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Congenital disorder of glycosylation due to *DPM1* mutations presenting with dystroglycanopathy-type congenital muscular dystrophy

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ABSTRACT

Congenital disorders of glycosylation (CDG) are rare genetic defects mainly in the post-translational modification of proteins via attachment of carbohydrate chains. We describe an infant with the phenotype of a congenital muscular dystrophy, with borderline microcephaly, hypotonia, camptodactyly, severe motor delay, and elevated creatine kinase. Muscle biopsy showed muscular dystrophy and reduced α-dystroglycan immunostaining with glycoepitope-specific antibodies in a pattern diagnostic of dystroglycanopathy. Carbohydrate deficient transferrin testing showed a pattern pointing to a CDG type I. Sanger sequencing of *DPM1* (dolichol-P-mannose synthase subunit 1) revealed a novel Gly > Val change c.455G > T missense mutation resulting in p.Gly152Val) of unknown pathogenicity and deletion/duplication analysis revealed an intragenic deletion from exons 3 to 7 on the other allele. DPM1 activity in fibroblasts was reduced by 80%, while affinity for the substrate was not depressed, suggesting a decrease in the amount of active enzyme. Transfected cells expressing tagged versions of wild type and the p.Gly152Val mutant displayed reduced binding to DPM3, an essential, non-catalytic subunit of the DPM complex, suggesting a mechanism for pathogenicity. The present case is the first individual described with DPM1-CDG (*CDG-Ie*) to also have clinical and muscle biopsy findings consistent with dystroglycanopathy.

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1. Introduction

Glycosylation is an important process of eukaryotic cells by which carbohydrate chains are attached to proteins and lipids. N-linked glycosylation is a post-translational protein modification that takes place in the endoplasmic reticulum (ER) and Golgi complex, involving a series of steps in which a tree of carbohydrates is assembled, attached covalently to the amide group of asparagines (thus termed N-linked), and trimmed. Congenital disorders of glycosylation (CDG) are a rapidly expanding and heterogeneous group of rare diseases originally defined as defects involving the N-glycosylation process. Now the CDG classification grows to include O-linked (glycan attachment to the hydroxyl group of threonine or serine) and lipid glycosylation defects [1].

Because glycosylation is essential to the function of many proteins, individuals with CDG type I (N-glycosylation process within the ER) typically present with a variety of multi-systemic manifestations, including seizures, psychomotor retardation, microcephaly, cerebellar atrophy, hypotonia, liver disease, coagulation abnormalities, protein-losing enteropathies, and subtle dysmorphic features. For some enzymatic defects, only a few cases have been described. Thus, the phenotypes associated with many of the subtypes have not been fully elucidated. Screening for N-linked glycosylation defects is usually performed by carbohydrate deficient transferrin analysis of serum [2].

O-mannosylation is a subtype of O-linked glycosylation involving the attachment of a mannose glycan to the nascent protein. Defects in the O-mannosylation of α-dystroglycan, an integral receptor for extracellular matrix proteins in neural and muscular tissues, can cause distinct subtypes of congenital muscular dystrophies called dystroglycanopathies (such as Walker–Warburg syndrome, muscle–eye–brain disease, Fukuyama syndrome, and also known

Abbreviations: CDG, congenital disorder of glycosylation; DPM, dolichol-P-mannose synthase; DLO, dolichol-linked oligosaccharides; ER, endoplasmic reticulum; CK, creatine kinase; MRI, magnetic resonance imaging; EEG, electroencephalogram; CGH, comparative genomic hybridization; Dol-P, dolichol-phosphate; GDP-Man, GDP-mannose; Dol-P-Man, dolichol-P-mannose; GPI, glycophosphatidyl inositol; GlcNAc, N-acetylglucosamine.

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as Muscular Dystrophy-Dystroglycanopathy, Type A, MIM 236670) [3,4]. Individuals with severe forms of dystroglycanopathy typically present with signs of muscular dystrophy (hypotonia and contractures) at birth or in infancy, along with brain and eye malformations.

Dolichol-P-Mannose (Dol-P-Man or DPM) synthase is a complex composed of 3 protein subunits (DPM1, DPM2, and DPM3) which catalyses the synthesis of dolichol-P-mannose from GDP-mannose and dolichol-phosphate [5]. Dol-P-mannose is subsequently used as a donor substrate in various glycosylation processes, including N-glycosylation and O-mannosylation, C-mannosylation and GPI anchor assembly [6]. DPM1 is the cytoplasmic catalytic subunit that is anchored to ER membrane by DPM3. DPM2, also an integral ER-membrane protein, acts to stabilize the complex [7] (Fig. 1).

Previously, defects in N-glycosylation and O-mannosylation were considered to cause separate groups of diseases, as described above. However, in 2009, Lefeber et al. reported one case that linked CDG type I with dystroglycanopathies: a female with a homozygous *DPM3* mutation (DPM3-CDG or *CDG-Io*) who has a mild muscular dystrophy, dilated cardiomyopathy, elevated transaminases, and experienced a stroke-like episode [8]. Transferrin isoelectric focusing showed a type 1 pattern consistent and immunohistostaining of the muscle revealed a dystroglycanopathy. Additionally, in 2011, Lefeber et al. identified 4 unrelated families in which children with homozygous *DOLK* mutations presented with dilated cardiomyopathy [9]. *DOLK* encodes dolichol kinase, an ER-resident enzyme that phosphorylates dolichol. A few affected children also had mildly elevated serum transaminases, mild hypotonia, mild developmental delays, and/or ichthyosis. They all had type 1 serum transferrin isoforms and O-mannosylation defects of α-dystroglycan. Most recently, Barone et al. reported the first 3 cases with *DPM2* mutations [10]. They presented with severe and early onset of microcephaly, seizures, and developmental delays, with elevated creatine kinase (CK), and early demise. All three cases had abnormal N-glycosylation serum transferrin, and one individual's muscle biopsy demonstrated an α-dystroglycanopathy.

Here, we describe an individual who initially presented in infancy with signs and symptoms that were mainly suggestive of a congenital muscular dystrophy and eventually developed a CDG phenotype. He was found to be compound heterozygous for a novel *DPM1* missense mutation and an intragenic deletion from exons 3 to 7. This illustrates another case of apparent CDG type I with clinical and muscle biopsy findings consistent with dystroglycanopathy. Notably, previously reported cases with *DPM1* mutations, classified as DPM1-CDG (*CDG-Ie*), have not been evaluated for a dystroglycanopathy [11–14].

2. Patient and methods

2.1. Patient

This full term male infant was noted to have camptodactyly at birth. Prenatal history was unremarkable. At 2 months of age, he was admitted for an episode of non-febrile seizures. Camptodactyly of the 3rd and 4th fingers was noted without other abnormal features. Length, weight, and head circumference were between the 50th and 75th centiles. An MRI of the brain and EEG were normal. He was seen in the genetics clinic at 6 months of age and was noted to have persisting camptodactyly, marked hypotonia (with normal reflexes), motor delay, and decreased head growth (3rd–10th percentile for age). He was cooing, tracking objects, but could not lift his head while prone, and could not roll over or sit up. He had no other episodes of seizures in the interim. There was no prior family history of seizures or neuromuscular disease. A karyotype, array CGH, testing for spinal muscular atrophy, and Prader–Willi syndrome were normal. An initial metabolic work-up (basic chemistry panel, serum transaminases, plasma amino acids, urine organic acids, acylcarnitine profile, very long chain fatty acid profile) was negative. However, CK was elevated (912-1288 U/L).

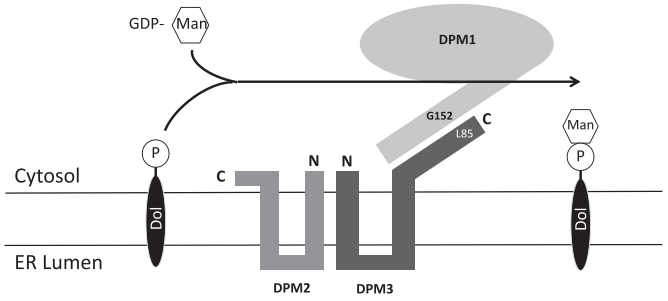

Fig. 1. The DPM complex. Dolichol-P-Mannose (Dol-P-Man or DPM) synthase is a complex composed of 3 protein subunits (DPM1, DPM2, and DPM3) which catalyses the synthesis of dolichol-P-mannose from GDP-mannose and dolichol-phosphate. DPM1 is the cytoplasmic catalytic subunit that is anchored to ER membrane by DPM3. DPM2, also membrane-bound, acts to stabilize the complex. As this and previous study suggest [8], positions G152 on DPM1 and L85 mutation on DPM3 likely reside at the DPM1-DPM3 binding interface.

2.2. Laboratory methods

2.2.1. Carbohydrate deficient transferrin testing

This was performed via liquid chromatography mass spectrometry as previously described by Lacey et al. [15].

2.2.2. Muscle biopsy, histology, and immunohistostaining for α-dystroglycan

A quadriceps muscle was performed at age 9 months and evaluated by standard histological and immunostaining methods. Additional immunohistostaining of the biopsy was performed in cryosections using two different α-dystroglycan glycol-epitope antibodies (IIH6 and VIA4-1) and a β-dystroglycan antibody (7D11). All dystroglycan antibodies were obtained from the Developmental Studies Hybridoma Bank, The University of Iowa.

2.2.3. Sequencing of the DPM1, DPM2, and DPM3 genes

This was performed via Sanger sequencing as previously described [16], with genomic DNA isolated from peripheral blood samples from the proband and parents.

2.2.4. Deletion/duplication analysis of DPM1

This was performed in a commercial laboratory (Emory Genetics Laboratory) via a customized array CGH.

2.2.5. Preparation of membrane fraction from primary dermal fibroblast cell cultures

Cell pellets were resuspended in 10 mM HEPES-OH, pH 7.4, 0.25 M sucrose, 1 mM DTT and disrupted by probe sonication (Kontes, 40% full power, 3 pulses, 15 sec) at 4 °C. The sonicates were centrifuged at 1000 ×*g* and the pellet discarded. The 1000 ×*g* supernatant was centrifuged at 100,000 ×*g*, the pellet was resuspended in homogenization buffer and stored at −20 °C until assayed for Dol-P-Man synthase activity.

2.2.6. Assay for Dol-P-Man synthase activity

Reaction mixtures for the analysis of Dol-P-Man synthase activity contained 50 mM Tris–HCl (pH 8.0), 1 mM 5′-AMP, 0.1% CHAPS, 10 mM $MgCl_2$, membrane fraction prepared from CDG or normal primary epithelial cell cultures (12–15 μg membrane protein), 2.5 to 50 μM Dol-P (dispersed in 1% CHAPS by sonication) and 0.5 to 5 μM GDP-[^{3}H]Man (2475 dpm/pmol) in a total volume of 0.05 ml. Following incubation at 37 °C for 2–10 min, the reactions were stopped by the addition of 2 ml $CHCl_3/CH_3OH$ (2:1). The enzymatic transfer of [^{3}H]mannose into Dol-P-[^{3}H]Man was determined as described previously [17]. The reactions in which GDP-Man concentration was varied contained 40 μM Dol-P. The reactions in which Dol-P concentration was varied contained 5 μM GDP-Man.

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