

A novel missense mutation in *POMT1* modulates the severe congenital muscular dystrophy phenotype associated with *POMT1* nonsense mutations

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Abstract

Mutations in *POMT1* lead to a group of neuromuscular conditions ranging in severity from Walker–Warburg syndrome to limb girdle muscular dystrophy. We report two male siblings, ages 19 and 14, and an unrelated 6-year old female with early onset muscular dystrophy and intellectual disability with minimal structural brain anomalies and no ocular abnormalities. Compound heterozygous mutations in *POMT1* were identified including a previously reported nonsense mutation (c.2167dupG; p.Asp723Glyfs*8) associated with Walker–Warburg syndrome and a novel missense mutation in a highly conserved region of the protein O-mannosyltransferase 1 protein (c.1958C>T; p.Pro653Leu). This novel variant reduces the phenotypic severity compared to patients with homozygous c.2167dupG mutations or compound heterozygous patients with a c.2167dupG mutation and a wide range of other mutant *POMT1* alleles.

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Keywords: *POMT1*; Protein O-mannosylation; Dystroglycanopathy; Walker–Warburg syndrome; Congenital muscular dystrophy; Limb girdle muscular dystrophy

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1. Introduction

Alpha-dystroglycan is part of the dystrophin–glycoprotein complex. This oligomeric complex includes cytoplasmic proteins dystrophin and syntrophin, alpha- and beta-dystroglycan, and several sarcoglycans. The dystrophin–glycoprotein complex links dystrophin at the cell membrane to the extracellular matrix [1]. Alpha-dystroglycan is an extracellular protein that non-covalently binds to the transmembrane protein beta-dystroglycan, while in the extracellular matrix it binds to laminin and other tissue specific extracellular matrix proteins. Laminin binding depends on proper glycosylation of alpha-dystroglycan [2].

Dystroglycanopathies are a group of disorders with the predicted common pathogenic mechanism of abnormal post-translational modification of alpha-dystroglycan [3–5]. Mutations in the genes associated with dystroglycanopathy phenotypes (*POMT1*, *POMT2*, *POMGNT1*, *LARGE*, *FKTN*, *FKRP*, *ISPD*, *GTDC2*, *B3GALNT2*, *B3GNT1*, *TMEM5*, and *SGK196*) are specifically thought to disrupt proper glycosylation of alpha-dystroglycan [6–20], while mutations in genes involved in the synthesis of Dol-P-mannose (*DPM1*, *DPM2*, *DPM3*, *DOLK*, and *GMPPB*) cause congenital disorders of glycosylation and indirectly lead to dystroglycanopathy in some patients by reducing this essential mannose donor for *POMT1/2* [21–25]. The proteins encoded by *POMT1* and *POMT2* carry out the first step of O-mannosylation of alpha-dystroglycan [12]. Multiple glycans are proposed to build on this O-mannose. *SGK196* phosphorylates the 6-position of O-mannose [26] and *LARGE* transfers xylose and glucuronic acid to produce the specific, high affinity glycan receptor for extracellular matrix proteins [27]. The glycosyltransferase functions of *POMGNT1* [20], *GTDC2*, and *B3GALNT2* have also been identified [20,26]. However, the exact mechanism by which proteins encoded by *FKTN*, *FKRP*, *ISPD*, *TMEM5*, and *B3GNT1* disrupt alpha-dystroglycan glycosylation is still unclear.

Dystroglycanopathy gene mutations lead to a range of phenotypes [28,29]. The Walker–Warburg syndrome (WWS) is the most severe phenotype but patients with milder mutations in each of these genes may present with milder forms of congenital muscular dystrophy (CMD) or with limb girdle muscular dystrophy (LGMD) [5]. Refining the genotype-phenotype correlations has proven difficult. We report two male siblings and an unrelated female with early onset muscular dystrophy and intellectual disability with minimal structural brain anomalies and no ocular abnormalities. Compound heterozygous mutations in *POMT1* were identified including a previously reported nonsense mutation (c.2167dupG) associated with WWS [17] and a novel missense mutation in a highly conserved region of the protein O-mannosyltransferase 1 protein (c.1958C>T; p.Pro653Leu). This second variant reduces the severity of the phenotype.

2. Patients

Patient 1: This patient is a 19 year old Caucasian male born to nonconsanguineous parents. Pregnancy was complicated by decreased fetal movements and he was delivered at 41 weeks gestation by vacuum assisted delivery. Gross motor delay was noted when he was not sitting at 9 months. Additional delayed milestones included walking at 42 months, beginning to use single words at 4 years, and toilet training at 5 years. At 7 years, his creatine kinase was elevated at 9162 IU/L. At 10 years, he had dysarthria, dyspraxia, frequent drooling and inability to whistle. His face was narrow with a high arched palate. He had atrophy of the shoulder and hip girdle musculature with winging of the scapulae and poor grip strength. He wrote with difficulty and had trouble eating with a fork, but he was able to fasten snaps and tie his shoes. Strength in the lower extremities was 4/5 without evidence of calf hypertrophy, and he rose from the floor without a Gowers' maneuver. He was independently mobile with moderate ataxia, and he was able to ascend stairs without difficulty, ride a bike and ski. By 13 years, he developed a progressive thoracolumbar scoliosis unresponsive to bracing, increasing intention tremor and decreased endurance with walking. At 14 years, height and head circumference were at the 10th to 25th centile and he had a normal ophthalmology exam. His brain MRI scan showed a mildly hypoplastic cerebellar vermis, no cerebellar cysts, mild ventriculomegaly particularly involving the trigones, mild prominence of the sulci for age, and no definite cortical abnormality (Fig. 1). By 16 years, severe scoliosis and progressive weakness led to restrictive lung disease and difficulty with ambulation requiring a walker. At 19 years, he required BiPAP for moderate restrictive lung disease; he was able to ambulate independently, but at times used a walker. An evaluation with cardiology, including an EKG and echocardiogram, was normal. He has not had seizures and an EEG has not been performed. He was on an individualized education plan that included speech therapy for mild difficulties with enunciation. He recently graduated from high school and entered a transitional program that focuses on life skills training.

Patient 2: The 14 year-old brother of patient 1 was the product of an uncomplicated pregnancy and delivery. He had hypotonia and delay in motor milestones from birth. Independent sitting occurred at 10 months, crawling at 14 months, and at 18 months he was not yet walking independently or using any words. He presented at 18 months with a viral respiratory illness associated with a deterioration of motor function with refusal to crawl or sit. Height and head circumference were at the 3rd to 5th centile and his physical examination showed proximal and distal hypotonia without calf hypertrophy. Creatine kinase was 16,800 IU/L. At 22 months, a right vastus lateralis muscle biopsy revealed mild type 1 fiber predominance with scattered degenerating and

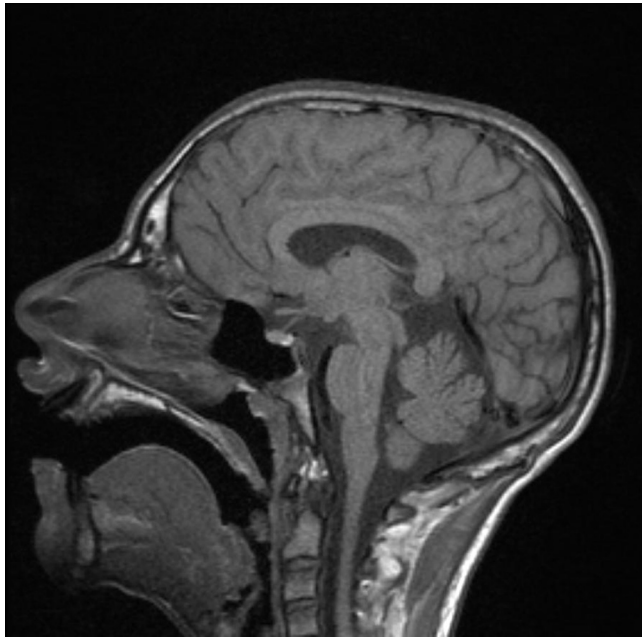


Fig. 1. Brain MRI for patient 1 at 14 years. This mid-sagittal section scan demonstrates a mildly hypoplastic cerebellar vermis, no cerebellar cysts, mild ventriculomegaly and slight prominence of the sulci for age.

regenerating muscle fibers (Fig. 2A) and increased perimysial fibrofatty tissue, suggesting a CMD. A mild degree of endomysial lymphocytic inflammation with expression of MHC class I and complement C5b-9 deposition was also present. Merosin, dystrophin and sarcoglycan immunohistochemistry was normal. At 24 months, he was walking independently and using minimal specific words. At 9 years, he continued to require help with dressing, had significant speech delay and severe constipation. At 11 years, he had 4/5 strength in the upper and lower extremities, and he could climb stairs and ascended from the floor without a Gowers' maneuver. He has no history of seizures and has not had an EEG. The severity of his cognitive and language delays were greater than his older brother, patient 1, and impaired his ability to follow multi step commands. At age 14, he continued to receive speech, occupational and physical therapy in a special education classroom. Given the progressive scoliosis seen in his older brother, this patient's original muscle biopsy was further evaluated by electron microscopy, which identified occasional fibers with marked sarcomeric disorganization corresponding to the degenerative fibers without structural changes diagnostic of a specific myopathy. Immunofluorescence analysis with an expanded panel of antibodies revealed normal appearance of dystrophin, beta-dystroglycan, sarcoglycans, spectrin, caveolin-3, collagen VI, and emerin. A few regenerating fibers expressed embryonic myosin heavy chain and had greatly reduced sarcolemmal dysferlin along with increased cytoplasmic dysferlin. Dysferlin expression was normal in other fibers. Staining for alpha-dystroglycan using two different glycoepitope

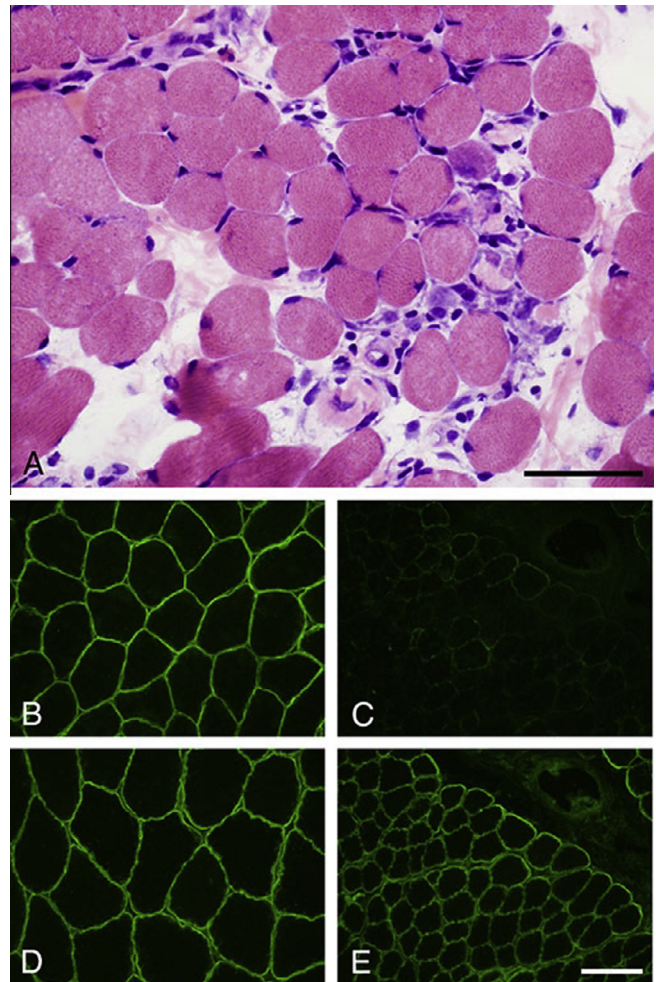


Fig. 2. Histopathology of the muscle biopsy from patient 2. (A) This cryosection H and E from patient 2 shows the classic dystrophic features of myonecrosis, regeneration, and mild endomysial fibrosis. There is increased variation in fiber size. (B and D) Control muscle biopsy immunostained for alpha- and beta-dystroglycan, respectively. (C) The patient's muscle shows greatly reduced staining for α -dystroglycan using the glycoepitope-dependent antibody, VIA4-1. Similar, but less dramatically reduced staining of alpha-dystroglycan was seen with the glycoepitope-dependent antibody IIH6. (E) Beta-dystroglycan staining is normal in the patient's biopsy. The photomicrographs in (C) and (E) are from adjacent sections.

antibodies (IIH6 and VIA4-1) ranged from negative to nearly normal; reduced staining with IIH6 was milder than with VIA4-1 (Fig. 2B–E). There was a mild degree of reduced staining for merosin using an antibody against an 80 kDa fragment of laminin alpha2, while merosin appeared normal using an antibody against a 300 kDa fragment. Sarcolemmal neuronal nitric oxide synthase (nNOS) was nearly negative.

Patient 3: This 6 year old Caucasian girl was first evaluated at 13 months because of congenital microcephaly and global developmental delay. She was born at 39 weeks gestation by normal spontaneous vaginal delivery to nonconsanguineous parents. The pregnancy was uncomplicated, and normal fetal movement was noted at 6 months gestation (similar to

her normal 6 year old male sibling). She was diagnosed with failure to thrive at 4 months. She was a slow, picky eater without choking, but she did not chew well and had persistent drooling. Hypotonia was noted at 12 months. She sat and crawled at 15 months and walked at 30 months. Presently at 6 years she has good endurance, and she can ride a bicycle with training wheels. She said her first words at 30 months, and at 6 years she had a vocabulary of several hundred words with several short word phrases. She has good receptive language, but does not recognize letters, and she receives regular speech therapy. She is presently enrolled in a special education kindergarten program which includes occupational and adaptive physical therapies. She has not fully developed bowel and bladder continence, and she has never experienced seizures.

At 6 years of age her weight was at the 10th centile, height at the 8th centile, and head circumference less than the 2nd centile. She had a small chin, normal tongue, moderately high palate, and frequent drooling. The abdomen and calves were prominent, and she had flat feet and mild hypotonia. Muscle strength in the upper extremities was normal, and she had a fine appendicular tremor but normal appendicular coordination. She could jump on both feet, and she easily ascended from the floor without a Gowers' maneuver. Her gait was narrow based with a tendency to swing the hips with poor hip flexion suggestive of pelvic girdle weakness.

At 2 years the patient had a normal audiogram and brain MRI, and at 3½ years- of-age she had a normal EKG and echocardiogram. Elevated transaminases were present from infancy and at 3 years-of-age the AST and ALT were 121 and 111 U/L, respectively and the CPK was elevated at 3411 IU/L.

A vastus intermedius muscle biopsy at age 3½ years showed severe dystrophic changes. Immunofluorescence studies of dystrophin, beta-dystroglycan, sarcoglycans, merosin, collagen VI, caveolin-3 and dysferlin were non-diagnostic. Staining for alpha-dystroglycan using two different glycoepitope antibodies (IIH6 and VIA4-1) ranged from negative to nearly normal in a pattern very similar to that described above for patient 2. Sarcolemmal nNOS also ranged from negative to nearly normal. MHC class I was weakly positive across the biopsy with focal regions of more intense immunostaining. The greater intensity myocyte MHC class I corresponded to small foci with endomysial lymphocytic inflammation. A mild degree of complement C5b-9 deposition was also noted.

3. Genetic testing results

By direct sequencing of the *POMT1* gene, all three patients were found to be heterozygous for a single nucleotide duplication in exon 20 (c.2167dupG) predicted to lead to a frame-shift and premature protein

termination (p.Asp723Glyfs*8) [6], and a previously unreported mutation in exon 19 (c.1958C>T) that is predicted to result in substitution of a highly conserved proline residue with a leucine residue at position 653 (p.Pro653Leu) (Fig. 3). Parental testing for the two siblings (patients 1 and 2) revealed that the c.1958C>T mutation was of paternal origin and the c.2167dupG mutation was of maternal origin. The parents of patient 3 were not tested.

4. Fibroblast assays

Cultured fibroblasts derived from a skin biopsy of patient 2 were evaluated by western blotting as described by Willer et al. [19]. Alpha-dystroglycan expressed by this patient was reduced in molecular weight using a core peptide antibody. It was not detectable in a laminin overlay assay, while binding to the glycoepitope antibody IIH6 was greatly reduced. The molecular weight, binding to IIH6, and binding to the basement membrane ligand laminin were all rescued with adenovirus-mediated gene transfer of *POMT1* (Fig. 4). Adenovirus-mediated gene transfer of *POMT1* also rescued IIH6 binding in cultured fibroblasts derived from patient 3 using an on-cell western blot assay [19], data not shown.

5. Discussion

POMT1 encodes protein O-mannosyltransferase-1, a glycosyltransferase necessary for proper post-translational processing of alpha-dystroglycan. Protein O-mannosyltransferases 1 and 2 (POMT1 and POMT2) are responsible for the first step in O-glycosylation of alpha-dystroglycan by transfer of a mannosyl residue from

			p.P653L
HsPOMT1	645	GGWAVNYL	PFFLMEKTLFL
RnPOMT1	645	GGWALNYL	PFFLMERMLFL
MmPOMT1	645	GGWALNYL	PFFLMERVLFLL
GgPOMT1	623	GGWVVNYL	PFFLMEKTLFL
XtPOMT1	623	GGWAVNYL	PFFLMEKTLFL
DrPOMT1	618	GGWAVNYL	PFFLMEKTLFL
OaPOMT1	623	GGWAVNYL	PFFMMEKTLFL
SpPOMT1	658	S-WFVNYE	PFYFMERTLFL
DmPOMT1	715	MGYVMHYI	PFYFCVDRTLFL
ScPmt1p	637	LGFAVHYA	PSFLMQRQMFLL
HsPOMT2	645	LGWTLHYE	PFFLMGRVLYF
MmPOMT2	645	LGWMLHYE	PFFLMGRILYF

Fig. 3. Proline 653 of POMT1 is highly conserved within the POMT protein family. The POMT1 p.P653 residue, highlighted in red, is conserved among humans (Hs), rat (Rn), mouse (Mm), chicken (Gg), frog (Xt), zebrafish (Dr), platypus (Oa), sea urchin (Sp) fruit fly (Dm) and baker's yeast (Sc). The proline at 653 is also the first amino acid in a 5 residue contiguous sequence shared by POMT1 and POMT2.

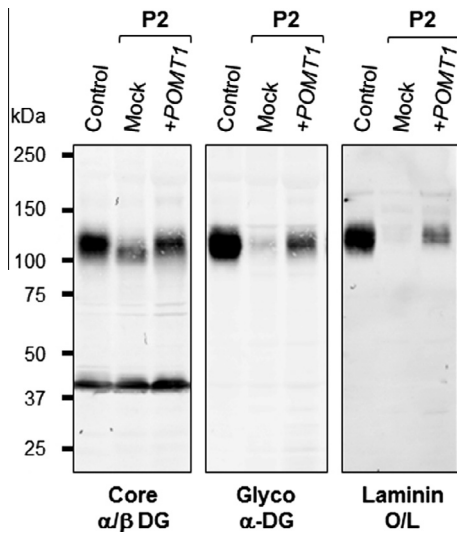


Fig. 4. Adenovirus expressing *POMT1* rescues alpha-dystroglycan glycosylation. The molecular weight of alpha-dystroglycan is reduced in patient 2 as compared to control fibroblasts. The molecular weight, binding to IIH6, and binding to laminin were all rescued by infection with adenovirus-*POMT1*, but not with mock infection. Core α DG is a polyclonal antibody that binds the peptide of α -dystroglycan. Glyco α DG is the glycoepitope specific monoclonal antibody IIH6. β DG is used to assess loading. Laminin O/L is a laminin overlay assay.

Dol-P-Man to serine and threonine residues [13,30]. Several additional genes contribute to the glycosylation of alpha-dystroglycan, and mutations in each of these cause a variety of dystroglycanopathy subtypes ranging in severity from WWS to LGMD [5,7,10,11,14,15,18–20,31]. Mutations in *POMT1* that create premature stop codons within a small region of the C-terminus or occur within two highly conserved regions, protein mannosyltransferase (PMT) and mannosyl-IP3R-RyR (MIR), severely disrupt mannosyltransferase activity and are associated with more severe phenotypes. Conversely, mutations in *POMT1* that are associated with a higher level of residual *POMT1* activity are associated with the milder phenotype. The type of mutation and amount of residual enzyme activity may be more predictive of severity than the specific gene involved [19,29,32].

All three patients described in detail in this report are heterozygous for a previously reported *POMT1* mutation in exon 20 (c.2167dupG) which is predicted to result in a frame-shift and premature protein termination (p.Asp723Glyfs*8). This mutation has been documented to be causative for WWS [6]. A second heterozygous mutation found in all three patients is a previously unreported *POMT1* (c.1958C>T) nucleotide change. This second variant is suspected to reduce the severity of the phenotype as compared to WWS patients who are either homozygous c.2167dupG [17] or compound heterozygous c.2167dupG and other mutant *POMT1* alleles [6,33–35]. The *POMT1* (c.1958C>T) nucleotide change is predicted to result in the amino acid substitution p.Pro653Leu. The p.Pro653 residue is conserved among the POMT protein family from human, non-primate mammals, chicken,

frog, zebrafish, invertebrates and yeast (Fig. 3). Alignment of the *POMT1* protein to that of the *POMT2*-encoded *POMT2* protein shows a region of conservation beginning at p.Pro653 and extending five contiguous residues to p.Met657. The c.1958C>T variant is registered in the NHLBI-ESP variant server database (rs149682171; <https://esp.gs.washington.edu/drupal/>), having been detected once in an exome capture screen of 4552 individuals (9104 chromosomes). From these limited data, the calculated frequency of the minor (T) allele is approximately 1/10,000, a reasonably low frequency for a rare disease allele in a healthy population. The computational models PolyPhen-2 [36] and SIFT [37] predict the *POMT1* p.Pro653Leu substitution to be deleterious and not tolerated, respectively. The pathogenicity of this novel mutation is further supported by cell culture studies in which adenovirus-mediated gene transfer of *POMT1* was able to rescue functional glycosylation of alpha-dystroglycan in fibroblasts from patient 2 (Fig. 4).

Homozygous c.2167dupG (Asp723Glyfs*8) *POMT1* mutations result in a severe WWS phenotype (see Table 1 and [6]). Fig. 6 illustrates a previously unreported WWS patient who is homozygous for c.2167dupG (Asp723Glyfs*8); muscle biopsy evaluation and molecular genetic testing were done at the University of Iowa. This mutation results in the deletion of the C-terminal end with an insertion and the new reading frame ending in a stop codon at amino acid 731. Two patients with homozygous *POMT1* mutations resulting in the deletion of the C-terminal end and creation of a new stop codon at amino acid 730 or 731 also presented with the severe, WWS phenotype [6,38]. One patient with the WWS phenotype was found to have compound heterozygous mutations c.2167dupG (Asp723Glyfs*8) and c.1983_1984delCT (Leu661Leufs*69) resulting in a deletion of the C-terminal end and a new stop codon at amino acid 730. The introduction of a stop codon within a short segment of the C-terminus appears to be associated with the severe phenotype (Fig. 5). In addition, compound heterozygous individuals with one *POMT1* mutation in loop 1, loop 5, or a transmembrane helix and a second mutation at c.2167dupG (Asp723Glyfs*8) present with WWS. Loop 5 shares sequence similarities with the catalytic domains of yeast protein O-mannosyltransferases (Pmtp) and three MIR motifs [39]. The shared severe phenotype associated with mutations involving loop 1, loop 5 and the C-terminus suggests that mutations affecting regions of *POMT1* within the lumen or membrane of the endoplasmic reticulum significantly disrupt catalytic activity. Individuals with one mutation at c.2167dupG (Asp723Glyfs*8), and a second missense or in-frame mutation within the cytoplasmic loops 4 or 6, including the Pro653Leu mutation reported in our 3 patients, present with a milder phenotype of CMD or LGMD. *POMT1* missense mutations within the cytoplasmic

Table 1
Dystroglycanopathy patients with c.2167dupG *POMT1* mutations. Also, see Fig. 5 for topology mapping.

Exon	Allele 1	Exon	Allele 2	Phenotype [‡]	Phenotypic details	Citations
<i>Homozygous cases</i>						
20	c.2167dupG p.Asp723Glyfs*8	20	c.2167dupG p.Asp723Glyfs*8	WWS	Intellectual disability, motor delays, microcephaly, hydrocephalus, cortical, white matter and cerebellar abnormalities, microphthalmia, retinal dysplasia	[17]
20	c.2167dupG p.Asp723Glyfs*8	20	c.2167dupG p.Asp723Glyfs*8	WWS	Hypotonia at birth with hydrocephalus, cobblestone lissencephaly, pontocerebellar hypoplasia, cloudy corneas, fusion of corneas and irises (see Fig. 6)	*
<i>Compound heterozygous cases</i>						
20	c.2167dupG p.Asp723Glyfs*8	2	c.81dupA p.Leu28Thrfs*16	WWS	None provided	†
20	c.2167dupG p.Asp723Glyfs*8	2	c.89G>A p.Arg30Gly	WWS	“Polymicrogyria” (likely cobblestone lissencephaly), posterior encephalocele, cervical spinal cord syrinx, coloboma	*
20	c.2167dupG p.Asp723Glyfs*8	3	c.130G>A p.Glu44Lys	WWS	Hydrocephalus, cobblestone lissencephaly	†
20	c.2167dupG p.Asp723Glyfs*8	5	c.291delC p.Ser97Argfs*26	WWS	Hydrocephalus, lissencephaly, cerebellar dysplasia, cataracts, cleft lip/palate	[43]
20	c.2167dupG p.Asp723Glyfs*8	5	c.385delT p.Ser129Leufs*11	WWS	None provided	†
20	c.2167dupG p.Asp723Glyfs*8	5	c.418_420del p.Met140del	MEB	Intellectual disability, non ambulatory, mild microcephaly -2.5 SD, cortical dysplasia, flat pons, cerebellar hypoplasia, myopia	[17,29,35]
20	c.2167dupG p.Asp723Glyfs*8	6	c.517_523delinsG p.Phe173_Asn175delinsAsp	WWS	No specific clinical features provided	†
20	c.2167dupG p.Asp723Glyfs*8	11	c.1097G>A p.Cys366Tyr	WWS	Brain and ocular involvement, seizures, contractures, polydactyly	[34]
20	c.2167dupG p.Asp723Glyfs*8	13	c.1261_1262del p.Leu421Gluufs*12	WWS	Aborted fetus with cobblestone lissencephaly	[33]
20	c.2167dupG p.Asp723Glyfs*8	13	c.1283T>A p.Val428Asp	WWS	Intellectual disability, non ambulatory, mild microcephaly -2.5 SD, myopia	[6,12]
20	c.2167dupG p.Asp723Glyfs*8	17	c.1723delC p.Leu575Trpfs*23	WWS	None provided	†
20	c.2167dupG p.Asp723Glyfs*8	19	c.1958C>T p.Pro653Leu	CMD	Intellectual disability, ambulatory, mild cerebellar hypoplasia, mild ventriculomegaly, normal eye exam	Cases 1 and 2 from current paper [†]
20	c.2167dupG p.Asp723Glyfs*8	19	c.1958C>T p.Pro653Leu	CMD	Motor and speech delays, microcephaly, normal brain MRI	Case 3 from current paper [†]
20	c.2167dupG p.Asp723Glyfs*8	19	c.1983_1984delCT p.Leu661Leufs*69	WWS	Hydrocephalus, cerebellar hypoplasia, bilateral glaucoma, optic nerve hypoplasia, anal atresia, bilateral hydronephrosis	*
20	c.2167dupG p.Asp723Glyfs*8	19	c.2005G>A p.Ala669Thr	CMD	None provided	[12]
<i>Presumed compound heterozygous cases – second mutant allele unknown</i>						
20	c.2167dupG p.Asp723Glyfs*8	?	Unknown	WWS	None provided	†
20	c.2167dupG p.Asp723Glyfs*8	?	Unknown	WWS	Hydrocephalus, cobblestone lissencephaly, posterior encephalocele, no eye exam reported	*

* Sequencing performed in the Molecular Pathology Laboratory, Department of Pathology, University of Iowa Health Care, Iowa City, IA. This case has not been previously reported and is not currently listed in the Leiden Muscular Dystrophy pages database.

† Sequencing performed at PreventionGenetics, Marshfield, WI, and mutations reported to Leiden Muscular Dystrophy pages (<http://www.dmd.nl/>) by T.L. Winder.

‡ Abbreviations: WWS: Walker–Warburg syndrome; MEB: muscle eye brain disease; CMD: congenital muscular dystrophy.

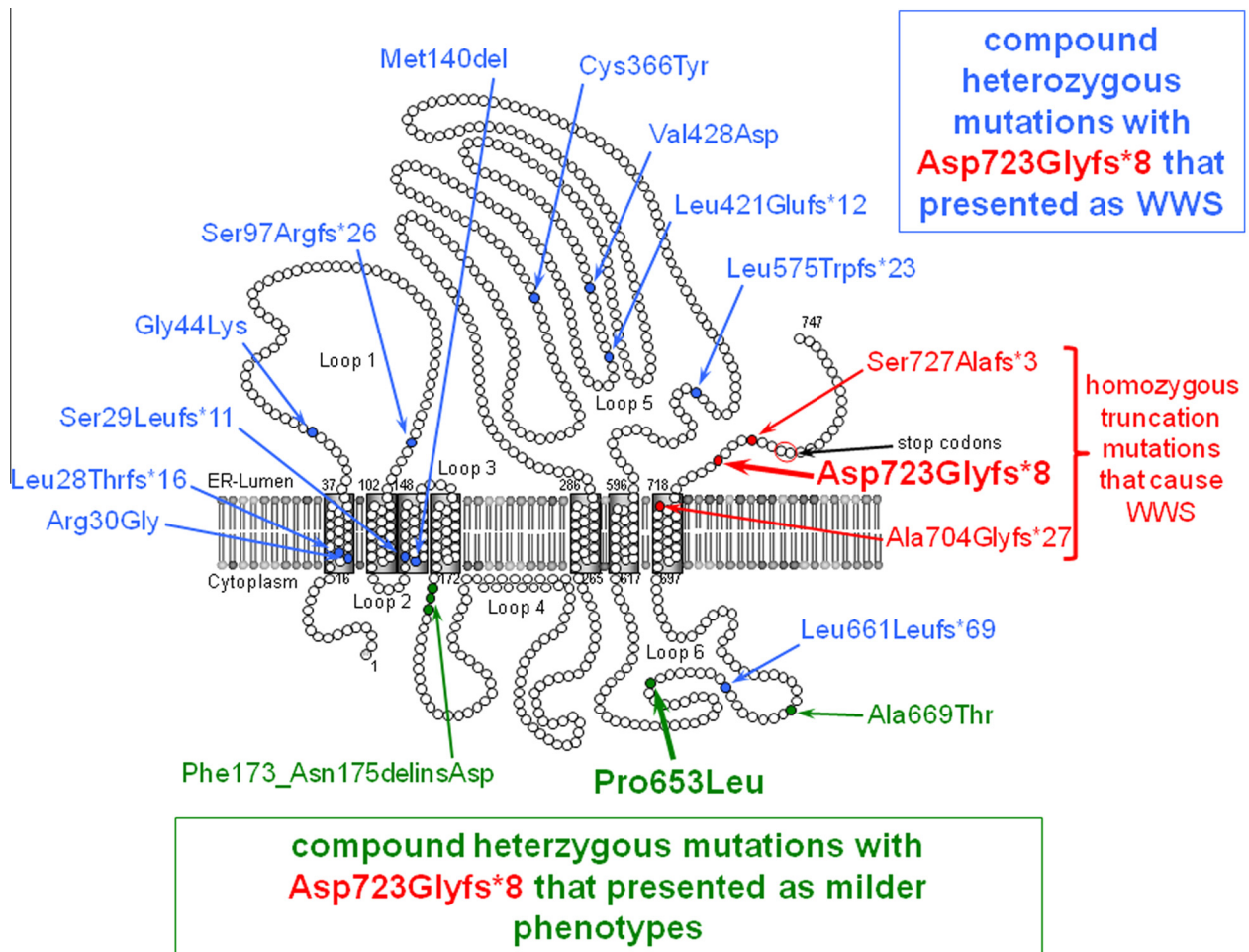


Fig. 5. *POMT1* mutations in combination with c.2167dupG. Homozygous c.2167dupG and two additional homozygous mutations that truncate 17 or 18 amino acids from the C-terminus of *POMT1* result in a WWS clinical phenotype (red lettering and red arrows). Nine additional mutations in combination with c.2167dupG also cause severe phenotypes, most described as WWS (blue lettering and blue arrows). The novel mutation presented here (c.1958C>T) and two additional mutations lead to milder phenotypes, CMD to LGMD (green lettering and green arrows).

domains may be better tolerated and result in higher residual O-mannosyltransferase activity.

This genotype phenotype correlation is further supported by reports of patients on the milder end of the spectrum that do not harbor a severe mutation affecting the carboxy terminus. Individuals with homozygous or compound heterozygous mutations only affecting the loops within the cytoplasm are more likely to present with the milder phenotype of LGMD [38,40]. Mutations affecting the transmembrane domains and loop 1 and loop 5 within the lumen of the endoplasmic reticulum tend to be associated with the more severe presentation of CMD [17,35,38,41,42]. There is mild clinical variability reported in patients with identical mutations, preventing a precise prediction of phenotype [38,40]. Identifying specific mutations in additional patients may clarify this correlation and lead to a more accurate prognosis in young patients with *POMT1* mutations.

The cases presented here illustrate the important relationship between the mutation type, its location and

the severity of clinical features, and demonstrate the prognostic value of genetic testing as an added tool in the care of these patients. As more information is learned about the glycosylation process, critical regions of each enzyme, and mutation prevalence in specific populations, specific testing strategies based on phenotype may be developed.

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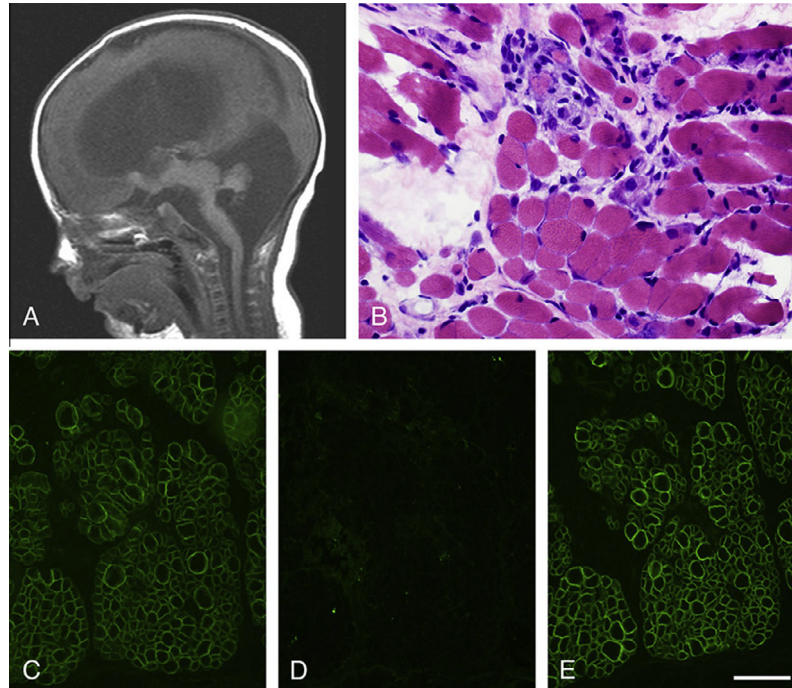


Fig. 6. Walker–Warburg Syndrome phenotype in a patient homozygous for *POMT1* c.2167dupG mutations. (A) Hydrocephalus, cobblestone lissencephaly, kinked midbrain, and pontocerebellar hypoplasia are evident in this midsagittal MRI. (B) The cryosection of his muscle biopsy stained with H and E shows myonecrosis, regeneration, increased variation in fiber size, and mild endomysial fibrosis. Immunofluorescence stains of the muscle biopsy show normal dystrophin (C) and β -dystroglycan (E), while α -dystroglycan (D) is nearly negative. The anti- α -dystroglycan used here is IH6, which is specific for the glycoepitope necessary for laminin binding to α -dystroglycan. The size bar is 40 μ m in panel B and 50 μ m in panels C–E.

patient 1. Joel Carl assisted with the preparation of Figs. 2 and 6.

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