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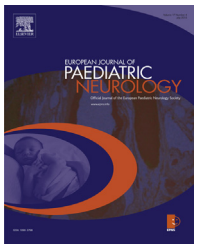

Case study

A fourth case of POMT2-related limb girdle muscle dystrophy with mild reduction of α-dystroglycan glycosylation

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ABSTRACT

Background: *POMT2* mutations have been identified in Walker–Warburg syndrome or muscle–eye–brain-like, but rarely in limb girdle muscular dystrophy (LGMD).
Results: Two *POMT2* mutations, one null and one missense, were found in a patient with LGMD and mild mental impairment, no brain or ocular involvement, minor histopathological features, and slight reduction of α-dystroglycan (α-DG) glycosylation and α-DG laminin binding.
Conclusions: Our case, the fourth LGMD *POMT2*-mutated reported to date, provides further evidence of correlation between level of α-DG glycosylation and phenotype severity.

1. Introduction

Altered glycosylation of α-dystroglycan (α-DG) is associated with congenital muscle disorders characterized by muscle weakness since birth, dystrophic changes at muscle biopsy, and variably severe central nervous system and eye abnormalities.[1] In the last few years the phenotypic manifestations of α-DG defects have emerged as being rather broad, ranging from severe conditions similar to Walker–Warburg syndrome (WWS) or Fukuyama congenital muscular dystrophy (CMD), through classic muscle–eye–brain disease (MEB), to the relatively mild limb girdle muscular dystrophy (LGMD) phenotype.[1] They are also heterogeneous at the genetic level: to date at least 18 causative genes have been identified in these disorders,[2–14] and more are going to be found by the next generation sequencing techniques.

Mutations in the *POMT2* were first identified in patients with WWS and have been found also in patients with MEB-like phenotype or with milder LGMD features.[15–22]

The *POMT2* gene encodes the protein O-mannosyltransferase 2 that forms a complex with O-mannosyltransferase 1 (POMT1) responsible for the catalysis of the first

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step in O-mannosyl glycan synthesis.[23,24] We describe a patient with mild LGMD associated with normal brain MRI, progressive facial involvement and very mild reduction of α-DG glycosylation, in whom we detected two, one null and one missense, *POMT2* mutations.

2. Case report

The proband, now 18 years old, is a female, third child of healthy nonconsanguineous Italian parents. Psychomotor development was referred to have been normal. At the age of 5 years a marked elevation of muscle enzymes was noticed during routine pre surgery blood evaluation: CK = 1600 UI/L (normal value < 185 Ul), AST = 93 (normal value < 31 U/l), ALT = 69 (normal value < 31 U/l). Neurological examination was referred as normal. The patient, when first examined at our Institute at 11 years, showed elongated face, open mouth, sloping shoulders, normal strength and normal deep tendon reflexes. Brain MRI and EEG were normal; EMG revealed myopathic signs; and mild cognitive impairment (IQ = 72) was observed with the revised Wechsler intelligence scale for children (WISC-R 2004). During the following years she developed progressive facial weakness, nasal voice, mild upper limb girdle weakness and wasting, while muscle strength remained normal in the lower limbs. She also complained of recurrent myalgias and cramps in the proximal lower limb muscles. At 16 years ECG was normal, and echocardiogram showed mild mitral and tricuspid valve deficiency; both cardiac features have remained stable ever since.

A muscle biopsy revealed mild perimysial connective tissue increase and fibre diameter variation, but no fibre degeneration or inflammation. Immunohistochemistry of dystrophin, α-, β-, γ-, and δ-sarcoglycan, caveolin 3 and laminin α2; as well as immunoblot of calpain 3 and dysferlin, showed normal expression of these proteins; α-DG was slightly reduced by immunostaining, using VIA4-1 and IIH6C4 antibodies (both from Upstate Biotechnology, Charlottesville, VA, USA) (Fig. 1(A)), and appeared as a smeared band by western blotting using IIH6C4 antibody (Fig. 1(B)). Laminin binding to α-DG was tested using a solid phase assay modified from Michele et al.[25] on protein extracts of patient myoblasts. Briefly, 96-wells microplates were coated with myoblast solubilized protein extracts (previously enriched with WGA – agarose beads; Vector Laboratories, Burlingame, CA, USA), diluted 1:250 in TBS for 48 h at 4 °C. Plates were washed with laminin binding buffer (LBB: 10 mM triethanolamine, 140 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, pH 7.6) and blocked with 3% BSA for 2 h in LBB. EHS laminin (Sigma Aldrich, St. Louis, MO, USA) diluted in LBB, was applied for 2 h. Wells were then washed with LBB, incubated for 30 min with 1:10.000 anti-laminin (Sigma), followed by incubation in alkaline phosphatase-anti-rabbit IgG (Jackson ImmunoResearch Inc, West Grove, PA, USA). Plates were developed with an alkaline phosphatase substrate kit (Bio-Rad Life Science, Hercules, CA, USA) and read on a microplate reader at 405 nm absorbance. Sample reads were expressed as percentage of mean reads obtained in wells that had only laminin coating. The assay was performed in triplicate for each myoblast extract. In the patient (Pt 1 in Fig. 1(C)), the laminin assay showed a binding lower than in controls, including a DMD patient, but higher that in another *POMT2*-mutated patient with MEB-like phenotype (Pt 2 in Fig. 1(C)).

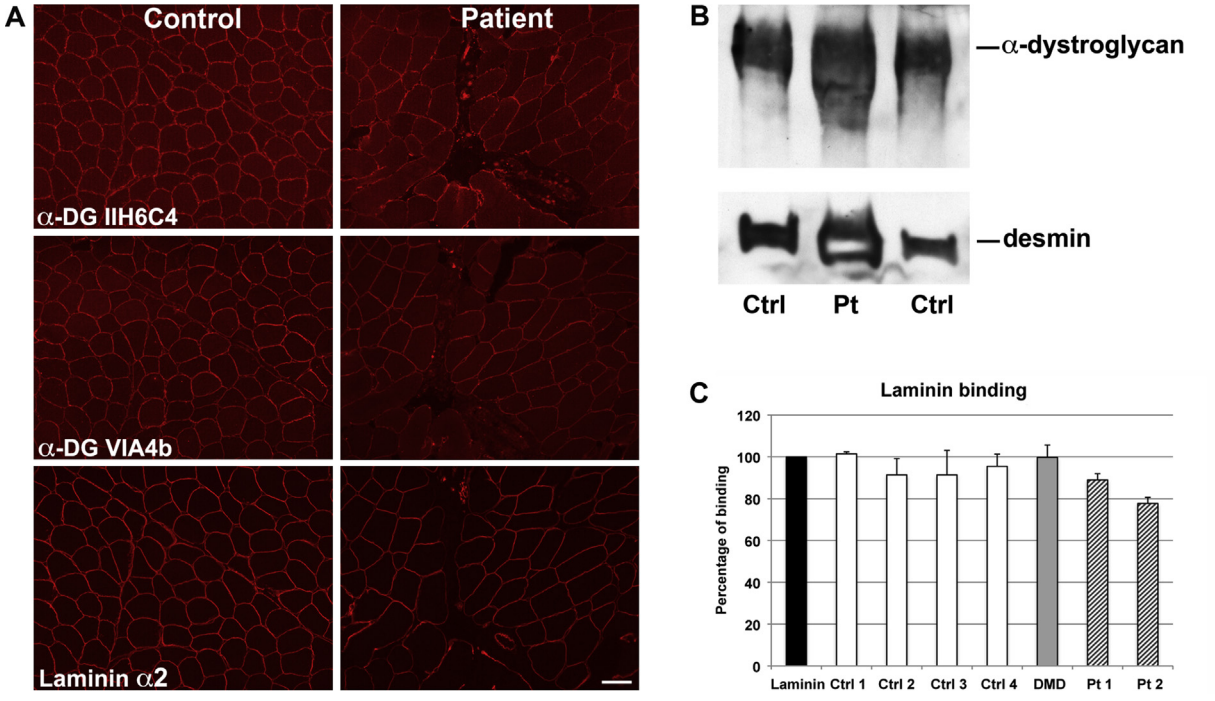

Fig. 1 – (A) Immunohistochemistry showing mild reduction of α-DG and normal expression of laminin α2 chain (scale bar = 50 μm); (B) Western blot showing a smeared band in the patient; (C) laminin binding assay showing mild reduction of binding in the patient (Pt1) compared to controls and to another *POMT2*-mutated patient (Pt2).