogeneous group of dystrophies, characterised by early onset of hypotonia and weakness, and a progressive muscular dystrophy with abnormalities of the muscle basement membrane [20]. In some forms of the disease there are also defects in structures within the brain, which may lead to mental retardation. Laminin is required for formation of the muscle basement membrane, and laminin α2 is the predominant α chain in muscle and peripheral nerve [21]. Not surprisingly then, mutations in the *LAMA2* gene encoding the laminin α2 chain are associated with CMD and account for ~40% of cases [22–24]. Clinically, this group of CMD patients are relatively homogeneous, with a severe form of the disease commonly referred to as merosin-deficient CMD or MDC1A. Deficiency or abnormality of integrin α7 or of the α2 chain of collagen VI also results in CMD phenotypes [25, 26], indicating the importance of the extracellular matrix. The remaining CMD patients are divided between several clinically and genetically distinct forms of the disease; those relevant to the subject of this review are discussed below.

Fukuyama-type congenital muscular dystrophy FCMD

Fukuyama-type congenital muscular dystrophy (FCMD) is an autosomal recessive disorder that produces a severe, congenital muscular dystrophy in association with characteristic brain malformations (cerebral and cerebellar cortical dysplasia), profound mental retardation and ophthalmologic abnormalities [27]. FCMD shows geographical differences in prevalence, being most common in Japan [27]. Onset is usually before the age of 9 months, most patients are never able to walk and most die by the age of 20. Skeletal muscle biopsies show necrosis and fibre regeneration. Immunostaining shows a significant reduction in laminin α2, thought to be a secondary event [28], while electron microscopy shows the muscle basal lamina to be thin and disrupted, reminiscent of that seen in merosin-deficient CMD [29].

A positional cloning strategy identified the gene mutated in FCMD as *fukutin* [30]. Mutation analysis showed over 80% of Japanese disease-associated chromosomes are derived from a single ancestral founder and carry a 3-kb retrotransposal insertion into the 3′ noncoding region of *fukutin*, explaining the increased prevalence of FCMD in this population. Northern blotting demonstrated that the effect of this founder mutation is to reduce the level of *fukutin* mRNA expression [30]. Patients who have only one copy of the insertion carry a point mutation in the other allele [30, 31]. Genotype/phenotype analysis of over 100 FCMD families [31] showed that typical mild phenotypes are associated with homozygosity for the founder retrotransposon insertion. Compound heterozygotes tended to have a much more severe phenotype, presumably because of adverse effects on fukutin protein function in addition to a reduction in the amount of mRNA.

Kobayashi et al. expressed GFP-tagged fukutin in cos cells, and showed that the protein was localised to the Golgi body and to secretory granules [30]. However, the first suggestion that fukutin may play a role in carbohydrate modification came from a bioinformatics study which showed it to have significant amino acid similarity to bacterial proteins involved in polysaccharide/phosphorylcholine modification and a yeast protein involved in mannosyl phosphorylation of oligosaccharides [32].

The brains of FCMD patients display type II lissencephaly (or cobblestone cortex), a disruption of the normal folding of the cortex [27]. This arises from neurons or

neuronal precursors migrating out of the developing brains through breaches in the superficial neural basal lamina, resulting in a bumpy or 'cobblestone' appearance of the surface of the cortex. Thus, fukutin appears also to play a role in neuronal migration. Consistent with this, in situ hybridisation showed *fukutin* mRNA to be expressed in the cell body of neuronal cell lineages, but not in glial cells, within the developing human cerebrum and cerebellum [33]. Western blotting and immunohistochemistry using a polyclonal antibody raised against fukutin peptides agrees with the in situ hybridisation data, with the protein showing primarily neuronal expression in prenatal human brain [34].

A recent study has now shown that, as in the *myd* mouse, α-dystroglycan may be involved in the pathogenesis of FCMD. Skeletal muscle and brain tissue showed loss of α- but not β-dystroglycan on immunoblots and immunohistochemistry [35]. Although they do not show the data, these authors also report a reduction in muscle sarcolemmal staining of α-dystroglycan with the same polyclonal antibody we used [35].

Muscle-eye-brain disease MEB

Clinically, muscle-eye-brain disease (MEB) resembles FCMD: congenital muscular dystrophy in combination with severe mental retardation, ocular abnormalities and a neuronal migration disorder of the brain [36, 37]. This disease is rare with the majority, but not all, of the cases reported arising in Finland. Muscle pathology in MEB is quite varied, although the presence of regenerative fibres and mild variation in fibre size are common features [38]. Initial investigation of the molecular pathology of skeletal muscle in MEB showed that β-dystroglycan, α- and γ-sarcoglycan, and dystrophin levels showed only a slight reduction compared to controls [38]; α-dystroglycan was not examined in this study. There was a clear reduction in the amount of laminin α2 chain, with irregular staining around the sarcolemma, and concomitant increases in the β1 and β2 chains. Genetic mapping studies showed that at least one form of MEB is distinct from FCMD and maps to chromosome 1p32-p34 [39].

The gene for *O*-mannose β-1,2-*N*-acetylglucosaminyltransferase (*POMGnT1*) was cloned by homology searching and mapped to the same interval on chromosome 1p as MEB [10]. Yoshida et al. therefore screened MEB patients and identified mutations in five Turkish and one French family. No mutation analysis of the Finnish patient cohort has been published so far, so it is not known whether there is a founder mutation effect in this population. In contrast to fukutin, the enzyme activity of POMGnT1 has been characterised; two groups independently demonstrated that the protein is an *N*-acetylglucosaminyltransferase requiring a mannose peptide as an acceptor [10, 40]. POMGnT is therefore a candidate enzyme for formation of the GlcNAcβ1 $\rightarrow$ 2Man linkage in *O*-mannosyl glycans. This unusual linkage is known to be contained within a large proportion of the *O*-glycans present on α-dystroglycan [41, 42], suggesting the protein is modified by POMGnT1. Consistent with this, there is a significant loss of VIA4$_1$ α-dystroglycan immunoreactivity by both immunoblotting and immunohistochemistry in MEB muscle [43].

Walker-Warburg syndrome WWS

Walker-Warburg syndrome (WWS) is a form of CMD that shares with FCMD and MEB the combination of muscular dystrophy, ocular abnormalities and brain malformations characteristic of a neuronal migration defect. WWS and MEB, in particular, show significant clinical overlap, and unequivocal diagnosis in individual patients can be difficult [44]. Therefore, it seems plausible that WWS may also be due to a defect in a glycosylation enzyme. The initial mapping of MEB to chromosome 1p32-p34 [39], and now the identification of POMGnT1 as the causative gene [10] has helped to differentiate between these two diseases. Although genetic studies of WWS have been difficult as the disease is very rare and most patients die very young [44], linkage analysis of a large collection of 19 families with either WWS or MEB showed that most families with clinically defined WWS were inconsistent with linkage to the MEB locus [44]. Given the difficulty of identifying the gene using a standard positional cloning approach, it seems likely that the gene for WWS will be identified on the basis of candidate gene screening and the human *LARGE* gene represents one plausible contender.

CMD, type MDC1C

Brockington et al. used database searching to identify human genes encoding proteins closely related to fukutin and identified the gene for fukutin-related protein (FKRP) [8]. Although fukutin and FKRP show significant similarity only within their predicted active-site regions, FKRP has characteristics of a glycosyltransferase with a hydrophobic potential membrane-spanning region followed by a stem region and a putative catalytic domain. The enzyme activity of the protein has not yet been established. Northern blot analysis shows expression of *FKRP* mRNA in most tissues, with highest levels in skeletal muscle, placenta and heart [8].

Brockington et al. then screened a series of muscular dystrophy patients for mutations in *FKRP* and found them in seven families with a severe form of CMD, now termed MDC1C [8]. Onset of this disease occurs in the first few weeks after birth, and affected children do not achieve independent ambulation. There is also respiratory involvement, with patients often requiring ventilation. In contrast

to FCMD, intelligence of affected individuals is normal with no evidence of brain abnormalities [8]. Again, α-dystroglycan appears abnormal, as immunoblotting with monoclonal antibody $VIA4_1$ showed a reduction in the size of the protein in skeletal muscle from affected individuals, while immunohistochemistry showed a variable reduction in sarcolemmal α-dystroglycan staining [8].

Limb girdle muscular dystrophy 2I

Unlike CMD, where the onset of symptoms is at birth or within the first 6 months of life, LGMD onset generally occurs from late childhood onwards. Genetically, LGMD is also very heterogeneous, with at least 14 genetic loci identified [45]. Inheritance can be either autosomal dominant (LGMD type 1) or recessive (LGMD type 2). Mutations in genes encoding components of the DGC often produce a limb girdle phenotype, although causative genes are not restricted to this class [45]. The locus for LGMD type 2I maps to the same chromosomal region as *FKRP*, 19q13.3 [46]. Therefore, Brockington et al. screened the *FKRP* gene in patients consistent with linkage to this region and found mutations in 17 families [9]. LGMD2I and MDC1C are therefore allelic disorders.

The range of phenotypic severity is extremely large in LGMD 2I [9]. Severely affected individuals present within the first 2 years of life with hypotonia. Muscle hypertrophy is found predominantly in the legs, but often is also present in the tongue. Loss of independent ambulation generally occurs in the early teens followed by development of cardiomyopathy. More mildly affected patients have a later onset and a less severe muscle phenotype, although tongue muscle hypertrophy remains a common feature. In common with MDC1C, intelligence and brain MRI are normal. Brockington et al. identified a recurrent missense mutation, C826A, in LGMD2I [9]. Homozygosity for this change produced a mild phenotype, whereas compound heterozygotes tended to have a more severe phenotype. Interestingly, in 6 of the 10 compound heterozygotes for C826A, they did not identify the mutation on the other allele, suggesting that mutations exist outside the *FKRP* coding region, perhaps in regulatory regions.

Abnormal expression or immunoreactivity of α-dystroglycan is also a feature of LGMD 2I; immunohistochemistry showed a reduction of α-, but not β-dystroglycan, in all biopsies studied [9]. Patients at the severe end of the phenotypic scale also showed a mild deficiency of laminin $\alpha 2$ on immunohistochemistry, while most mildly affected individuals had normal amounts of laminin $\alpha 2$ [9].

Hereditary inclusion body and Nonaka myopathy

These two recessive myopathies have recently been reported to result from mutations in UDP-*N*-acetylglucosamine-2-epimerase/*N*-acetyl-mannosamine kinase (UDP-GlcNAc 2-epimerase or GNE), an enzyme involved in sialic acid production [47, 48]. Sialic acid is commonly found as a terminal modification of glycan chains. Sialic acids are synthesised in the cytosol from UDP-*N*-acetylglucosamine. The first two steps in this pathway are rate limiting and are catalysed by GNE, which is a bifunctional enzyme. Mutations in GNE have also been described in sialuria, an inborn error of metabolism characterised by dominant inheritance, hepatosplenomegaly and urinary excretion of very high levels of sialic acid. In this disorder dominant missense mutations in the epimerase domain lead to a loss of feedback inhibition, resulting in overproduction of free sialic acid [49]. In mice, GNE is essential as inactivation by gene targeting results in early embryonic lethality around E8.5 [50].

Hereditary inclusion body myopathy (HIBM) onset usually occurs after the age of 20 years. Affected individuals show a progressive development of muscle weakness and wasting, affecting proximal and distal muscles of both upper and lower limbs. Muscle pathology includes characteristic cytoplasmic inclusions of 15- to 18-nm filaments and rimmed vacuoles. Many vacuolated muscle fibres showed immunoreactivity to neural cell adhesion molecule, a fetal muscle antigen [51]. Nonaka is a distal myopathy that shares clinical features with HIBM, including rimmed vacuoles [48]. The differences in phenotype between sialuria and myopathy may be explained by the nature of the mutations. The missense mutations associated with sialuria have a dominant effect, resulting in overproduction of sialic acid. The recessive mutations in HIBM and Nonaka myopathy are likely to result in reduced enzyme activity, as HIBM patients showed no detectable overproduction of urinary sialic acid [47]. A high proportion of HIMB and Nonaka myopathy mutations are missense, and no patients have been described with two truncating mutations, suggesting that homozygous loss of function of GNE might be embryonic lethal in humans as in mice. It is unknown why mutation of this enzyme should produce such a specific phenotype, but it is possible that skeletal muscle is particularly susceptible to reduction of levels of free sialic acid.

Conclusions and perspectives

While there is clearly still a long way to go before we understand how mutations in the genes mentioned in this review result in the muscular dystrophy phenotypes, in many cases α-dystroglycan appears to be a key protein. Dystroglycan plays an important role in muscle formation and maintenance, but also has important functions in nonmuscle tissues including brain, kidney and peripheral nerve [52–55]. Null mutants of dystroglycan are lethal in

the mouse due to failure of embryos to develop beyond the egg cylinder stage; dystroglycan is required for formation of the Reichert's membrane, an early extraembryonic basement membrane [56]. α-Dystroglycan is extensively glycosylated in a tissue-specific manner; over half the mass of the protein may be carbohydrate [57–60]. The protein contains a central mucin-like domain rich in Thr, Ser and Pro residues; this region undergoes extensive O-glycosylation and probably keeps this region of the protein extended. Electron microscopy suggests cardiac muscle α-dystroglycan to assume a central rod-shaped structure with globular domains at each end [61]. In addition to O-glycosylation, there are conserved sites for N-glycosylation and glycosaminoglycan attachment. It is likely that tissue-specific differences in posttranslational modification act to modify the function of dystroglycan [58]. There may also be different forms within a single tissue; McDearmon et al. identified three α-dystroglycan isoforms in skeletal muscle, based on lectin reactivity [62].

In most of the dystrophies described above, a selective reduction or loss of α-dystroglycan immunoreactivity has been reported. An important question is whether this is a direct effect due to loss of a carbohydrate epitope (most studies have used a single antibody) or secondary loss of the protein as is seen in some other forms of muscular dystrophy [1]. In MEB is seems likely that there is some loss of α-dystroglycan O-glycosylation, as POMGnT1 is involved in synthesis of *O*-mannosyl glycans, which are known to be present on the protein [10, 40]. A consequence of abnormal glycosylation might be loss of laminin binding, as this requires carbohydrate structures on α-dystroglycan; loss or abnormality of laminin results in muscular dystrophies with phenotypes similar to those arising from mutations in genes encoding components of the DGC [28, 63, 64].

Sialidase treatment and inhibitor studies suggest that sialylated structures are necessary for laminin binding [41, 65]. O-linked residues seem to be critical; although α-dystroglycan also carries N-glycosylation, enzymatic removal of these does not affect the laminin-binding activity of the protein [66]. α-Dystroglycan carries an unusual *O*-mannosyl-type oligosaccharide Siaα2→3Galβ1→4GlcNAcβ1→2Man→Ser/Thr. This is the major sialylated O-linked oligosaccharide of dystroglycan in bovine peripheral nerve [41] and is also present on the protein in rabbit skeletal muscle [67] and sheep brain [42]. POMGnT1, which catalyses a GlcNAcβ1-2Man linkage, is likely to be required for synthesis of this oligosaccharide [10, 40]. Loss of functional POMGnT1 results in muscular dystrophy, highlighting the importance of these *O*-mannosyl-type glycans. However, other O-linked glycans are also present, as rabbit skeletal α-dystroglycan also contains the core 1 structure Galβ1→3GalNAc [31, 66, 68].

Abnormal glycosylation of α-dystroglycan might not only affect laminin binding: laminin binds to α-dystroglycan via the LG domains on the laminin α1 and α2 chains [69, 70]. Additional extracellular matrix ligands for α-dystroglycan include agrin, neurexin and perlecan, all of which also bind in a carbohydrate-dependent manner via LG domains [5, 69, 71, 72]. Agrin is a basement-membrane associated proteoglycan required for correct neuromuscular junction formation [73], perlecan is a widely expressed basement membrane proteoglycan, while α- and β-neurexins are neuron-specific proteins [71].

Like many positional cloning stories, the studies described here have identified a new disease pathway; abnormal glycosylation now seems to be the mechanism for several types of muscular dystrophy. It is probable that the human diseases and animal models described here are not the only forms of muscular dystrophy due to mutations in genes involved in glycosylation pathways; no doubt more will be identified in the near future. For many of the genes mentioned in this review, investigation of the enzyme activity of the protein product and identification of target proteins is now an important priority. These diseases also represent new systems in which to investigate the functions of carbohydrates and generation of mouse mutants for these genes will provide useful tools for studying their function. Investigation of the molecular basis of muscular dystrophy now faces some major challenges that will require collaboration between molecular geneticists and glycobiologists.

Note added in proof. Two recent papers from Kevin Campbell's group in Iowa, USA, have very nicely confirmed a key role for α-dystroglycan in the pathogenesis of MEB and FCMD as well as the *myd* mouse. Using a polyclonal antibody to α-dystroglycan, they demonstrated a significant reduction in the mass of α-dystroglycan on Western blots in muscle tissue from MEB and FCMD patients and in muscle and brain of *myd* mice, consistent with hypoglycosylation. Using overlay assays, they also showed loss of laminin, agrin and neurexin binding by dystroglycan in patient and *myd* tissues [74]. In the accompanying elegant paper they used a cre-lox system to selectively delete dystroglycan from the CNS of mice. These animals display neuronal migrations defects strikingly similar to those seen in the *myd* mouse and in MEB and FCMD patients [75]. Together, these two papers support a central role for abnormal glycosylation of dystroglycan in the pathogenesis of these glycosylation-deficient forms of muscular dystrophy.

Acknowledgements. Work in the authors' laboratory is supported by the Wellcome Trust, the Biotechnology and Biological Sciences Research Council and the Muscular Dystrophy Association, USA.

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