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Case report

Uniparental disomy unveils a novel recessive mutation in *POMT2*

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Abstract

Mutations in *POMT2* are most commonly associated with Walker–Warburg syndrome and Muscle-Eye-Brain disease, but can also cause limb girdle muscular dystrophy (LGMD2N). We report a case of LGMD due to a novel mutation in *POMT2* unmasked by uniparental isodisomy. The patient experienced proximal muscle weakness from three years of age with minimal progression. She developed progressive contractures and underwent unilateral Achilles tenotomy. By age 11, she had borderline low left ventricular ejection fraction and mild restrictive lung disease. Muscle biopsy showed mild dystrophic changes with selective reduction in α-dystroglycan immunostaining. Sequencing of *POMT2* showed a novel homozygous c.1502A>C variant that was predicted to be probably pathogenic. Fibroblast complementation studies showed lack of functional glycosylation rescued by wild-type *POMT2* expression. Chromosomal microarray showed a single 15 Mb copy number neutral loss of heterozygosity on chromosome 14 encompassing *POMT2*. RNAseq verified homozygosity at this locus. Together, our findings indicate maternal uniparental isodisomy causing LGMD2N.

Keywords: *POMT2*; LGMD; α-dystroglycan; Dystroglycanopathy; Uniparental disomy.

1. Introduction

The dystroglycanopathies are a group of autosomal recessive muscular dystrophies characterized by absent or reduced functional glycosylation of α-dystroglycan due to mutations in one of 18 known causative genes [1]. Alpha-dystroglycan is a major extracellular component of the dystrophin-glycoprotein complex [2] that undergoes post translational O-mannose-linked glycosylation. Protein O-mannosyltransferase 2, encoded by *POMT2*, along with its homolog POMT1 catalyze an early step in the functional glycosylation pathway of α-dystroglycan [3,4]. Mutations in *POMT2* were initially described in patients who had Walker–Warburg Syndrome, a severe form of congenital muscular dystrophy [5]. Subsequently, *POMT2* mutations have been reported in individuals across the entire dystroglycanopathy phenotypic spectrum from Walker–Warburg Syndrome to mild limb girdle muscular dystrophy (LGMD2N) [6].

Uniparental disomy, the inheritance of a single chromosome or chromosomal segment from only one parent, was initially described by Engel in 1980 [7]. In this early work, Engel predicted that isodisomy, the inheritance of two copies

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of the same chromosome from a single parent, could lead to similar outcomes as parental consanguinity. Over time, understanding the mechanisms of uniparental disomy has expanded, and with it, the disease implications [8]. Although not disease causing intrinsically, uniparental disomy has the potential to unmask recessive disorders or lead to abnormal genetic imprinting.

Here, we report a girl with LGMD caused by a novel c.1502A>C mutation in *POMT2*. Homozygosity for this recessive mutation was due to uniparental isodisomy at the *POMT2* locus on chromosome 14.

2. Case report

The patient is a female born to non-consanguineous parents with no family history of muscular dystrophy. Pregnancy was complicated by maternal tobacco use in the first trimester. She was born at 38 weeks with no complications during delivery and met early developmental motor milestones though onset of walking occurred at 18 months. At two years of age, parents became concerned about toe-walking and frequent falls. When evaluated by her primary care physician at age 3, her CK was elevated at 9558 U/L. Initial neurologic exam at 3 1/2 years showed proximal muscle atrophy, generalized hypotonia, weakness of shoulder girdle with slip through when held suspended, bilateral winging of the scapulae, and proximal muscle weakness greater than distal. Reflexes were reduced throughout. There was mild bilateral tightness of the Achilles tendons though passive dorsiflexion to neutral was achieved. She was noted to toe-walk, and gait was waddling. Gowers maneuver was present. Follow-up examinations revealed stable to minimally progressive proximal weakness and evidence of mild intellectual disability requiring special education in reading and mathematics. She developed progressive contractures involving the neck, elbows, knees, and most prominently in the bilateral Achilles tendons. At age 5, she underwent bilateral serial casting with restoration of ankle dorsiflexion to 10 degrees past neutral. Due to continued progression of the contractures and increasing difficulty in walking, she underwent left sided Achilles tenotomy at age 10. She did not regain ambulation after surgery. At the age of 13, the patient remains non-ambulatory. She has weakness of proximal

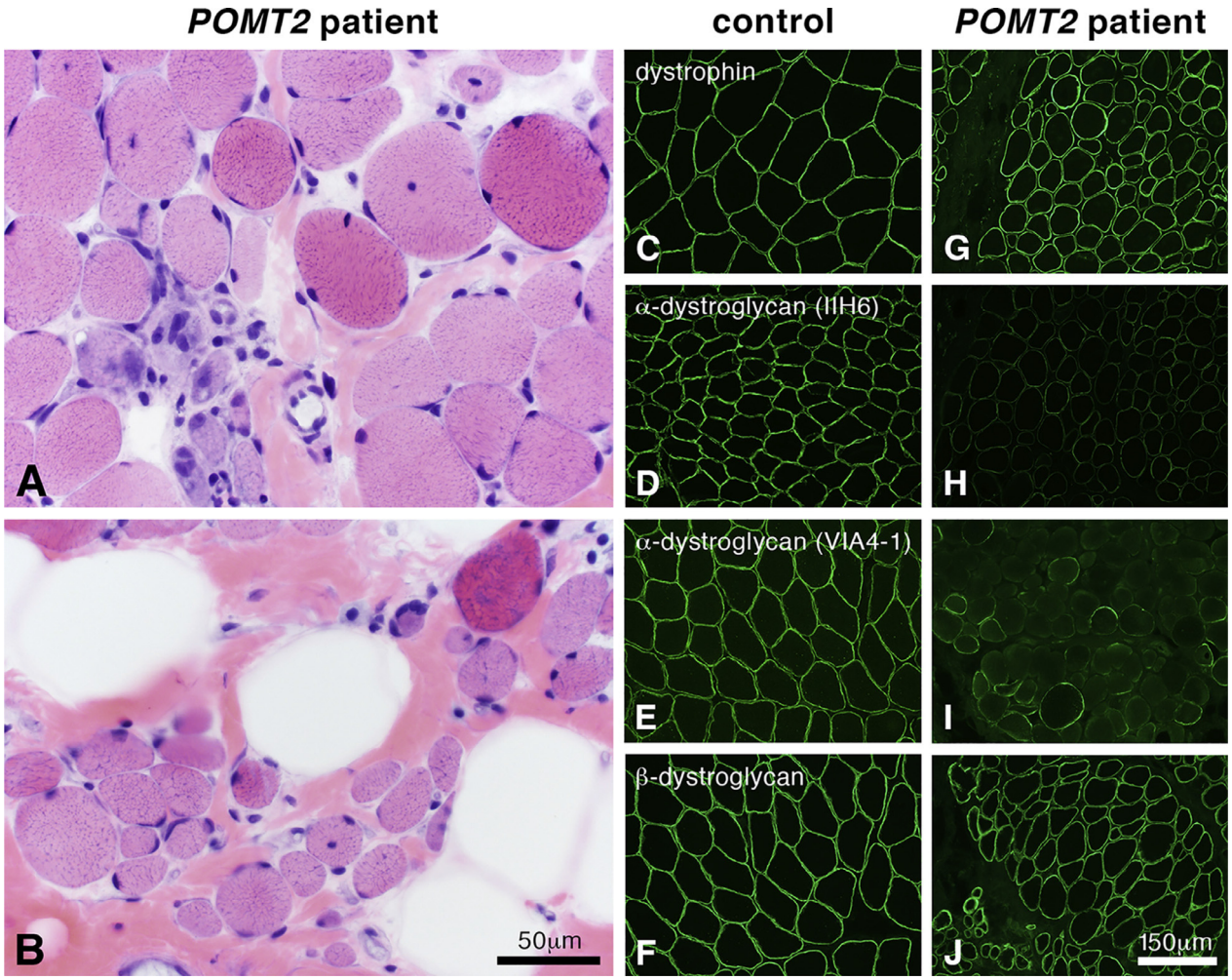

Fig. 1. Muscle biopsy. The H&E photomicrographs (A and B) are representative of the dystrophic pathology found in the *POMT2* patient. Immunofluorescence images from a non-dystrophic control patient (C–F) and the *POMT2* patient (G–J) illustrate reduced staining for functionally glycosylated α-dystroglycan, while dystrophin and β-dystroglycan appear normal.

muscles most severe in the bilateral hip flexors, absent deep tendon reflexes, and progressive contractures.

Throughout follow up, cardiopulmonary function was screened using echocardiogram, electrocardiogram, and pulmonary function tests. Initial electrocardiogram and echocardiogram at age 5 showed normal sinus rhythm, normal cardiac anatomy, and normal ejection fraction. Initial pulmonary function testing at age 9 revealed normal pulmonary function. No abnormal electrical conduction patterns were noted on follow-up electrocardiogram. By age 11, echocardiogram showed borderline low ejection fraction at 51 percent and pulmonary function tests showed mild restrictive lung disease with forced vital capacity at 68 percent predicted. She was treated with enalapril for management of cardiomyopathy.

A vastus lateralis muscle biopsy was obtained at age 10 while anesthetized for the tenotomy procedure. Frozen sections from the biopsy were evaluated by routine hematoxylin and eosin (H&E), immunostaining for slow and fast myosin, and enzyme histochemical stains, as well as by a panel of immunofluorescence stains as reported previously [9]. The antibodies used for the immunostaining reported here include anti-dystrophin (ab15277; Abcam), anti-α-dystroglycan [IIH6 and VIA4-1; Developmental Studies Hybridoma Bank (DSHB)], and anti-β-dystroglycan (7D11; DSHB). The biopsy showed wide variation in fiber size attributable to scattered atrophic and hypertrophic fibers, type I fiber predominance, scattered or groups of fibers undergoing necrosis or regeneration, increased internally placed nuclei, mild to absent endomysial fibrosis, and focal, mild fatty replacement (Fig. 1A and B). No lymphocytic inflammation, inclusion bodies, vacuoles, or ragged-red fibers were identified. Immunostaining for dystrophin and β-dystroglycan showed normal expression of these proteins (Fig. 1G and J). There was selective reduction in staining for functionally glycosylated α-dystroglycan using glycoepitope-specific antibodies IIH6 and VIA4-1 (Fig. 1H and I).

Due to the pattern of muscular weakness, sequencing of several LGMD genes was performed. No pathogenic variants were identified in *FKRP, caveolin 3, alpha/beta/gamma-sarcoglycan, calpain-3,* or *LMNA*. Genetic testing for FSHD and *dystrophin* duplication/deletion and sequencing were normal. Sequencing of *POMT2* identified an apparently homozygous c.1502A>C (p.E501A) variant that has not previously been reported. A large-scale deletion on the homologous chromosome could not be ruled out. Maternal DNA was heterozygous for the same *POMT2* c.1502A>C variant; paternal DNA was not available. The glutamic acid at this position is conserved down to yeast *Saccharomyces cerevisiae* (*Sc*Pmt1p) and is also conserved in *POMT1* (Fig. 2A). Bioinformatic analysis identified this change to have a Blosum62 score of −1 and to be Probably Damaging (PolyPhen) or Not Tolerated (SIFT). In topography mapping of the POMT2 protein, E501 is present within the endoplasmic reticulum (ER) lumen facing loop 5 (Fig. 2B).

To verify homozygous expression of *POMT2*, and to exclude non-coding variants and splice changes, RNA sequencing at a depth of 90 million paired end reads was performed

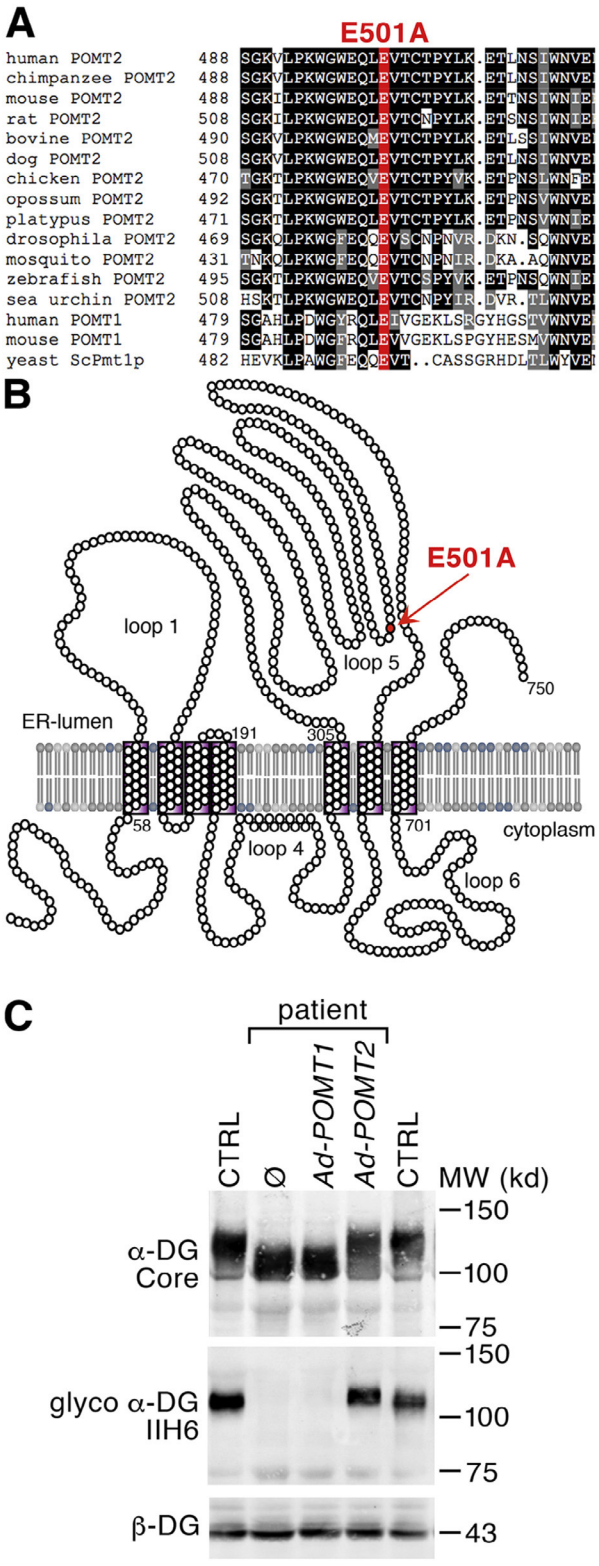

Fig. 2. Evaluation of *POMT2* variant. (A) Multiple amino acid sequence alignment of *POMT2* showing high conservation of the E501 across species including yeast (*Saccharomyces cerevisiae*) *Sc*Pmt1p and conservation in POMT1. (B) A topology map of POMT2 shows that the E501A substitution is located in the ER lumen facing loop 5. (C) Successful complementation of cultured fibroblast α-dystroglycan with adenovirus-*POMT2* (Ad-*POMT2)* mediated gene expression but not adenovirus-*POMT1* (Ad-*POMT1*) is shown by western blotting. The α-dystroglycan core antibody is sheep 174. CTRL = healthy control human fibroblasts.

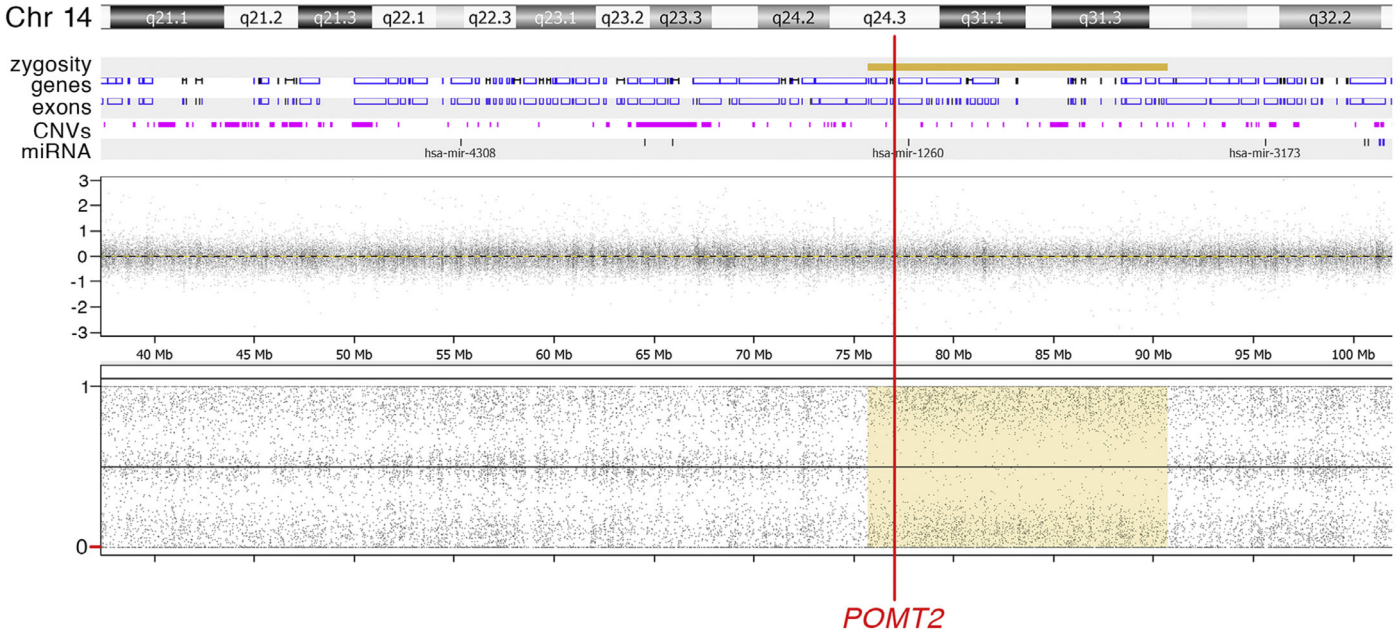

Fig. 3. Chromosomal microarray. Chromosomal microarray results showing copy-neutral loss of heterozygosity encompassing the *POMT2* gene. Top: Chromosome 14 ideogram indicating chromosomal band positions. Middle: Log-ratio showing copy number neutral state. Bottom: B-allele frequency showing loss of heterozygosity (yellow box).

on patient derived fibroblasts. Analysis focused on transcripts from all known muscle disease genes. The c.1502A>C substitution was supported by 154 of 154 reads at the locus. A long run of homozygosity was confirmed for several adjacent transcripts on chr14. No other changes were detected in the *POMT2* transcript, and no changes in expression or processing were noted in any other known muscular dystrophy gene.

To further determine if the E501A substitution was pathogenic, complementation studies were performed on cultured skin fibroblasts [10]. First, the glycosylation status of α-dystroglycan in subject fibroblast cultures was determined by On-cell Westerns to be reduced; it was partially and selectively rescued by adenovirus-*POMT2* mediated gene expression (data not shown). Then western blots using WGA enriched fibroblast cell lysates according to published protocols [10] showed decreased molecular weight of α-dystroglycan and a lack of functional LARGE glycosylation that were both rescued by expression of a wild-type copy of *POMT2*, but not by *POMT1* (Fig. 2C).

Chromosomal microarray was performed to differentiate between a large deletion encompassing *POMT2* and a true homozygous mutation. The array was completed using Affymetrix CytoScan HD h19 (NCBI build 37) whole genome array and scanned using the Affymetrix 2000DX.2 system. The data were extracted and processed using Affymetrix ChAS software (Affymetrix, version 1.2.2) and Nexus Copy Number (BioDiscovery, version 7) software. The patient's chromosomal microarray showed a large region of homozygosity on chromosome 14 (chr14: 75,699,438–90,713,574) approximately 15 Mb in length encompassing the *POMT2* gene (Fig. 3). This region of homozygosity did not extend to the imprinting locus at 14q32.2.

3. Discussion

The majority of reported mutations in *POMT2* are associated with severe congenital muscular dystrophy such as Walker–Warburg Syndrome [6]. Less frequently, *POMT2* mutations lead to milder LGMD type 2N without brain or eye involvement except for mild intellectual disability [6,11–14]. In a recent series of 12 cases of LGMD2N [14], all twelve had cognitive impairment, two had decreased left ventricular ejection fraction, and the eight who were tested had decreased forced vital capacity. The case we describe here displays a phenotype similar to previously reported cases of LGMD2N including mild cognitive impairment, decreased left ventricular ejection fraction, decreased forced vital capacity, and relatively stable muscular weakness. A majority of mutations reported for cases of LGMD2N have been missense mutations, though a few frameshift mutations have been discovered. These mutations have been distributed throughout the coding region of *POMT2*, leading to the hypothesis that there is little mutational resilience within *POMT2* [14]. The homozygous c.1502A>C (p.E501A) mutation lies in a domain essential for POMT enzyme activity [15,16] thus, together with our complementation data, strongly indicates this is a novel pathogenic mutation.

Uniparental disomy has historically been detected through microsatellite testing and methylation studies in trios to detect both uniparental iso- and heterodisomy, the inheritance of both parental homologues. More recently, studies have shown the clinical utility of using array data for detection and

diagnosis of uniparental isodisomy [17–20]. Together these studies show that SNP microarray studies can identify large regions of homozygosity. Uniparental heterodisomy would not be detected by this approach, as there would be no region of homozygosity [19]. In instances where regions of homozygosity are distributed across multiple chromosomes, parental relatedness is likely the causative mechanism. A large region of homozygosity isolated to a single chromosome suggests uniparental isodisomy. Homozygous blocks of ≥13 Mb [19] and 13.5 Mb [18] have been suggested as the threshold that should suggest uniparental disomy and when present, parental testing might not be required to confirm the diagnosis [18,19]. The patient presented here has a 15 Mb region of homozygosity present on chromosome 14 without regions of homozygosity throughout the remainder of her chromosomes. Although paternal DNA was not available for testing, these results argue strongly in favor of uniparental isodisomy unveiling a pathologic recessive mutation in *POMT2*.

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