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Case report

A homozygous *DPM3* mutation in a patient with alpha-dystroglycan-related limb girdle muscular dystrophy

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Abstract

Defects of O-linked glycosylation of alpha-dystroglycan cause a wide spectrum of muscular dystrophies ranging from severe congenital muscular dystrophy associated with abnormal brain and eye development to mild limb girdle muscular dystrophy. We report a female patient who developed isolated pelvic girdle muscle weakness and wasting, which became symptomatic at age 42. Exome sequencing uncovered a homozygous c.131T > G (p.Leu44Pro) substitution in *DPM3*, encoding dolichol-P-mannose (DPM) synthase subunit 3, leading to a 50% reduction of enzymatic activity. Decreased availability of DPM as an essential donor substrate for protein O-mannosyltransferase (POMT) 1 and 2 explains defective skeletal muscle alpha-dystroglycan O-glycosylation. Our findings show that *DPM3* mutations may lead to an isolated and mild limb girdle muscular dystrophy phenotype without cardiomyopathy.

Keywords: Limb girdle muscular dystrophy; Alpha-dystroglycan; Dolichol-P-mannose synthase; *DPM3*

1. Introduction

Limb girdle muscular dystrophies (LGMD) represent an increasingly large and heterogeneous group of autosomal dominant and recessive disorders. In many patients, the molecular origin remains unknown and next generation sequencing has become a very important tool to hasten the genetic diagnosis and to identify variants and mutations in genes not previously associated with LGMD. Here, we report an adult female patient with autosomal recessive LGMD (LGMD2), in whom exome sequencing by inclusion in the MYO-SEQ project (Newcastle upon-Tyne, UK) revealed a homozygous substitution in *DPM3*, encoding dolichol-P-mannose (DPM) synthase subunit 3.

2. Case report

The patient's medical history was uneventful. Early motor developmental milestones normally acquired and the patient started walking at the age of 1 year. At age 30 years, the patient presented with right-sided painful brachial plexopathy. Otherwise, the clinical neurological examination was normal. The neurological work-up was indicative of an inflammatory origin and the patient was diagnosed with neuralgic amyotrophy. She recovered within a few weeks. Surprisingly, serum creatine kinase (CK) activity was elevated at 4310 IU/L (N < 200). A deltoid muscle biopsy only showed mild nonspecific myopathic changes. At age 42, the patient had difficulty rising from a chair and developed an unsteady gait with tendency to fall. Because of persistingly high CK levels (2732 IU/L), a quadriceps muscle biopsy was performed, which showed a dystrophic pattern and alpha-dystroglycan (aDG) deficiency as demonstrated by immunoblotting with a IIH6C4 antibody at 1:500 dilution (Millipore SA, Overijse, Belgium)

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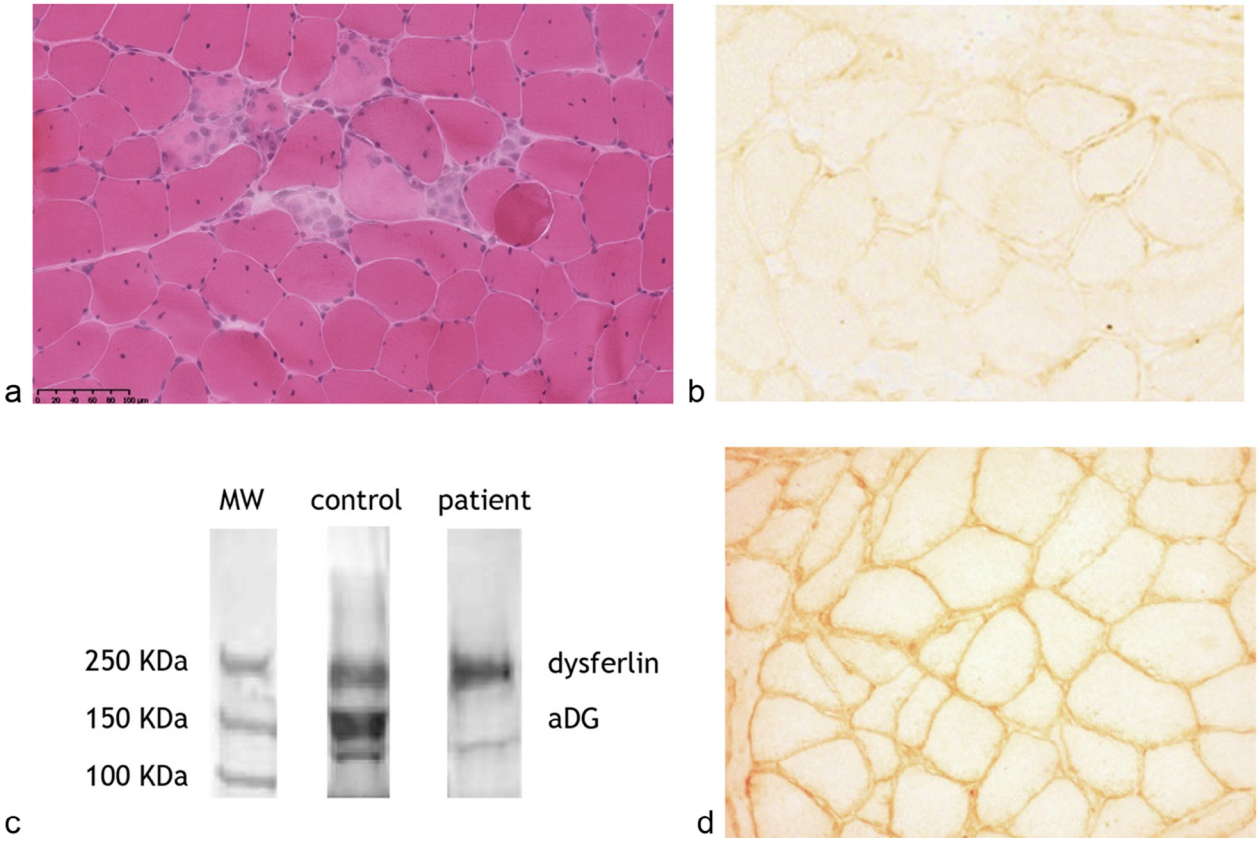

Fig. 1. Left quadriceps muscle biopsy. (A) HE showing muscle fibre necrosis, central nuclei, increased fibre size variability. aDG immunohistochemistry using IIH6C4 antibody (Millipore SA, Overijse, Belgium, DAB) showing almost complete absence of sarcolemmal staining (B) but not in a control muscle biopsy (D). (C) Western blot confirming absence of aDG (IIH6C4 antibody, 1/500 dilution).

(Fig. 1). This led to the diagnosis of aDG-related LGMD. No mutations in *FKRP* were found. At age 57, proximal lower limb weakness had clearly progressed. The patient could not get up from a chair without using her hands and had difficulty going upstairs. Manual muscle testing (MRC grades) showed the following abnormalities: gluteus maximus 0/5; iliopsoas and quadriceps 3/5; gluteus medius and adductors 2/5; hamstrings 4/5, tibialis anterior, tibialis posterior, peroneus longus, and triceps surae 3/5. Gowers sign was positive. Deep tendon reflexes, cranial nerves and sensation were normal. Brain MRI, respiratory, cardiac and ophthalmologic work-up were unremarkable. Nerve conduction studies were normal. EMG showed brief small amplitude polyphasic motor unit action potentials with early recruitment in the iliopsoas muscle. Muscle MRI results are shown in Fig. 2. There is no family history of neuromuscular disease. The mother died at age 94 of pancreas carcinoma. The father is healthy at age 95. One brother died at age 58 of intestinal cancer, one sister at age 39 of a brain tumour. Two other sisters and 2 sons are healthy. Serum CK levels were normal in the father and the 2 sisters.

3. Molecular analysis

Exome sequencing by inclusion into MYO-SEQ (Newcastle University, Newcastle upon-Tyne, UK) was performed at the Broad Institute's Genomics Platform, using Illumina exome capture. A homozygous c.131T > G (p.Leu44Pro) substitution was identified in *DPM3* (gene coverage 95%). *DPM3* encodes DPM synthase subunit 3. This change is extremely rare in the control population (MAF = 0.00084%) and only found in the heterozygous state. Leu44 is an evolutionary highly conserved amino acid and a change to proline is predicted to be pathogenic by *in silico* tools (Mutation Taster, PolyPhen, UMD-Predictor and SIFT). Segregation studies identified heterozygosity in the father and in one of the two sisters. Maternal DNA was not available. Transferrin isoelectric focusing as well as mass spectroscopy of transferrin N-glycans in serum [1] were normal, indicating that N-glycosylation was well preserved in liver and serum. DPM synthase activity was analysed according to Barone et al [2] by measuring the formation of radio-active DPM in cultured fibroblasts and was reduced by 50% as compared to a healthy control.

4. Discussion

We report a patient with mild LGMD2 and without central nervous system involvement, caused by a homozygous substitution in *DPM3*. This gene encodes DPM synthase subunit 3 and we found that the enzymatic activity of DPM synthase was reduced by 50%. DMP synthase is an enzyme complex composed of 3 protein subunits, DPM1, DPM2, and DPM3. Whereas DPM2 stabilises the complex, DPM3 tethers

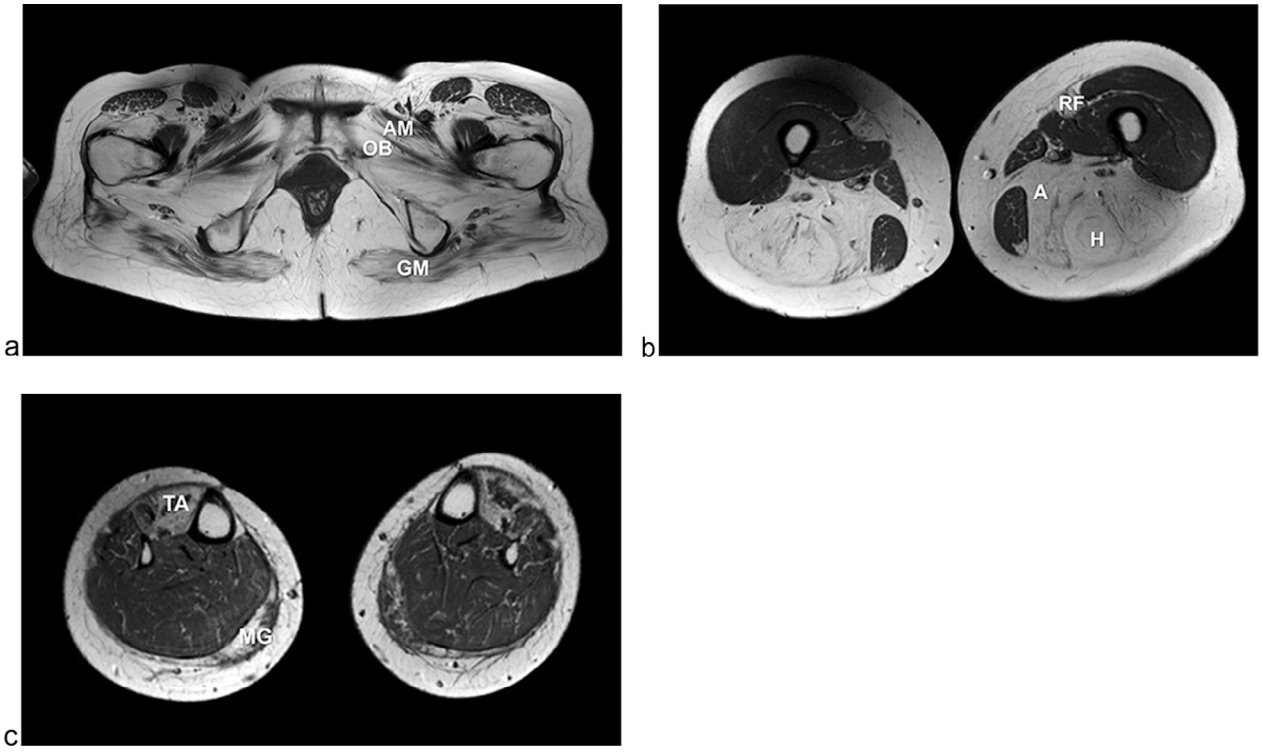

Fig. 2. T1 axial MRI sections showing increased signal intensity in (A) the gluteus maximus (GM), adductor magnus (AM) and obturatorius externus (OB) muscles, (B) in the hamstring (H), adductor (A) and rectus femoris (RF) muscles, and (C) in the tibialis anterior (TA) and medial gastrocnemius (MG) muscles.

the catalytic subunit (DPM1) to the endoplasmic reticulum membrane [3]. DPM synthase catalyses the synthesis of DPM from GDP-mannose and dolichol phosphate. As DPM is an essential donor substrate required in different glycosylation pathways (N-glycosylation, C- and O-mannosylation, GPI-anchor formation) [4,5], it is not surprising that O-mannosylation of aDG, catalysed by protein O-mannosyltransferase (POMT) 1 and 2, is compromised when one of the subunit-encoding genes is mutated. This was first reported in an 11year-old female patient with mild LGMD2 and a 254T > C (p.L85S) mutation in *DPM3* [6]. At age 20, she developed dilated cardiomyopathy and at age 21, she had a stroke-like episode involving the right temporo-parietal region with normal brain MRI. In addition to abnormal N-glycosylation, deficient O-mannosylation of aDG was confirmed in a muscle biopsy and the disorder was classified as DPM3-CDG. Later, Barone et al [2] reported 3 children from 2 families with *DPM2* mutations with profound developmental delay, intractable seizures, microcephaly, and early fatal outcome. The patients had aDG-deficient congenital muscular dystrophy. *DPM1* mutations have been reported in 7 cases with various degrees and combinations of early onset encephalopathy, seizures, microcephaly, dysmorphic features, developmental delay, optic atrophy, and cerebellar dysfunction [7–10]. These were classified as Congenital Disorders of Glycosylation (CDG) type I (DPM1-CDG) due to abnormal N-glycosylation. In 5 of these patients, CK levels were elevated but evidence of muscular dystrophy was not reported. In 2013, Yang et al [11] reported an infant with *DPM1* mutations and showed deficient O-mannosylation of aDG as well, presenting with aDG-deficient congenital muscular dystrophy with seizures but otherwise minimal central nervous system involvement on MRI only. Our findings show that abnormal aDG O-mannosylation related to *DPM3* mutations may lead to LGMD2 phenotype without cardiomyopathy or central nervous system involvement and with presumably normal N-glycosylation.

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