Glycomarkers for muscular dystrophy

Jane E. Hewitt
Centre for Genetics and Genomics, School of Biology, Queen’s Medical Centre, University of Nottingham, Nottingham NG7 2UH, U.K.

Abstract
During the last 10 years it has become apparent that a significant subset of inherited muscular dystrophy is caused by errors in the glycosylation of α-dystroglycan. Many of these dystrophies are also associated with abnormalities of the central nervous system. Dystroglycan has to be fully glycosylated in order bind to its ligands. To date, six genes have been shown to be essential for functional dystroglycan glycosylation and most, if not all, of these genes act in the formation of O-mannosyl glycans. Genetic heterogeneity indicates that other genes are involved in this pathway. Identification of these additional genes would increase our understanding of this specific and essential glycosylation pathway.

Aberrant glycosylation of α-dystroglycan causes muscular dystrophy

DG (dystroglycan) is an essential component of the DGC (dystrophin-associated glycoprotein complex) that links the muscle cell cytoskeleton to the extracellular matrix [1]. Initially synthesized as a precursor molecule, the protein is cleaved into α- and β-subunits that remain non-covalently associated [1]. The α-subunit (α-DG) binds ECM (extracellular matrix) proteins such as laminin, agrin, perlecan, neurexin and pikachurin [1]. All of these proteins bind α-DG via laminin-G domains [2] and require the presence of specific O-glycans within the mucin-like region of the protein.

Not surprisingly, abnormalities in DG are associated with genetic forms of muscular dystrophy. However, rather than mutations in the protein itself, it is the glycosylation of α-DG that is altered in these disorders. In the mouse, complete gene knockouts of DG are embryonic lethal [3], and it is presumed that the human protein is essential for normal embryonic development. DG undergoes extensive, heterogeneous, tissue-specific O-glycosylation. We now know that the addition of essential O-linked glycans required for ligand binding is controlled by a specific set of at least six genes. For a detailed overview of the glycobiology of DG, readers are referred to an excellent review [4].

Earlier biochemical and MS studies identified unusual O-mannosyl structures on DG [4]. One particular sialylated O-linked α-mannose structure, Neu5Acα2–3Gal(β1–4)GlcNAc(β1–2)Man-Ser/Thr has been proposed to be directly involved in laminin binding [5]. More recent MS analysis of α-DG purified from rabbit or human muscle has confirmed the presence of these O-mannosyl glycans and also allowed glycopeptide assignment [6,7].

Key words: central nervous system, dystroglycan, glycosylation, monoclonal antibody, muscular dystrophy.

Abbreviations used: CMD, congenital muscular dystrophy; CNS, central nervous system; DG, dystroglycan; FKRP, fukutin-related protein; LARGE, like glycosyltransferase; MEB, muscle-eye-brain disease; POMGnT1, protein O-linked mannose β1, POMT1, protein O-mannosyltransferase 1; POMT2, protein O-mannosyltransferase 2, WWS, Walker–Warburg syndrome.

1To whom correspondence should be addressed (email jane.hewitt@nottingham.ac.uk).

To date, recessive mutations in six genes [POMT1 (protein O-mannosyltransferase 1), POMT2 (protein O-mannosyltransferase 2), POMGnT1 (protein O-linked mannose β1), Fakutin, FKRP (fukutin-related protein) and LARGE (like glycosyltransferase)] have been shown to cause genetic forms of muscular dystrophy that is a consequence of hypoglycosylation of α-DG [8]. Together, these disorders are known as dystroglycanopathies. The clinical severity of the dystroglycanopathies is very variable: while most present as severe forms of CMD (congenital muscular dystrophy) that are associated with ocular abnormalities and CNS (central nervous system) defects [e.g. WWS (Walker–Warburg syndrome) and MEB (muscle-eye-brain disease)], some gene mutations are associated with milder forms, such as limb girdle muscular dystrophy or CMD with no CNS involvement; however, it has been difficult to draw clear genotype/phenotype correlations. Although a plausible explanation might be that the disease severity is likely to be related to the extent of α-DG hypoglycosylation, molecular analysis of patient cohorts do not always support this hypothesis [9].

It is clear that these six genes are not the only ones that are required for functional glycosylation of α-DG. For example, WWS is genetically extremely heterogeneous: whereas a subset of patients have point mutations in either the POMT1 or POMT2 gene, there are several additional mapped loci for which the gene is currently unknown [10]. Identification of these genes, perhaps through the recent development of whole exome-sequencing strategies [11], will probably provide important insights into the biochemical pathways that act on α-DG glycosylation.

Diagnosis of dystroglycanopathy: reliance on antibody immunoreactivity

Diagnosis of dystroglycanopathy relies on a combination of clinical assessment and immunohistochemistry. The hypoglycosylation of α-DG can be detected as a loss of immunoreactivity on muscle biopsies using either of two commercially available monoclonal antibodies, VIA4; and IIH6 [1]. A polyclonal antibody raised against a hypoglycosylated
form of α-DG identifies a reduced molecular mass form of the protein on Western blots containing skeletal muscle protein from dystroglycanopathy patients [12] and can be useful in diagnosis, although this antibody is not commercially available. For molecular diagnosis, the overlap in the spectrum of phenotypes associated with different genes means that systematic screening for mutations is often required; for example, see [9].

The VIA41 and IIH6 monoclonal antibodies recognize unknown epitopes that are present only on the fully glycosylated form of α-DG. These antibodies can be difficult to use, with significant variation in affinity between different lots of antibody, and there is also a high level of variability in staining seen between patients [13]. The development of better-characterized and more reliable antibodies would aid diagnosis of these disorders.

Key steps in α-DG glycosylation

Strong support for an essential role for O-mannosyl glycans in ligand binding came from the identification of CMD patients with mutations in the POMT1 or POMT2 genes. These genes encode proteins that add a mannose from a dolichol phosphate donor to a serine/threonine residue of a protein [14]. POMT1 and POMT2 act as a co-complex [15] and are responsible for the initiation of O-mannosyl glycan formation [14].

O-mannosylation is rare in mammals and has only been identified in a limited number of glycoproteins (including α-DG) in brain, nerve and skeletal muscle [16]. Mutations in POMT1 were first identified in WWS [17]. Subsequently, mutations in POMT2 were also shown to be causative for WWS [18]. Given that these two proteins act together, such an association was unsurprising. Although most WWS cases have missense mutations or deletions within highly conserved domains [10], it has previously been difficult to define clear genotype/phenotype correlations [20]. However, a recent study compared enzyme activity with DG glycosylation status in patient dermal fibroblasts and concluded that the severity of the clinical phenotype of the patients analysed is inversely correlated with POMT activity [21].

POMGnT1 encodes protein O-linked mannose β1,2 N-acetylglucosaminytransferase 1, which catalyses the transfer of N-acetylglucosamine from UDP-GlcNAc to O-mannosyl glycoproteins within the Golgi [22]. POMGnT1 therefore acts downstream of POMT1/2 in the synthesis of O-mannosyl structures on α-DG. Mutations in this gene were first identified in MEB, which is similar in clinical presentation to WWS, although generally not as severe. Most MEB mutations produce POMGnT1 proteins that are non-functional when assayed in vitro [22]. The high prevalence of mutations in POMT1/2 or POMGnT1 in the dystroglycanopathies is strong evidence for an essential role of O-mannosyl structures for functional α-DG.

Thus far, POMT1, POMT2 and POMGnT1 are the only dystroglycanopathy loci for which biochemical activities of the encoded proteins have been identified. The functions of the three additional genes required for functional glycosylation of α-DG (Fukutin, FKRP and LARGE) have not yet been defined, but they are presumed to act in the same biochemical pathway. Mutations in Fukutin are mainly associated with FCMD (Fukuyama CMD) [23]. This gene encodes a Golgi protein with similarity to phosphoryl-sugar transferases [24]. Although no enzymatic activity has been identified for Fukutin, co-immunoprecipitation experiments suggest there is a physical interaction with POMGnT1, indicating that Fukutin may act as a chaperone [25].

Mutations in a gene encoding a closely related protein (FKRP) are frequent in dystroglycanopathy. Mutations in FKRP are usually associated with forms of CMD without mental retardation and with limb-girdle muscular dystrophy [26,27], although more severe phenotypes have been described [28]. However, despite the high frequency of mutations in FKRP and its clinical importance, the role of this protein is currently little understood.

Interest in my own group has focused on LARGE, which encodes a putative bifunctional glycosyltransferase. A loss-of-function mutation in this gene produces a muscular dystrophy phenotype and CNS defects in the myd (myo-dystrophy) mouse mutant that are associated with hypoglycosylation of α-DG [12,29,30]. However, only a few mutations in LARGE have been reported in human dystroglycanopathy patients, making it difficult to draw conclusions about genotype/phenotype correlations [31–33]. Again, little is known about the biochemical activity of this protein, although it is highly conserved, with orthologues in almost all animal genomes, including sponges and cnidarians [34].

A unique property of LARGE, compared with the other five dystroglycanopathy genes, is that when exogenously expressed in cultured cells the protein induces expression of IIH6 and VIA41 epitopes on DG and these correlate with induction of laminin-binding activity [35–39]. Furthermore, LARGE can induce these properties even in cells carrying loss-of-function mutations in other genes in this pathway such as POMT1 or POMGnT1 [35]. Additionally, studies using Lec mutants of CHO (Chinese hamster ovary) cell lines indicated that neither O-mannose nor high-mannose N-glycans were required as a prerequisite for LARGE activity [39].

This ability of LARGE to induce IIH6 immunoreactivity in these mutant cells might simply reflect promiscuity and an ability to modify (either directly or indirectly) a wide range of glycan acceptors. Alternatively, LARGE may induce a rare, uncharacterized glycan structure on α-DG that is not absolutely dependent on the presence of O-mannosyl glycans. In either case, these experiments indicate that up-regulation of LARGE could be a therapeutic strategy for a wide range of dystroglycanopathies [40].

The relationship between α-DG glycosylation and laminin-binding activity

The genetic data discussed above demonstrate an essential role for O-mannosyl glycans on α-DG in vivo. This is in agreement with biochemical data suggesting a role for
The diagram represents the known partial structure of the α-linked phosphomannose glycan on α-dystroglycan.

The POMT1/2 complex is known to add the mannose to dystroglycan. Genetic evidence indicates that LARGE acts downstream of this structure and is likely, but not proved, to function as a glycosyltransferase. The functions of the other proteins implicated in this pathway are unknown.

**Implications for other diseases**

Changes in α-DG glycosylation may also play a role in cancer progression [45]. In addition, α-DG has been previously identified as a cellular receptor for several arenaviruses including LCMV (lymphocytic choriomeningitis virus) and the human pathogen Lassa fever virus [46]. As for laminin binding, functional glycosylation of α-DG is necessary and virus binding seems to require the same gene pathways and glycan structures as laminin binding [47]. This close relationship between α-DG glycosylation and viral co-receptor activity is likely to be an explanation for the high frequency of some hypomorphic dystroglycanopathy mutations such as an L276I substitution in FKRP in Caucasian populations [27].

**Future perspectives**

Although there have been many recent significant advances in our understanding of this subset of muscular dystrophies, much remains unknown. In particular, the nature of the glycan(s) responsible for ligand binding remains unknown, as does the relationship between LARGE and these structures; however, it seems reasonable to suppose that LARGE plays a significant (perhaps direct) role in the addition of these structures. A better understanding and structural definition of this DG glycan will help in development of diagnostic reagents, such as specific antibodies, and therapeutic strategies.

**Funding**

This work is funded by the Muscular Dystrophy Association (MDA) and the Biotechnology and Biological Sciences Research Council.

---

**References**


18 Reference deleted


Received 17 October 2010
doi: 10.1042/BS0390336

©The Authors Journal compilation ©2011 Biochemical Society