Available online at www.sciencedirect.com

ScienceDirect

Neuromuscular Disorders 29 (2019) 497–502

www.elsevier.com/locate/nmd

Case report

Dilated cardiomyopathy and limb-girdle muscular dystrophy-dystroglycanopathy due to novel pathogenic variants in the *DPM3* gene

J. Svahn[a,*], P. Laforêt[b], C. Vial[a], N. Streichenberger[c], N. Romero[d], C. Bouchet-Séraphin[e], A. Bruneel[e], T. Dupré[e], N. Seta[e], R. Menassa[f], L. Michel-Calemard[f], T. Stojkovic[g]

[a] *Electroneuromyography and Neuromuscular Department, Hospices Civils de Lyon, Lyon, France*
[b] *Centre de référence des maladies neuromusculaires Nord/Est/Ile de France, Service de Neurologie, Hôpital Raymond-Poincaré, Garches, AP-HP/ INSERM U1179, END-ICAP, Université Versailles Saint-Quentin-en-Yvelines, Montigny-le-Bretonneux, France*
[c] *Department of Neuropathology, Hospices Civils de Lyon, Lyon, France*
[d] *Myology Institute, Neuromuscular Morphology Unit, Groupe Hospitalier Universitaire La Pitié-Salpêtrière, Sorbonne Universités UPMC Univ Paris 06, Paris, France*
[e] *APHP, Bichat Hospital, Biochemistry, Paris, France*
[f] *Department of Molecular Endocrinology and Rare Diseases, Hospices Civils de Lyon, Lyon, France*
[g] *Myology Institute, Neuromuscular Pathology Reference Center, Groupe Hospitalier Universitaire La Pitié-Salpêtrière, Sorbonne Universités UPMC Univ Paris 06, Paris, France*

Received 6 April 2018; received in revised form 14 April 2019; accepted 7 May 2019

Abstract

Deficiency of Dolichol-P-mannose synthase subunit 3 (DPM3) affects the N-glycosylation and O-mannosylation pathways that are respectively involved in congenital disorders of glycosylation (CDG) and alpha-dystroglycanopathies. Herein, we describe novel pathogenic variants in the *DPM3* gene in two unrelated male patients. They developed dilated cardiomyopathy in their late teens, limb-girdle muscular dystrophy - one patient in childhood and the other in adulthood. In both patients, next generation sequencing found in the *DPM3* gene a heterozygous deletion and a heterozygous pathogenic missense mutation in exon 2 (c.41T>*C*, p.Leu14Pro). Electrophoresis of serum transferrin found an abnormal N-glycosylation profile suggestive of CDG type 1 (decreased tetrasialotransferrin, increased disialo- and asialotransferrin).

Only two cases of *DPM3* gene mutations with limb-girdle muscular dystrophy-dystroglycanopathy have been reported previously. The present study highlights several aspects related to *DPM3* gene mutations such as mild to moderately severe limb-girdle muscular dystrophy, dilated cardiomyopathy, and abnormal N-glycosylation profile suggestive of CDG type 1.
© 2019 Elsevier B.V. All rights reserved.

Keywords: Alpha-dystroglycanopathies; Congenital disorders of glycosylation; Dilated cardiomyopathy; Dolichol-P-mannose (DPM) synthase subunit 3 (DPM3); Limb-girdle muscular dystrophy.

1. Introduction

Abnormalities of the N-glycosylation and O-mannosylation pathways are respectively involved in congenital disorders of glycosylation (CDG) and alpha-dystroglycanopathies [1–4]. Dolichol-P-mannose (DPM) is produced by the DPM synthase and plays an important role, as a mannosyl donor, in four different glycosylation pathways (N-glycosylation, C-mannosylation, glycosyl-phosphatidylinositol anchor assembly, and O-mannosylation) [1]. DPM synthase is composed of three subunits; DPM3 anchors the cytoplasmic catalytic subunit DPM1 to the endoplasmic reticulum membrane, and DPM2 stabilizes the complex [5–7].

As patients presenting with DPM3 mutations are very rare, there is a need to describe the clinical features and genotypes

* Corresponding author.
E-mail address: juliette.svahn@chu-lyon.fr (J. Svahn).

https://doi.org/10.1016/j.nmd.2019.05.004
0960-8966/© 2019 Elsevier B.V. All rights reserved.

encountered in novel cases to expand the knowledge about this disorder. Herein, we describe novel pathogenic variants in the *DPM3* gene previously unreported in the literature in two unrelated male patients presenting limb-girdle muscular dystrophy (LGMD) and dilated cardiomyopathy.

2. Case reports

2.1. Clinical description

2.1.1. Patient 1

A 22-year-old male patient was referred to our clinic for limb-girdle weakness since childhood and dilated cardiomyopathy diagnosed in early adulthood. He was born at term following an uneventful pregnancy to healthy unrelated parents and has a healthy sister. He started walking at 18 months of age but had difficulty participating in sports. He attended high school without developmental delay. At 20 years of age he developed exertional dyspnea. Electrocardiogram showed signs of left ventricular hypertrophy. Echocardiographic examination revealed dilated, hypokinetic left ventricle with a reduced left ventricular ejection fraction (46%). Twenty-four-hour Holter monitoring was unremarkable and respiratory tests were normal.

Neurological examination found Gowers' sign, calf hypertrophy and mild scapular winging. Manual muscle testing (MRC grades) found the following abnormalities: deltoid 4+/5, biceps and triceps 4/5, gluteus medius 4/5, psoas 3+/5, adductors 4/5, hamstrings and quadriceps 5/5 without facial weakness. At 26 years of age both proximal and distal lower limb weakness had worsened and he complained of difficulties in climbing stairs. Serum creatine kinase (CK) was elevated (4054 IU/L, normal <200 IU/L). Nerve conduction studies were normal while electromyography showed myopathic changes in deltoid and quadriceps muscles. Brain imaging and ophthalmological examination were normal.

2.1.2. Patient 2

A 33-year-old male patient was referred to our clinic for persistently increased CK (4700 IU/L) with moderate limb girdle muscle weakness and dilated cardiomyopathy. He was the second child of healthy unrelated parents. His eldest sister died from cardiomyopathy of undetermined cause at the age of 7 years. Like her brother, she presented calf hypertrophy. The patient reported no muscle weakness or cognitive impairment in childhood.

At 18 years of age he was diagnosed with dilated cardiomyopathy, which led rapidly to terminal heart failure with successful heart transplant. Electrocardiogram found left anterior hemiblock and right bundle-branch block. Histological examination of the explanted heart confirmed dilated cardiomyopathy with myocyte hypertrophy.

The patient suffered from left frontal arteriovenous malformation bleeding at 21 years of age, with residual right hemiparesis and partial seizures. Unfortunately, a few years later, he presented a left parietal ischemic stroke with transient hemianopsia.

Neurological evaluation at 33 years of age found right hemiparesis with pyramidal signs, mild scapular winging, and calf hypertrophy. Manual muscle testing (MRC grades) found the following abnormalities: deltoids 4/5, biceps 4/5, psoas 3/5, quadriceps 4/5, hamstrings 4/5, left tibialis anterior 4/5, and right tibialis anterior 2/5. Both proximal and distal limb muscle weakness progressively worsened leading to inability to walk without assistance or to climb stairs; there was no facial or oculomotor weakness. Laboratory investigations showed elevated CK level (4700 IU/L, normal <200 IU/L) with normal coagulation tests and complete blood count. Respiratory investigations were unremarkable. At 48 years of age he developed syncope and needed pacemaker placement with atrial flutter ablation.

2.2. Muscle and cardiac imaging

2.2.1. Patient 1

Cardiac magnetic resonance imaging (MRI) confirmed dilated cardiomyopathy with reduced left ventricular ejection fraction, measured at 39% (normal ejection fraction >50%) (Fig. 1A). Muscle MRI and muscle computed tomography scan showed severe fatty degeneration of adductor, biceps femoris, and semimembranosus muscles, as well as moderate fatty degenerative changes in the right medial gastrocnemius muscle (Fig. 1B–D).

2.2.2. Patient 2

Muscle computed tomography scan showed diffuse fatty degeneration in gluteus maximus, gluteus medius, rectus femoris, adductor, hamstring, and triceps surae muscles (Fig. 1E–H).

2.3. Muscle biopsy: histological and immunohistological results

The deltoid muscle biopsies of both patients found moderate muscle dystrophy with fiber-size variation, multiple internal nuclei, and interstitial fibrosis (Fig. 2A and E). Immunolabeling of αDG (VIA4-1 antibody; Millipore, Billerica, MA) showed reduced alpha-dystroglycan (DG) (αDG) immunostaining in patient 1 and thin and irregular sarcolemmal staining in patient 2 (Fig. 2B, C, F, G). Immunolabeling of Laminin alpha-2 did not find any abnormality in both patients (Fig. 2D). Western blotting, performed only in patient 1, confirmed αDG hypoglycosylation (Fig. 2H).

2.4. Transferrin glycosylation pattern

In both patients, capillary zone electrophoresis of serum transferrin found abnormal N-glycosylation profile suggestive of CGD type 1 with loss of complete N-glycan chains (decreased tetrasialotransferrin, increased disialo- and asialotransferrin compared to control; Fig. 3A–D). Two

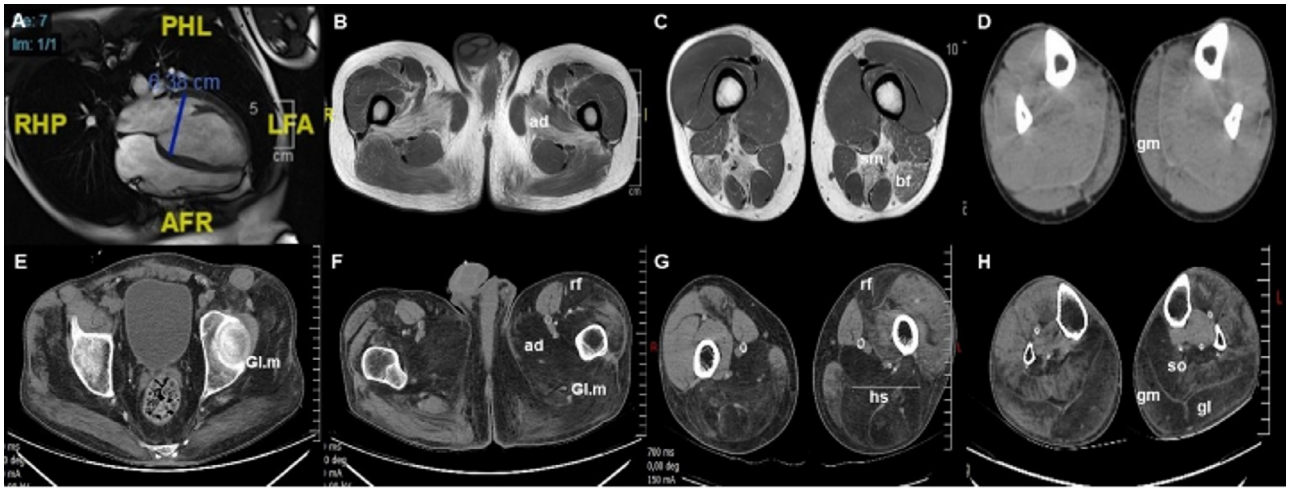

Fig. 1. Muscle and cardiac imaging.
Patient 1: (A) Cardiac MRI highlighting a dilated cardiomyopathy with increased left ventricle end-diastolic diameter (64 mm).
(B, C, D) Axial T1-weighted MRI sections showing severe fatty degeneration of adductor (ad) (B), biceps femoris (bf), semimembranosus (sm) muscles (C), and moderate fatty degenerative changes in right medial gastrocnemius (gm) muscle on axial muscle computed tomography (D).
Patient 2: (E, F, G, H) Axial muscle computed tomography images showing diffuse fatty degeneration in gluteus maximus, gluteus medius (Gl.m), rectus femoris (rf) (E, F), adductor (ad), hamstring (hs; F, G) and triceps surae (gm, gl, so) muscles (H).

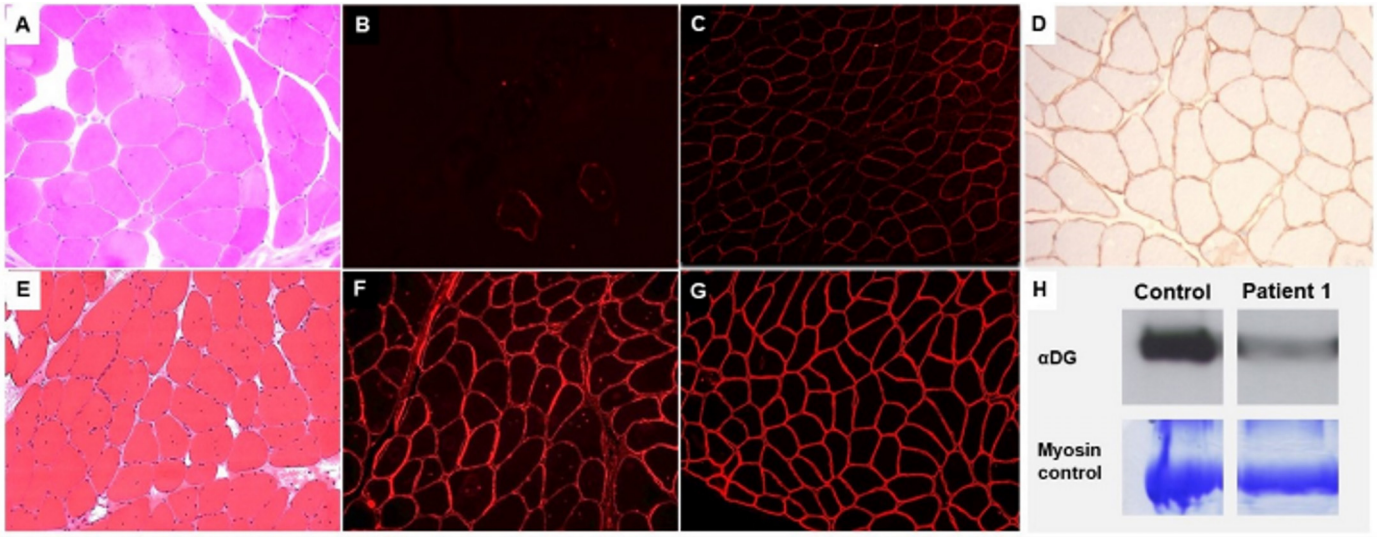

Fig. 2. Histological, immunohistochemical analysis of deltoid muscle biopsy (Patient 1 and 2). Western blot analysis of αDG (Patient 1).
Patient 1. Hematoxylin and eosin stain showing moderate muscle dystrophy with muscle fiber necrosis, central nuclei, increased fibre size variation, mild endomysial and perimysial fibrosis (A). αDG immunolabeling (VIA4-1 antibody; Millipore, Billerica, MA) showing almost complete absence of sarcolemmal staining (B) compared to control (C). Immunolabeling of Laminin alpha-2 did not find any abnormality (D).
Patient 2. Hematoxylin and eosin stain showing moderate muscle dystrophy with fiber-size variation, multiple internal nuclei, and interstitial fibrosis (E). Immunolabelling of αDG (VIA4-1 antibody; Millipore, Billerica, MA) showing defective glycosylation with thin and irregular sarcolemmal staining (F), compared to control (G).
Original magnification: A, $D = X20$; B, C, $E = X10$; F, $G = X16$
Western blot analysis of αDG (H): note the reduced expression of αDG compared to myosin (VIA4-1 antibody, Millipore, 1/400 dilution).

dimensional gel electrophoresis of other serum glycoproteins found similar N-glycosylation defects in patient 1 (patient 2 not available).

2.5. Molecular analysis

Analyses of *FKRP* (Fukutin related protein), *DES* (desmin), *LMNA* (lamin A/C), and *DMD* (dystrophin) genes performed over the last decade did not find any mutation in both patients. In 2016, next generation sequencing of 18 alpha-dystroglycanopathies genes (*B3GALNT2*, *B3GNT1*, *DAG1*, *DOLK*, *DPM1*, *DPM2*, *DPM3*, *FKRP*, *FKTN*, *GMPPB*, *ISPD*, *LARGE*, *POMGNT1*, *POMGNT2*, *POMK*, *POMT1*, *POMT2*, *TMEM5*) was performed using HaloPlex technology. In both patients, it uncovered in the *DPM3* gene (1q22) a heterozygous deletion (Fig. 3E) confirmed

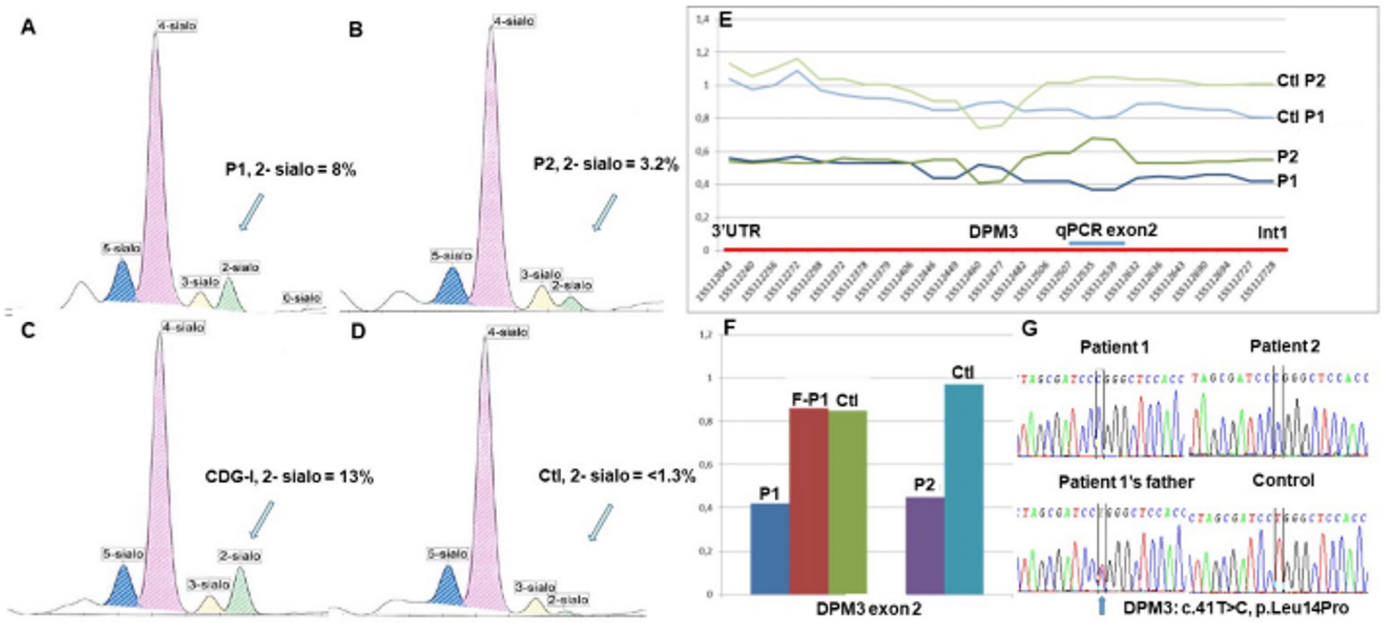

Fig. 3. Capillary Zone Electrophoresis profiles of Serum Transferrin (A, B, C, D) and molecular genetic analysis (E, F, G).
Transferrin profile is normally composed of one major peak (4-sialoglycoform) and three minor peaks (5-sialo-, 3-sialo and 2-sialo-glycoforms). A, *B*= Profiles of Patient 1 (P1) and Patient 2 (P2) showed increased 2-sialotransferrin (8% and 3.2%) as typically encountered in CDG type 1 (C) (13%). The control (serum from a healthy individual) (Ctl) is presented on panel D.
In both patients, next generation sequencing found a heterozygous deletion in the *DPM3* gene (1q22) (E) that was confirmed by Real-time quantitative PCR (qPCR) (F) and a heterozygous missense variant in exon 2 (c.41T>*C*, p.Leu14Pro) that was confirmed by Sanger sequencing (G). Direct DNA sequencing of patient 1′s father (F-P1) identified the same heterozygous missense mutation (G).

by real-time quantitative PCR (qPCR) (Fig. 3F) and a heterozygous missense mutation in exon 2 (c.41T>*C*, p.Leu14Pro) confirmed by Sanger sequencing (Fig. 3G). Real-time quantitative PCR was done using a couple of qPCR primer pairs designed in the *DPM3* exon 2 and intron 1 and a 7500 Fast Real-Time PCR system analysis (Applied Biosystems, Thermofisher). To normalize qPCR results for standard diploid DNA, we amplified *RB1* (Chr 13) and *MYH9* (Chr 22) genes and used genomic DNA samples from a healthy individual as a control. Results of relative quantification were expressed as a normalized ratio comparison between controls and patients (a ratio around 1.0 means that two DPM3 alleles have been amplified, while a ratio of 0.5 corresponds to the amplification of only one DPM3 allele). The breakpoints of the deletion were not exactly defined but the deletion takes away at least the region between the first intron and the 3′UTR *DPM3* gene (Fig. 3E). It is possible that other genes beside *DPM3* were also deleted.

In silico analysis predicted the missense variant to be pathogenic (SIFT, Polyphen, Panthern AlignGVGD, mutation t@ster). Direct DNA sequencing of patient 1′s father identified the same heterozygous missense mutation (Fig. 3G); DNA from patient 1′s mother and patient 2′s parents were not obtained.

3. Discussion

We describe herein novel pathogenic mutations in the *DPM3* gene in 2 unrelated male patients. The patients presented with alpha-dystroglycanopathy associated with dilated cardiomyopathy, and abnormal N-glycosylation of serum proteins. Defective αDG O-mannosylation was observed on muscle biopsy and they presented a phenotype of LGMD without any associated congenital brain or eye malformation. Progressive deterioration of their physical condition during adulthood was observed. As already described, the spectrum of alpha-dystroglycanopathies is extended, and includes in descending order of severity Walker-Warburg syndrome, congenital muscular dystrophy (CMD), LGMD, and isolated exercise intolerance or elevated CK [8]. To the best of our knowledge, only a LGMD phenotype has been described in cases of alpha-dystroglycanopathy associated with *DPM3* mutations [1,9]. Interestingly, the 2 previously reported cases present clinical, biological and morphological features similar to those of the patients presented herein. For instance, the first reported patient presented at 11 years of age mild LGMD, and mild calf pseudohypertrophy. Later, at 20 years of age, she developed dilated cardiomyopathy and at the age of 21 years she had a stroke-like episode without structural brain or eye anomalies on brain MRI [1]. The second case developed at 42 years of age isolated pelvic girdle muscle weakness. Her muscle imaging revealed severe fatty replacement in the adductor, hamstring, and rectus femoris muscles that was less marked in the medial gastrocnemius muscle [9]. However, these female patients had, respectively, a homozygous missense mutation (c. 254T>*C*, p.Leu85Ser) [1] and a homozygous c.131T>*G* (p.Leu44Pro) substitution

[9], whereas the patients presented herein both had a heterocompound mutation with an unlimited *DPM3* gene deletion. Surprisingly, these two unrelated male patients had the same novel pathogenic *DPM3* missense mutation (c.41T>*C*, p.Leu14Pro).

Another important point to note is that 3 of these *DPM3* patients had a mildly abnormal N-glycosylation profile on electrophoresis of serum transferrin suggestive of CDG type 1 pattern (DPM3-CDG) with decreased tetrasialotransferrin, increased disialo- and asialotransferrin [1]. CDG are a group of inherited metabolic disorders caused by alterations in protein glycosylation, affecting largely the N-glycosylation pathway. CDG type 1 pattern indicates defects in the endoplasmic reticulum during assembly of the lipid-linked oligosaccharide and transfer to nascent protein chains [10,11]. However, to date patients with *DPM3* mutations have not been reported to display multi-systemic manifestations classically described in CDG such as epilepsy, psychomotor disability, microcephaly, cerebellar atrophy, hypotonia, liver disease, coagulation abnormalities, protein losing enteropathies, and dysmorphic features [10,11]. To better explain this discrepancy, Lefeber et al. investigated the consequences of decreased DPM3 activity on the four DPM-dependent glycosylation pathways [1]. They observed marked impairment of O-mannosylation, mild N-glycosylation defects and normal C-mannosylation and glycosyl-phosphatidylinositol anchor assembly. The reasons underlying the effect of decreased DPM3 activity on O-mannosylation, and N-glycosylation but not on the other glycosylation pathways is yet to be elucidated [1]. This is particularly interesting since mutations in genes coding for the other subunits, *DPM2* and *DPM1*, have been associated with more severe phenotypes with multi-systemic features. For instance, *DPM1* mutations have been reported in 8 cases presenting with early onset encephalopathy, seizures, microcephaly, dysmorphic features, developmental delay, optic atrophy, and cerebellar dysfunction [12–16]. In 6 of these patients, CK levels were elevated with evidence for αDG-deficient CMD in one patient [12]. Barone et al. described 3 children from 2 families with *DPM2* mutations, profound developmental delay, intractable seizures, microcephaly, and early fatal outcome [7]. These *DPM2* patients had αDG-deficient CMD. The more severe clinical outcome and the multi-systemic features observed in these cases of CDG due to *DPM1* and *DPM2* mutations could be related to N-glycosylation and glycosyl-phosphatidylinositol synthesis defects.

As observed for the first *DPM3* patient reported by Lefeber et al. and those reported herein, dilated cardiomyopathy could be one of the manifesting features of this disease [1]. Dilated cardiomyopathy is a frequent feature in alpha-dystroglycanopathies due to *FKRP* gene mutations but this was ruled out early in the diagnostic process. Moreover, in alpha-dystroglycanopathies associated with *FKRP* mutations and dilated cardiomyopathies, no abnormal profile of serum transferrin glycosylation profile is expected. Mutations of *DOLK*, the dolichol kinase responsible for the formation of Dolichol-P, were also ruled out because this may cause similarly autosomal recessive forms of dilated cardiomyopathy with defective αDG O-mannosylation and deficient protein N-glycosylation [17].

In conclusion, the present observations highlight several aspects related to *DPM3* mutations in adult patients such as mild to moderately severe LGMD predominating in the pelvic girdle, early dilated cardiomyopathy, and abnormal N-glycosylation of serum proteins. These observations confirm the importance of investigating abnormal N-glycosylation of transferrin and DPM synthase defect in unsolved patients with alpha-dystroglycanopathies.

Acknowledgment

We thank Dr Philip Robinson (DRCI, Hospices Civils de Lyon, France) for help in manuscript preparation.

References

[1] Lefeber DJ, Schönberger J, Morava E, Guillard M, Huyben KM, Verrijp K, et al. Deficiency of Dol-P-Man synthase subunit DPM3 bridges the congenital disorders of glycosylation with the dystroglycanopathies. Am J Hum Genet 2009;85:76–86.

[2] Muntoni F, Torelli S, Wells DJ, Brown SC. Muscular dystrophies due to glycosylation defects: diagnosis and therapeutic strategies. Curr Opin Neurol 2011;24:437–42.

[3] Godfrey C, Foley AR, Clement E, Muntoni F. Dystroglycanopathies: coming into focus. Curr Opin Genet Dev 2011;21:278–85.

[4] Bouchet-Séraphin C, Vuillaumier-Barrot S, Seta N. Dystroglycanopathies: about numerous genes involved in glycosylation of one single glycoprotein. J Neuromuscul Dis 2015;2:27–38.

[5] Maeda Y, Tanaka S, Hino J, Kangawa K, Kinoshita T. Human dolichol-phosphate-mannose synthase consists of three subunits, DPM1, DPM2 and DPM3. EMBO J 2000;19:2475–82.

[6] Ashida H, Maeda Y, Kinoshita T. DPM1, the catalytic subunit of dolichol-phosphate mannose synthase, is tethered to and stabilized on the endoplasmic reticulum membrane by DPM3. J Biol Chem 2006;281:896–904.

[7] Barone R, Aiello C, Race V, Morava E, Foulquier F, Riemersma M, et al. DPM2-CDG: a muscular dystrophy-dystroglycanopathy syndrome with severe epilepsy. Ann Neurol 2012;72:550–8.

[8] Sparks SE, Quijano-Roy S, Harper A, et al. Congenital muscular dystrophy overview 2001 Jan 22 [Updated 2012 Aug 23] GeneReviews® [Internet]. Adam MP, Ardinger HH, Pagon RA, et al., editors, SeattleWA: University of Washington, Seattle; 2018. 1993-Available from: https://www.ncbi.nlm.nih.gov/books/NBK1291/.

[9] Van den Bergh PYK, Sznajer Y, Van Parys V, van Tol W, Wevers RA, Lefeber DJ, et al. A homozygous DPM3 mutation in a patient with alpha-dystroglycan-related limb girdle muscular dystrophy. Neuromuscul Disord 2017;27:1043–6.

[10] Péanne R, de Lonlay P, Foulquier F, Kornak U, Lefeber DJ, Morava E, et al. Congenital disorders of glycosylation (CDG): quo vadis? Eur J Med Genet 2017. doi:10.1016/j.ejmg.2017.10.012.

[11] Schiff M, Roda C, Monin M-L, Arion A, Barth M, Bednarek N, et al. Clinical, laboratory and molecular findings and long-term follow-up data in 96 French patients with PMM2-CDG (phosphomannomutase 2-congenital disorder of glycosylation) and review of the literature. J Med Genet 2017;54:843–51.

[12] Yang AC, Ng BG, Moore SA, Rush J, Waechter CJ, Raymond KM, et al. Congenital disorder of glycosylation due to DPM1 mutations presenting with dystroglycanopathy-type congenital muscular dystrophy. Mol Genet Metab 2013;110:345–51.

[13] Imbach T, Schenk B, Schollen E, Burda P, Stutz A, Grunewald S, et al. Deficiency of dolichol-phosphate-mannose synthase-1 causes congenital disorder of glycosylation type Ie. J Clin Invest 2000;105:233–9.

[14] Kim S, Westphal V, Srikrishna G, Mehta DP, Peterson S, Filiano J, et al. Dolichol phosphate mannose synthase (DPM1) mutations define congenital disorder of glycosylation Ie (CDG-Ie). J Clin Invest 2000;105:191–8.

[15] García-Silva MT, Matthijs G, Schollen E, Cabrera JC, Sanchez del Pozo J, Martí Herreros M, et al. Congenital disorder of glycosylation (CDG) type Ie. A new patient. J Inherit Metab Dis. 2004;27:591–600.

[16] Dancourt J, Vuillaumier-Barrot S, de Baulny HO, Sfaello I, Barnier A, le Bizec C, et al. A new intronic mutation in the DPM1 gene is associated with a milder form of CDG Ie in two French siblings. Pediatr Res 2006;59:835–9.

[17] Lefeber DJ, de Brouwer APM, Morava E, Riemersma M, Schuurs-Hoeijmakers JHM, Absmanner B, et al. Autosomal recessive dilated cardiomyopathy due to DOLK mutations results from abnormal dystroglycan O-mannosylation. PLoS Genet 2011;7:e1002427.