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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  $\alpha$ -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.455 G>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,

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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.

## Keywords

congenital disorder of glycosylation; dystroglycanopathy; congenital muscular dystrophy; *DPM1*; DPM1-CDG; CDG-Ie; mutation

## 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  $\alpha$ -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 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] (Figure 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  $\alpha$ -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  $\alpha$ -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 3–7. 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 50<sup>th</sup> and 75<sup>th</sup> 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).

### 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  $\alpha$ -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  $\alpha$ -dystroglycan glycol-epitope antibodies (IIH6 and VIA4-1) and a  $\alpha$ -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<sub>2</sub>, 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-[<sup>3</sup>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<sub>3</sub>/CH<sub>3</sub>OH (2:1). The enzymatic transfer of [<sup>3</sup>H]mannose into Dol-P-[<sup>3</sup>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.

**2.2.7. Interaction analysis of mutant DPM1 to DPM3**—293T cells were transfected with plasmids as indicated in Figure 6 and as previously described [5]. Whole cell extracts were prepared 48 hours post-transfection. A portion of the extract was used for affinity purification of GST-DPM3 using GST-Sepharose beads. Binding of FLAG-DPM1 was determined by Western blotting with monoclonal M2-FLAG antibody. To ensure cells were transfected with each FLAG-DPM1 construct, 20 µg of whole cell extract was used for FLAG Western blotting. The same extract was used for both experiments to be sure results were valid. DPM constructs were obtained from Taroh Kinoshita.

### 3. RESULTS

Serum transferrin glycan profile analysis showed a pattern consistent with CDG-I, with elevated mono-oligo/di-oligo ratio (0.334; normal 0.000–0.100) and normal a-oligo/di-oligo ratio. Normal phosphomannose mutase 2 and phosphomannose isomerase activities ruled out the most common types of CDG I, namely PMM2-CDG and MPI-CDG.

Histopathology of the muscle biopsy, performed at age 9 months, was consistent with a muscular dystrophy, showing wide variation in fiber size and several fibers undergoing necrosis or regeneration on haematoxylin and eosin staining (Figure 2A). There were no internalized nuclei, rimmed vacuoles, ragged red fibers, abnormal storage material or inclusions. Staining for dystrophin (c-terminus, mid-rod domain), sarcoglycans ( -, -, -, and -), merosin, and dysferlin were normal.

The reported patient with *DPM3* mutation showed evidence of concurrent N-linked glycosylation and O-mannosylation defects [8]. Therefore, additional immunohistochemical staining of the muscle biopsy was performed using two  $\alpha$ -dystroglycan glycoepitope antibodies (IIIH6 and VIA4-1). This revealed a pattern of reduced  $\alpha$ -dystroglycan immunostaining that

is characteristic of dystroglycanopathies (Figure 2B–G). Staining for  $\alpha$ -dystroglycan was normal.

By Sanger sequencing, no mutation was found in *DPM2* or *DPM3*, but sequencing of *DPM1* revealed a novel, seemingly homozygous c.455 G>T (p.Gly152Val) variant (Figure 3). This amino acid is highly conserved across nearly all organisms (human NP\_003850.1, mouse NP\_034202.1, zebra fish NP\_001003596.1, fruit fly NP\_609980.1, nematode NP\_499931.2, and budding yeast NP\_015509.1).[18] Sequencing in the phenotypically normal parents revealed that the father was a heterozygous carrier for the p.Gly152Val variant and the mother was not a carrier. *DPM1* deletion/duplication testing revealed an intragenic deletion spanning exon 3 to exon 7, with approximate breakpoints at nucleotide positions g.49656245 in intro 2 and g.49556461 in intron 7, approximately 100kb in genomic size, and would result in a 302bp deletion in the transcript. (Figure 4) The intragenic deletion was not previously detected on the initial commercial array CGH due to lack of probe coverage for the *DPM1* gene. Neither of these mutations was present in dbSNP or the NHLBI GO Exome Sequencing Project which consists of exome data from 6500 individuals. The mother has not yet been tested for the intragenic deletion.

*In vitro* enzyme analysis of fibroblast membrane fractions from the affected showed an apparent 80% deficiency in *DPM1* activity compared to wild type.  $V_{max}$  is consistently lower than control whether varying [GDP-Man] or [Dol-P]. The apparent  $K_m$  for GDP-Man is virtually identical to control. The apparent  $K_m$  for Dol-P is slightly lower than in control, implying an increased affinity for Dol-P as a substrate compared to control. Thus, a lower  $V_{max}$  in the presence of normal to decreased  $K_m$  suggest a decrease in the amount of active enzyme (Table 1 and Figure 5). Transfection of the p.Gly152Val variant plasmid into cultured human 293T cells was performed. Unlike the wild type protein, the mutant *DPM1*-G152V was unable to associate with *DPM3* based on Western Blot analysis (Figure 6). This further suggests that the p.Gly152Val variant is pathogenic.

#### 4. PATIENT FOLLOW-UP

At age 11 months, after diagnosis, the individual was still seizure-free and a repeat EEG was normal. An echocardiogram was normal. Coagulation studies showed normal protein C and S, but anti-thrombin III level was low (44–72%, normal 80–120%), and D-dimer (0.81, normal <0.50  $\mu\text{g/mL}$  FEU) was mildly elevated. Coagulation profiles and all clotting factors, including factor XI, were normal. Serum transaminases were normal. At 17 months of age, he spoke no words, could sit up with support but could not bear weight on his legs, and eventually, leg braces were prescribed. He also had feeding problems and frequent respiratory infections. An audiology evaluation was normal. Around 19 months of age, his seizures recurred, and he currently experiences grand-mal and partial complex seizures every few months, despite being on anti-convulsants, zonisamide and levetiracetam. EEG findings suggested a diffuse cerebral dysfunction with focal epileptiform activity. At 32 months of age, a mild increase of serum transaminases occurred and persisted (ALT 136–229 U/L, AST 104–147 U/L). A liver ultrasound was normal. Protein C and S levels became abnormally low. Low anti-thrombin III levels and elevated D-dimers persisted. There have been no thrombotic events or stroke-like episodes. However, a second brain MRI study at age 34 months revealed mild cerebral volume loss, right hippocampal volume loss, delayed or abnormal myelination, and abnormal T2 hyperintensities in the hippocampus, amygdala, temporal lobe, and bilateral periauricular and occipital regions. An ophthalmology evaluation showed anisometry, but no atrophy. Hip X-ray revealed coxa valga deformity bilaterally, consistent with his neuromuscular disease status. Currently, at age 4, he speaks no words, smiles in response, can crawl, can bear weight on legs with braces and support, but cannot walk, and tolerates a soft and liquid diet.

Using the Nijmegen Pediatric CDG Rating Scale [19] retrospectively, clinical disease progressed from a moderate score of 15 at age 17 months to a moderate-severe score of 26 at age 32 months. At age 4, he has a severe score of 28.

## 5. DISCUSSION

We present a child with a combined O-mannosylation and N-glycosylation defect who harbors novel mutations in the *DPM1* gene and decreased Dol-P-Man synthase activity. Of note, the kinetic analysis implies that this individual's Dol-P-Man synthase has a lower apparent  $V_{\max}$ , despite a higher affinity (lower  $K_m$ ) for Dol-P as a substrate than control (Table 1 and Figure 5). This observation can sometimes be attributed to slower release of the product from the enzyme/product complex. Although the reduction in DPM1 activity in the patient is incomplete, reduced cellular Dol-P-Man levels may affect N-glycan biosynthesis by both incomplete mannosylation and by inefficient initiation of intermediates. Dol-P-Man is reported to stimulate UDP-GlcNAc:Dol-P GlcNAc-P-transferase, which catalyzes the formation of GlcNAc-P-P-Dol, initiating DLO intermediate biosynthesis. [20]

Due to high conservation of this position amongst species and further characterization of the variant, p.Gly152Val, *in vitro*, it appears to be pathogenic. Since this variant destroyed the ability of DPM1 to bind to DPM3, the mutation likely resides at the binding interface with DPM3. Previous work showed that the p.Leu85Ser mutation on DPM3 destroys binding to DPM1. [8] Thus, these mutations likely indicate the two complementary binding domains. (Figure 1) Our patient is compound heterozygous for the p.Gly152Val mutation and an intragenic deletion spanning exons 3–7 (Figure 4). The latter is predicted to cause a frame-shift and subsequent premature stop codon. Only the father was found to be a carrier for the p.Gly152Val mutation, and uniparental disomy was ruled out. The presence of the intragenic deletion in the patient caused the false appearance of homozygosity of the missense mutation on Sanger. (Figure 3)

Dolichol-P-mannose synthase has been shown to be involved in 4 different glycosylation processes; N-glycosylation, O-mannosylation, C-mannosylation, and glycosphosphatidyl inositol (GPI) anchor synthesis.[5] Lefeber et al. demonstrated that a defect in DPM3, can manifest with phenotypic features of both N-glycosylation and O-mannosylation. [8] Barone et al. identified 3 cases with *DPM2* mutations with severe neurological phenotype, elevated CPK, and combined N- and O- glycosylation defect. [10] Here we describe an individual, with a novel *DPM1* point mutation and intragenic deletion, exhibiting both features of CDG type I and a congenital muscular dystrophy consistent with  $\alpha$ -dystroglycanopathy. This case further supports the evidence that clinically, defects within any subunits of the DPM complex can cause a combined N-glycosylation and O-mannosylation deficiency. Although we did not assess GPI-anchor synthesis in our patient, deficient CD59 expression has been previously shown in fibroblasts of patients with DPM1-CDG, suggesting perturbation in GPI-anchor synthesis. [8,11]

Interestingly, of the 7 cases reported previously to have *DPM1* mutation[11–14] (classified as DPM1-CDG or *CDG-Ie*), 5 out of 7 also had elevated creatine kinase (Table 2), and a muscle biopsy was reportedly performed on one of these cases. [13] However, there has been no further analysis or characterization on a possible muscular dystrophy phenotype in these cases. In contrast, our case initially presented with a phenotype more consistent with a congenital muscular dystrophy (camptodactyly, hypotonia, elevated CPK, motor delay). Camptodactyly was the first indicator of a possible genetic disease. Subsequently, the patient developed additional features, such as speech delay, elevated serum transaminases, clotting factor deficiencies, and recurrent seizures, which were more consistent with other

described cases of DPM-CDG. Future characterization of the O-mannosylation defect in affected organ systems can expand the phenotypic spectrum of DPM-related disorders.

By applying the Nijmegen Pediatric CDG Rating Scale [19] to our case's clinical picture, we attempt to quantify his disease progression and worsening CDG characteristics from initial diagnosis to his current state. Lefeber *et al.* described a similar but slower disease progression in the DPM3 case, in which muscle weakness was the first presenting symptom at age 11, followed by cardiomyopathy at age 20, and a stroke-like episode at age 21. [8] Only 2 out of the 7 prior reported cases of DPM1 defects had some descriptions of disease progression. Dancourt *et al.* (2006) described a 14 year old male who had severe motor delay in infancy, with slow acquirement of additional symptoms through the years, including seizures at age 4, developmental delay, and ataxic gait and intention tremors in later years. [14] Garcia-Silva *et al.* (2004) presented a child with nystagmus, hypotonia, and seizures during infancy, thrombosis at age 8, and cerebellar signs at age 9. [13] The suspicion of a glycosylation defect will need to remain high in the evaluation of individuals presenting initially with only a few of the typical symptoms.

Finally,  $\alpha$ -dystroglycan is a heavily glycosylated protein that plays an integral part in the dystrophin complex, and its glycosylation is required for receptor function and ligand binding, e.g. laminin, agrin, neurexin, perlecan and pickachurin, within the extracellular matrix. [21] It is glycosylated mainly by three types of sugar linkage, N-glycosylation, O-linked mannosylation, and O-linked N-acetylgalactosaminylation (O-GalNAc). [22] Defects in the synthesis of the laminin binding O-mannosylglycan moiety leads to a group of disorders called dystroglycanopathy. In addition to the CDG-associated genes already discussed, another 11 genes have been implicated in this glycosylation defect (POMGNT1, POMT1, POMT2, LARGE, FKTN, FKR1, ISPD, TMEM5, 3GNT1, 3GALNT2, GTDC2). To date, there has been no dystroglycanopathy linked to defective O-GalNAc glycosylation. Mutations in the known genes are estimated to account for approximately 60% of the cases with dystroglycanopathy. [23] We propose that defects involving the DPM complex, the synthesis of dolichol [9], and/or the utilization of dolichol-P-mannose should also be investigated for muscular dystrophy and dystroglycanopathy.

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## Abbreviations

<b>CDG</b>	congenital disorder of glycosylation
<b>DPM</b>	dolichol-P-mannose synthase
<b>DLO</b>	dolichol-linked oligosaccharides
<b>ER</b>	endoplasmic reticulum

<b>CK</b>	creatine kinase
<b>MRI</b>	magnetic resonance imaging
<b>EEG</b>	electroencephalogram
<b>CGH</b>	comparative genomic hybridization
<b>Dol-P</b>	dolichol-phosphate
<b>GDP-Man</b>	GDP-mannose
<b>Dol-P-Man</b>	dolichol-P-mannose
<b>GPI</b>	glycophosphatidyl inositol
<b>GlcNAc</b>	N-acetylglucosamine

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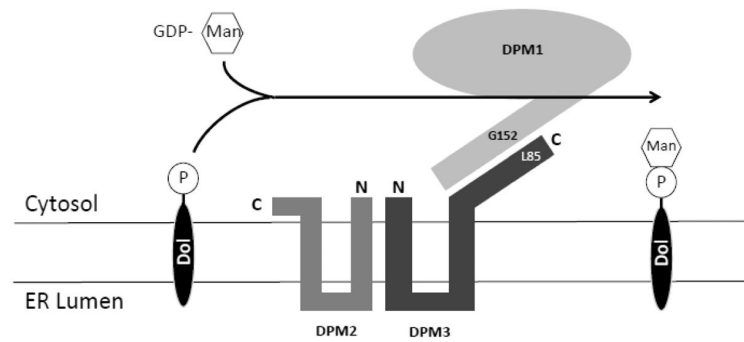


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**Highlights for**

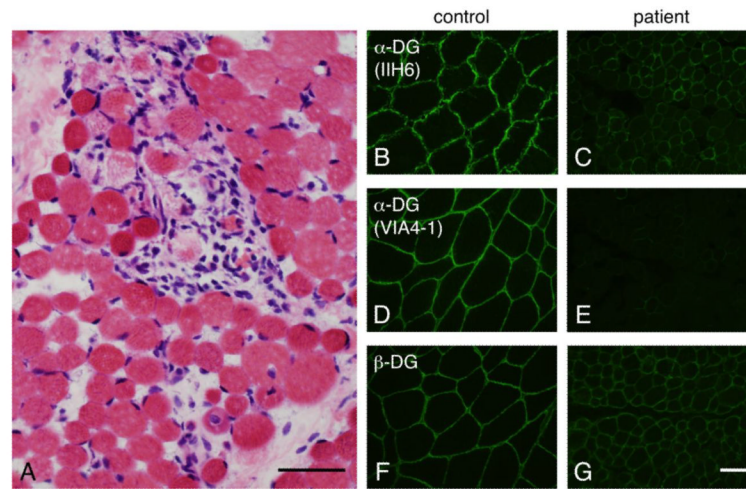
Congenital disorder of glycosylation due to DPM1 mutations presenting with dystroglycanopathy-type congenital muscular dystrophy

- Concomitant finding of dystroglycanopathy and N-glycosylation defect
- Novel missense mutation and an intragenic deletion in *DPM1*
- First description of an O-mannosylation defect in an affected individual with DPM1-CDG



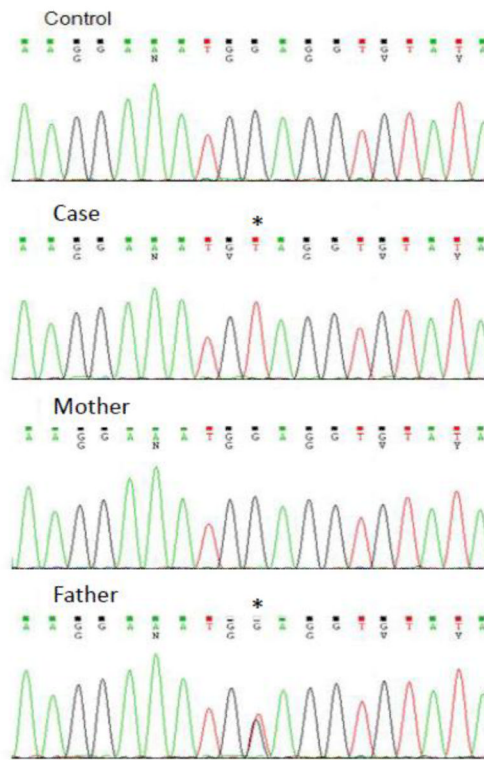
**Figure 1. The DPM complex**

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.



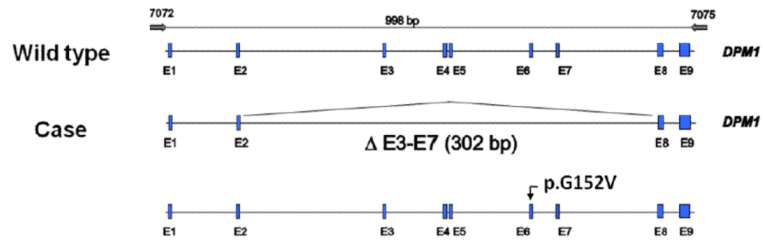
**Figure 2. Analysis of muscle sample**

(A) H & E staining reveals wide variation in fiber size. Several fibers are undergoing necrosis or regeneration. (B,D,F) Control muscle sample. (C,E,G) Muscle sample from the affected. Two different  $\alpha$ -dystroglycan glycoepitope antibodies (IIH6 and VIA4-1) show reduced staining.  $\beta$ -dystroglycan staining is essentially normal. This suggests a defect in O-mannosylation.

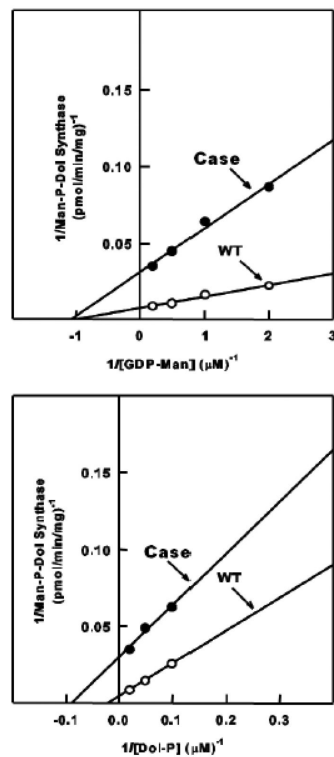


**Figure 3. Sequencing of DPM1**

In the proband, this revealed a homozygous novel Gly>Val change (c.455 G>T, p.G152V) in Exon-6. The father is a carrier for this mutation and the mother is not. This amino acid is highly conserved.

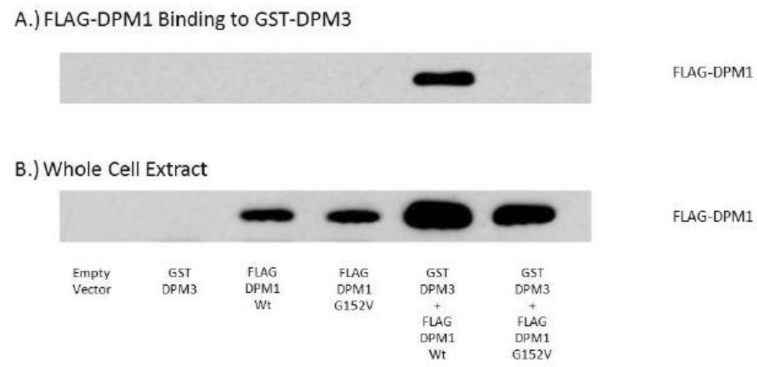


**Figure 4. Intragenic deletion spanning exon 3 to exon 7**  
 Deletion of exons 3 through 7 would cause a 302bp deletion in the mRNA resulting in a frame-shift.



**Figure 5. Dolichol-P-mannose synthase enzyme analysis**

This double reciprocal plot showed an apparent 80% reduction in DPM1 activity compared to control. Y-intercept is  $1/V_{\max}$  and X-intercept is  $-1/K_m$ .  $V_{\max}$  is consistently lower than control whether varying [GDP-Man] or [Dol-P]. The apparent  $K_m$  for GDP-Man is virtually identical in the two cell lines. The apparent  $K_m$  for Dol-P is slightly lower than in control.



**Figure 6. Physical Interactions were present between DPM3 and WT-DPM1, but not the G152V variant**

293T cells were transfected with indicated plasmids as previously described [5]. A.) A portion of whole cell extract was used for affinity purification of GST-DPM3 using GSTSepharese beads. Binding of FLAG-DPM1 was determined by Western blotting with monoclonal M2-FLAG antibody. B.) To ensure cells were transfected with each FLAG-DPM1 construct, 20ug of whole cell extract was used for FLAG Western blotting.



**Table 1**  
**Kinetic analysis of Dol-P-Man synthase from human microsomes**

Kinetic data represent apparent values determined as described in Materials and Methods.

Substrate	GDP-mannose		Dol-P	
	$K_m$ ( $\mu\text{M}$ )	$V_{\text{max}}$ ( $\text{pmol}/\text{min}/\text{mg}$ )	$K_m$ ( $\mu\text{M}$ )	$V_{\text{max}}$ ( $\text{pmol}/\text{min}/\text{mg}$ )
Control	0.95	113.6	55.6	200
Case	0.9	30.8	11.5	32

**Table 2**

Review of reported DPM1-CDG cases.

Age in case report	Genotype	Seizures	Age onset of seizures	Hypotonia	Nystagmus/Blindness/Optic atrophy	Psychomotor delay	Microcephaly	Abnormal brain imaging	Mildly high CK	Abnormal Clotting Factors	Increased serum transaminases
Kim et al, 2000	c.274C>G/c.274C>G (p.R92G)	+	1.5y/	+	+	+	+	Delayed myelination, frontal lobe atrophy	+	+	+
Imbach et al, 2000	c.274C>G/c.331_343del	+	Neonatal	+	+	+	+	Cerebellar atrophy	+	+	-
García-Silva et al, 2004	c.274C>G/c.628del	2/2	5w/6m	2/2	2/2	2/2	2/2	Frontal lobe atrophy	2/2	2/2	2/2
Dancourt et al, 2006	c.742T>C/c.742T>C (p.S248P)	+	Neonatal	+	+	+	+	Delayed myelination	+	+	-
Current case	c.373-5T>A/c.373-5T>A	1/2	1y/-	2/2	2/2	2/2	2/2	Ponto-cerebellar/cerebral atrophy	0/2	2/2	0/2
	c.455G>T (p.G152V)exon3-7 deletion	+	2m	+	-	+	+	+	+	+	+
		<b>7/8</b>		<b>8/8</b>	<b>7/8</b>	<b>8/8</b>	<b>8/8</b>	<b>8/8</b>	<b>6/8</b>	<b>8/8</b>	<b>4/8</b>