Human Molecular Genetics, 2003, Vol. 12, Review Issue 2 **R259–R264**
DOI: 10.1093/hmg/ddg272

Glycosylation defects: a new mechanism for muscular dystrophy?

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Received July 2, 2003; Accepted July 18, 2003

Recently, post-translational modification of proteins has been defined as a new area of focus for muscular dystrophy research by the identification of a group of disease genes that encode known or putative glycosylation enzymes. Walker–Warburg Syndrome (WWS) and muscle–eye–brain disease (MEB) are caused by mutations in two genes involved in *O*-mannosylation, *POMT1* and *POMGnT1*, respectively. Fukuyama muscular dystrophy (FCMD) is due to mutations in *fukutin*, a putative phospholigand transferase. Congenital muscular dystrophy type 1C and limb girdle muscular dystrophy type 2I are allelic, both being due to mutations in the gene-encoding fukutin-related protein (*FKRP*). Finally, the causative gene in the myodystrophy (*myd*) mouse is a putative bifunctional glycosyltransferase (Large). WWS, MEB, FCMD and the *myd* mouse are also associated with neuronal migration abnormalities (often type II lissencephaly) and ocular or retinal defects. A deficiency in post-translational modification of α-dystroglycan is a common feature of all these muscular dystrophies and is thought to involve *O*-glycosylation pathways. This abnormally modified α-dystroglycan is deficient in binding to extracellular matrix ligands, including laminin and agrin. Selective deletion of dystroglycan in the central nervous system (CNS) produces brain abnormalities with striking similarities to WWS, MEB, FCMD and the *myd* mouse. Thus, impaired dystroglycan function is strongly implicated in these diseases. However, it is unlikely that these five glycosylation enzymes only have a role in glycosylation of α-dystroglycan and it is important that other protein targets are identified.

INTRODUCTION

The isolation of dystrophin and subsequent advances in identification of the interacting proteins that form the dystrophin-associated glycoprotein complex (DGC) led to an explosion in characterization of the muscular dystrophies (1–5). Recently, the focus has shifted to post-translational modifications of proteins as genes encoding proteins involved in glycosylation have defined a new area of attention in muscular dystrophy research. As well as giving further insights into mechanisms underlying muscular dystrophy, study of these diseases may increase our understanding of the functions of protein glycosylation. Although the function of most glycosylation is poorly understood, many vertebrate proteins are post-translationally modified by carbohydrates and it has been estimated that 1% of human genes encode enzymes involved in oligosaccharide synthesis and function (6).

These types of muscular dystrophy appear to be distinct from the congenital disorders of glycosylation (CDGs), a group of diseases caused by defects in the well-characterized and highly conserved *N*-glycosylation pathways (7). Many CDGs are multisystemic due to defects in the modification of a wide range of proteins. In contrast, the glycosylation-deficient muscular dystrophies appear to involve the less well-defined *O*-glycosylation pathways and defective post-translation modification seems to be confined to a small number of proteins (of which only one has so far been identified; dystroglycan). In this review, we focus on those genes whose mutation appears to disrupt the function of the DGC.

GLYCOSYLATION GENES ASSOCIATED WITH INHERITED MUSCULAR DYSTROPHIES

POMT1

POMT1 is the human homologue of the *Drosophila rotated abdomen* (*rt*) gene (8). The human gene is widely expressed

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and encodes a predicted transmembrane protein with high homology to the yeast mannosyltransferases, suggesting a function in the first step in *O*-mannosylation of proteins. *O*-mannosylation is rare in mammals and has only been identified in a limited number of glycoproteins in brain, nerve and skeletal muscle (9). Homozygous *rt Drosophila* have abnormal embryonic muscle development (10), and Jurado *et al.* were the first to suggest that glycosylation might be important in the formation or maintenance of muscle (8). Mutations in *POMT1* cause Walker–Warburg Syndrome (WWS, MIM:236670), a very severe, recessive form of congenital muscular dystrophy (CMD) (11). Walker–Warburg Syndrome patients also have ocular and retinal abnormalities and brain defects including type II lissencephaly (12,13).

WWS is genetically heterogeneous as only about 20% of patients have point mutations in *POMT1* (14). Another candidate gene is *POMT2*, which encodes a closely related protein (15). However, *POMT2* is expressed at very low levels in skeletal muscle (15). Two WWS patients have recently been described with mutations in *fukutin* (see below), highlighting the clinical overlap of some of these diseases.

Although it is likely that both *POMT1* and *POMT2* encode mannosyltransferases, this catalytic activity has not yet been proven. Epitope-tagged POMT2 localizes to the endoplasmic reticulum membrane (15). Although *in vitro* assays failed to demonstrate an *O*-mannosyl transfer reaction for either *POMT1* or *POMT2*, this is likely to be due to technical difficulties (15).

POMGnT1

POMGnT1 encodes the protein *O*-linked mannose β1,2-*N*-acetylglucosaminyltransferase 1 (16,17). Mutations in *POMGnT1* have been described in muscle–eye–brain disease (MEB, MIM:253280), an autosomal recessive disorder characterized by congenital muscular dystrophy, brain malformations and ocular abnormalities (16,18). POMGnT1 catalyzes the transfer of *N*-acetylglucosamine from UDP-GlcNAc to *O*-mannosyl glycoproteins (16,17,19). Mutations in MEB produce proteins that are nonfunctional when assayed *in vitro* (16). This is the strongest evidence in support of *O*-mannosylation defects in these muscular dystrophies and the similarity between the phenotypes of WWS and MEB is consistent with POMT1 acting as an *O*-mannosyl transferase.

Fukutin

Fukutin mutations are associated with Fukuyama congenital muscular dystrophy (FCMD, MIM:253800) (20,21). In addition to severe muscle weakness, there is also always severe mental retardation, with seizures occurring in about half the cases (21). The disease is most prevalent in Japan, where patients carry at least one copy of an ancestral founder mutation (integration of a 3 kb retrotransposon element into the 3′ UTR) that results in a reduction in *fukutin* mRNA levels (21). Individuals who are homozygous for the founder mutation are less severely affected than compound heterozygotes with a point mutation on the other allele, presumably because they have a higher residual activity of fukutin (21). Recently, two non-Japanese individuals homozygous for truncating mutations have been reported with a WWS-type phenotype rather than FCMD (14,22). Clinically, it can be difficult to differentiate between severe FCMD and mild WWS cases (23) and this genetic overlap implicates a function for fukutin in the same pathway as POMT1.

Null mouse mutants of *fukutin* are embryonic lethal. However, fukutin-deficient chimeric mice generated using embryonic stem (ES) cells targeted for both *fukutin* alleles have a phenotype that resembles WWS (24). The mice have a severe muscular dystrophy and neuronal migration defects, including disorganized laminar structures and fusion of medial surfaces of the cerebral cortex. Striking ocular abnormalities include abnormal lens development and loss of laminar structure in the retina.

The primary sequence of fukutin shows similarity to microbial proteins involved in polysaccharide/phosphorylcholine modification and mannosyl phosphorylation (25). The protein contains an N-terminal hydrophic signal sequence but lacks a transmembrane domain and was initially suggested to be an extracellular protein (20). However, epitope-tagged protein has recently been reported to localize to the medial Golgi (20,26). No biochemical activity has yet been reported.

Fukutin-related protein (FKRP)

The gene for FKRP was cloned on the basis of homology to the putative catalytic domain of fukutin (27). Mutations in this gene were identified in a subgroup of CMD patients (MDC1C, MIM:606612), with severe muscle weakness and inability to stand unsupported, but no evidence of brain involvement (27). Mutations in *FKRP* also account for the milder limb girdle muscular dystrophy type 2I (LGMD2I, MIM607155), therefore this disease is allelic with MDC1C (28). The spectrum of LGMD2I phenotypes is quite wide; a large proportion of patients share a common mutation (C826A; Leu276Ile) and phenotypic severity is often correlated with the second allelic mutation (29). Although initially *FKRP* mutations were not associated with brain abnormalities (27–30), mutations in *FKRP* have recently been described in two patients with a pattern of muscle involvement identical to MDC1C but including mental retardation and cerebellar cysts (31).

FKRP is predicted to be a type II membrane protein and is targeted to the medial Golgi apparatus through its N-terminal and transmembrane domains (26). Overexpression of FKRP containing a missense mutation identified in MDC1C (P448L) caused the protein to be inefficiently trafficked to the Golgi apparatus (26). Although the function of the protein is unknown, like fukutin it is predicted to be a phospholigand transferase (27).

Large

Large encodes a putative, bifunctional glycosyltransferase (32,33). A loss of function mutation is responsible for the myodystrophy (*myd*) mouse (34). Although initial investigations of the *myd* phenotype highlighted the skeletal muscle aspect of the disease (35,36), we and others have shown that the heart, retinal, peripheral and central nervous systems are also involved (37–39). Homozygous *myd* mice display a severe, progressive muscular dystrophy and a mild cardiomyopathy. Neuronal migration abnormalities are present in the brain,

particularly the cortex and cerebellum (38,39). These resemble the brain abnormalities observed in fukutin-deficient mice (24). Although morphologically the eyes and retina appear normal, electroretinograms showed a normal a-wave but increased implicit times and decreased amplitudes of the b-wave, suggesting altered signal processing in the downstream retinal circuitry (38).

Large has an N-terminal transmembrane anchor, typical of glycosyltransferases. It also has a coiled coil motif and two putative catalytic regions (32,34). The two catalytic domains are not closely related to each other, each showing homology to a different family of glycosyltransferases. The proximal domain is related to bacterial α-glycosyltransferases involved in synthesis of lipopolysaccharides or lipooligosaccharides (40). The distal domain is most closely related to iGnT, required for synthesis of the poly-*N*-acetyllactosamine backbone $(Gal\beta1\rightarrow4GlcNAc\beta1\rightarrow3)_n$ found on *N*- and *O*-glycans and glycolipids (41).

ABNORMAL GLYCOSYLATION OF DYSTROGLYCAN

The overlapping phenotypes of these muscular dystrophies suggest that the glycosylation proteins might act in a common pathway. Using immunoblot assays of total protein extracts, we observed a selective deficiency of α-dystroglycan (α-DG) in the *myd* mouse, which we speculated might reflect altered glycosylation of the protein (34). A deficiency in α-DG is now known to be a common feature of all these glycosylation-deficient muscular dystrophies; in most cases this has been observed as a loss of immunoreactivity with one or both of the two commercially available monoclonal antibodies, VIA4-1 and IIH6 (11,27,28,34,42,43).

More recently, an antibody to hypoglycosylated α-DG generated by Kevin Campbell's group in Iowa was shown to identify a reduced molecular weight form of α-DG in skeletal muscle from FCMD and WWS patients and from *myd* mice (39). Again, this was interpreted as hypoglycosylation α-DG, although it is unclear why mutations in three different glycosylation enzymes should result in the same sized form of α-DG. It is possible that aberrantly modified α-DG is susceptible to proteolysis and the fragment is a common breakdown product.

Dystroglycan function

Dystroglycan is a central component of the DGC in the sarcolemmal muscle membrane (Fig. 1). Its main function is in maintaining sarcolemmal integrity by linking cytoskeletal actin (via dystrophin) to components of the extracellular matrix (via α-DG). Ligands for α-DG include the laminin-α1 and -α2 chains, agrin, perlecan and neurexin (44,45). 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 (1–5), although genetic forms of muscular dystrophy are not exclusively associated with the DGC (46). The complex is not confined to skeletal muscle, as some DGC components are associated with dystroglycan and dystrophin isoforms in

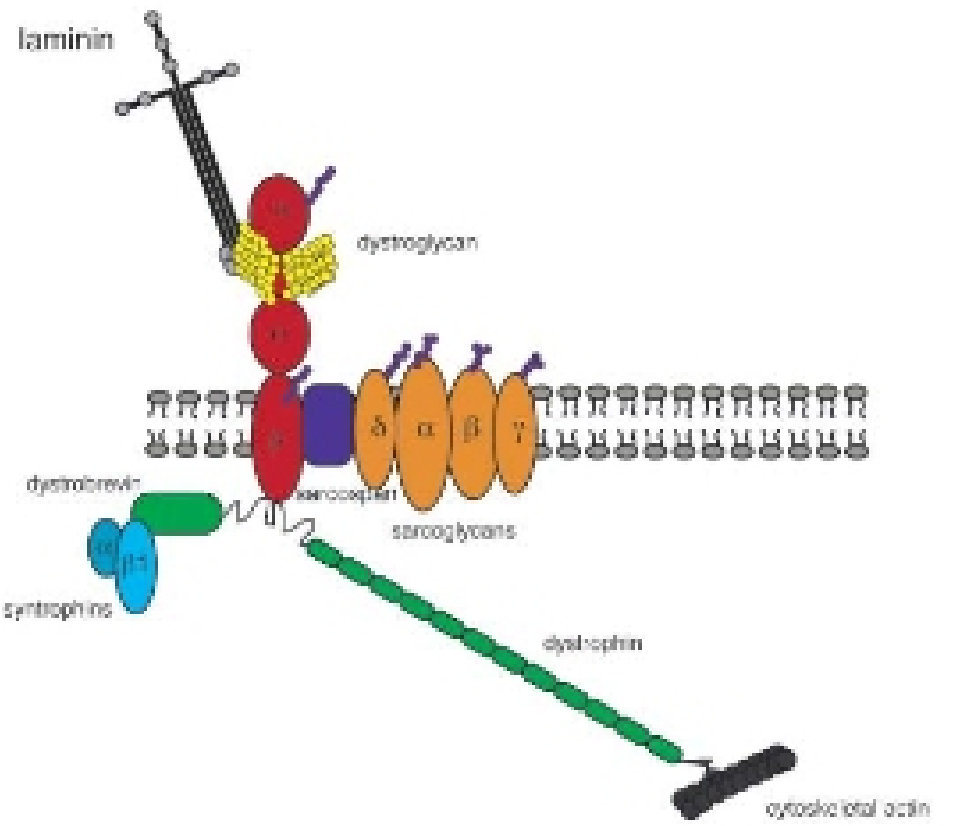

Figure 1. Schematic diagram of the skeletal muscle dystrophin-associated protein complex (DGC) (not drawn to scale). In skeletal muscle the DGC is composed of dystrophin, the dystroglycan complex of α- and β-subunits, the sarcoglycans and sarcospan, α and β1 syntrophins and dystrobrevin. *N*-glycosylation is indicated by purple circles, *O*-glycosylation by yellow circles. Up to one-half of the molecular mass of α-dystroglycan is accounted for by carbohydrate. Mutations in genes encoding most of these components results in muscular dystrophy phenotypes in human and/or animal models. Lamining consist 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, presumed to be part of the *O*-glycosylation.

other tissues including brain, peripheral nerve, retina and kidney (47–50).

Dystroglycan (*Dag1*) null mice are embryonic lethal due to very early defects in basement membrane assembly (51). Chimeric mice generated from $Dag1^{-/-}$ ES cells developed a progressive muscular dystrophy as the skeletal muscles lacked dystroglycan (52). Cre-LoxP technology has been used to produce conditional knockouts of dystroglycan. Loss of *Dag1* in differentiated skeletal muscle produced a relatively mild dystrophic phenotype as the satellite cells in this model are able to express dystroglycan successfully and regenerate muscle fibres (53). Elimination of dystroglycan from the CNS produced a phenotype with striking similarities to WWS, MEB, FCMD and the *myd* mouse with disruption of cortical layering, fusion of cerebral hemispheres and aberrant migration of granule cells (54). In combination with the evidence of altered glycosylation of α-DG, this implicates an important role for dystroglycan in the muscle and brain phenotypes of the glycosylation-deficient muscular dystrophies.

Dystroglycan glycosylation

Dystroglycan is post-translationally cleaved into α- and β-subunits (55,56). The 43 kDa β-subunit, which is *N*-glycosylated, migrates at the expected mass in all the glycosylation-deficient muscular dystrophies, indicating that the precursor protein is synthesized and cleaved normally. In