Mild POMGnT1 Mutations Underlie a Novel Limb-Girdle Muscular Dystrophy Variant

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Background: Mutations in protein-O-mannose-β1,2-N-acetylglucosaminyltransferase 1 (POMGnT1) have been found in muscle-eye-brain disease, a congenital muscular dystrophy with structural eye and brain defects and severe mental retardation.

Objective: To investigate whether mutations in POMGnT1 could be responsible for milder allelic variants of muscular dystrophy.

Design: Screening for mutations in POMGnT1.

Setting: Tertiary neuromuscular unit.

Patient: A patient with limb-girdle muscular dystrophy phenotype, with onset at 12 years of age, severe myopia, normal intellect, and decreased α-dystroglycan immunolabeling in skeletal muscle.

Results: A homozygous POMGnT1 missense mutation (c.1666G>A, p.Asp556Asn) was identified. Enzyme studies of the patient’s fibroblasts showed an altered kinetic profile, less marked than in patients with muscle-eye-brain disease and in keeping with the relatively mild phenotype in our patient.

Conclusions: Our findings widen the spectrum of disorders known to result from mutations in POMGnT1 to include limb-girdle muscular dystrophy with no mental retardation. We propose that this condition be known as LGMD2M. The enzyme assay used to diagnose muscle-eye-brain disease may not detect subtle abnormalities of POMGnT1 function, and additional kinetic studies must be carried out in such cases.

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panding the spectrum of diseases derived from mutations of this gene. This mutation led to a subtle abnormality in POMGnT1 enzyme activity not typical of that seen in MEB.

**METHODS**

**PATIENT**

A patient with limb-girdle muscular dystrophy was studied. The finding of abnormal glycosylation of α-DG in a muscle biopsy specimen was the criterion for inclusion in this study, which was approved by the Hammersmith Hospital Trust Ethics Committee (Research Ethical Committee 2000/5802).

**HISTOLOGIC ANALYSIS AND IMMUNOHISTOCHEMISTRY**

Frozen 10-µm muscle sections were stained with hematoxylin-eosin using a standard technique. Frozen 7-µm sections were incubated with mouse monoclonal antibodies to spectrin and dystrophin (spectrin DYS1, DYS2, and DYS3; Novocastra Laboratories Ltd, Newcastle upon Tyne, England); laminin-α2 (MAB1922; Chemicon International Inc, Temecula, California); the 80-kDa C-terminal fragment; β-dystroglycan (43DAG; Novocastra Laboratories Ltd); and an α-dystroglycan, IIH6; and with a sheep polyclonal antibody to the core protein of chick α-dystroglycan. Immunostaining was performed as previously described by Godfrey et al.11

**SINGLE-SECTION WESTERN BLOT AND OVERLAY ASSAYS**

Proteins from control and patient muscle biopsy specimens were extracted from sections using the method published by Cooper et al.12 In brief, sections were collected in sample buffer consisting of 4% sodium dodecylsulfate, 125mM Tris (pH 8.8), 40% glycerol, protease inhibitor cocktail, 100mM dithiothreitol, and bromophenol blue, and were boiled for 3 minutes. After centrifugation to sediment particulate material, the supernatant was used for Western blot analysis and for the laminin overlay. Samples were loaded into a NuPAGE Pre-Cast Gel System (4%-12% Bis-Tris gel; Invitrogen Corp, Carlsbad, California), then transferred electrophoretically to nitrocellulose membrane. Nitrocellulose strips were blotted with α-dystroglycan IIH6 antibody (Upstate Biotechnology, Waltham, Massachusetts) and β-dystroglycan antibody (Novocastra Laboratories Ltd) as described by Longman et al.13 The laminin overlay assay was performed as described by Longman et al.13

**MUTATION ANALYSIS**

Genomic DNA was isolated from total blood samples using standard extraction protocols. The complete coding regions of
FKRP, POMT1, POMT2, POMGnT1, LARGE, and fukutin were screened as described by Godfrey et al11 (GenBank Accession numbers: FKRP, NM_024301; POMT1, NM_007171.2; POMT2, NM_013382.3; POMGnT1, NM_017739.1; fukutin, NM_006731.1; and LARGE, NM_133642.2).

KINETIC ANALYSIS OF POMGnT1
ENZYME ACTIVITY

Kinetic analysis of POMGnT1 was carried out by a modification of a previously reported method.8,14 Fibroblasts from a control subject without congenital muscular dystrophy and from the patient were scraped off dishes without trypsin. Cells containing about 1 mg of protein were homogenized at 4°C in 0.2 mL of homogenizing buffer (2.0% Triton X-100, 0.2M sodium chloride in phosphate-buffered saline solution) containing one-fourth of a tablet of protease inhibitor cocktail (Roche Boehringer Mannheim, Basel, Switzerland). The assay incubations contained, in a total volume of 0.020 mL, 0.015 mL of fibroblast extract (approximately 0.05-0.07 mg of protein per assay), 75mM 2-(N-morpholino)ethanesulfonic acid buffer (pH 6.0), 10mM manganese chloride, 5mM adenosine monophosphate, 0.2M GlcNac, and 8 combinations of donor and acceptor concentrations: (1) 0.25, 0.5, 0.75, and 1.0mM uridine diphosphate (UDP)–(3H)GlcNac (New England Nuclear Corp, Boston, Massachusetts; 125 000 dpm/nmol) and 62.5mM Man1-O-benzyl (Toronto Research Chemicals Inc, North York, Ontario, Canada), and (2) 5, 10, 15, and 20mM Man1-O-benzyl and 0.3mM UDP–(3H)GlcNac. Incubations were carried out at 37°C for 2 hours. SepPak C18 cartridges (Waters Corp, Milford, Massachusetts) were used to determine the amount of radioactive product as previously described.15,16 Control assays were routinely carried out in the absence of an acceptor, and the value obtained was subtracted from all assays. All assays were carried out in duplicate.

RESULTS

CLINICAL DESCRIPTION

Our patient is one of 5 children born to healthy non-consanguineous Irish parents. She first developed proximal limb muscle weakness at the age of 12 years, with difficulty rising from a sitting position and climbing stairs. Her early motor milestones were normal. The weakness progressed rapidly, and by age 14 years was more proximal than distal, with the neck, hip girdle, and shoulder abductor muscles particularly affected. Her Gower sign was positive. There was hypertrophy of the calves and quadriceps and wasting of the hamstring and deltoid muscles. She had a lordotic stance and poor heel strike because of Achilles tendon tightening. Facial expression was normal. Progressive weakness resulted in loss of ambulation at age 19 years after a leg fracture. Her general health remains excellent. She has myopia (+6); at the age of 6 years, she underwent surgery to correct a convergent squint. Her intellect is normal, and she is attending a university and is 21 years old. Results of initial investigation included serum creatine kinase concentration consistently elevated between 5000 and 12 000 U/L and electromyographic findings suggestive of a myopathic process.

![Figure 2](https://archneur.jamanetwork.com/)

Figure 2. Single-section skeletal muscle analysis. A, Western blot analysis using the HH6 antibody (α-DG) shows that expression and molecular weight of patient muscle were similar to those of control muscle. β-Dystroglycan expression (β-DG) was similar to control and demonstrates equal loading between samples. B, Laminin overlay assay. The patient’s α-DG shows the same ability to bind laminin compared with the control muscle. α-DG and β-DG indicate α- and β-dystroglycan, respectively.

HISTOLOGIC ANALYSIS
AND IMMUNOHISTOCHEMISTRY

The muscle biopsy specimen exhibited dystrophy with abnormal variation in fiber size, necrosis, increased endomysial connective tissue and fat, and basophilic fibers, some of which were granular and had vacuoles (Figure 1A). Immunolabeling of β-spectrin, dystrophin, and laminin-α2 yielded normal findings (data not shown). A percentage of these fibers were also weaker with the anti-β-dystroglycan antibody and may correspond to basophilic fibers (Figure 1B). Labeling with HH6 antibody to the glycosylated epitope of α-DG was variable between fibers (Figure 1C). Some fibers showed a mild reduction; others were brightly labeled. Similarly, labeling with the core antibody was weaker on some small fibers (Figure 1D). Further examination of the biopsy specimen demonstrated normal findings at Western blot analysis of dystrophin, sarcoglycans, laminin-α2, caveolin, emerin, calpain 3, dysferlin, and telethonin.

SINGLE-SECTION WESTERN BLOT
AND OVERLAY ASSAYS

Western blot analysis of skeletal muscle showed an expression of α-DG similar to that in the control sample using an antibody that recognizes a glycosylated epitope (IH6; Figure 2A). β-Dystroglycan expression was normal and demonstrates equal protein loading. The laminin overlay assay showed that α-DG ability to bind laminin was similar to that in the control sample (Figure 2B).

MUTATION ANALYSIS

A novel homozygous point mutation, c.1666G>A, was detected in exon 20 of POMGnT1. This change is predicted to result in the substitution of a conserved aspartic acid at amino acid 556 to asparagine (p.Asp556Asn) in the POMGnT1 protein. No other sequence variations were detected in the other known dystroglycanopathy genes (FKRP, POMT1, POMT2, fukutin, or LARGE), and

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linkage to the MDC1B locus on 1q42 was excluded. Both parents were found to be carriers of the mutation, and none of the 4 unaffected siblings tested were homozygous for this change. This alteration was not detected in more than 100 disease control samples (data not shown).

**KINETIC ANALYSIS OF POMGnT1 ENZYME ACTIVITY**

Kinetic analysis of POMGnT1 activity at 4 different concentrations of UDP-GlcNAc (Figure 3A) showed apparent Michaelis-Menten constant ($K_m$) values of 2.2 and 6.1 mmol/L for control and patient fibroblasts, respectively. The respective apparent maximum velocity ($V_{max}$) values were 3.4 and 2.5 nmol/h/mg of protein (difference not statistically significant). It is impossible to obtain accurate $K_m$ and $V_{max}$ values for Manα1-O-benzyl with normal POMGnT1 because these parameters are both so large that a plot of enzyme rate vs acceptor concentration shows a straight line through the origin, up to the maximum soluble concentration of 60 mmol/L of Manα1-O-benzyl. However, a similar analysis of patient fibroblasts (Figure 3B) showed a qualitative change from a straight line to a classic hyperbolic Michaelis-Menten plot (apparent $K_m$ and $V_{max}$ values of 9 mmol/L and 0.1 nmol/h/mg of protein, respectively).

**COMMENT**

To date, the identification of POMGnT1 mutations has been restricted to patients with congenital muscular dystrophy and brain abnormalities. Most of these patients have a phenotype consistent with MEB, although some cases resembling Walker-Warburg syndrome have also been reported. One of the unanswered questions about the genotype-phenotype correlation for POMGnT1 mutations is whether it can cause mild as well as severe phenotypes, as noted in other dystroglycanopathy genes, most notably FKRP and more recently also fukutin. The p.Asp556Asn mutation reported herein is predicted to be located in the substrate-specific region of the catalytic domain. Several lines of evidence indicate that this alteration is pathogenic. First, the patient's skeletal muscle biopsy specimen demonstrated findings consistent with a muscular dystrophy and suggestive of dystroglycanopathy. No mutations were detected in the other dystroglycanopathy genes screened. The reduction of α-DG labeling was subtle at immunocytochemistry, and no substantial reduction in molecular weight was observed at Western blot analysis. This highlights the difficulty in detecting mild α-DG abnormalities and has been previously observed with mild FKRP mutations in limb-girdle muscular dystrophy type 2I. Second, the mutation segregated with the disease in this large family. Third, the mutation causes a reversal of amino acid polarity in the substrate-specific domain of POMGnT1, which may be expected to have functional consequences. In addition, the detailed enzyme studies offer support for this theory. The POMGnT1 activity is observed but differs in its kinetics from that of control samples. The apparent $K_m$ for UDP-GlcNAc is substantially higher and the apparent $V_{max}$ for Manα1-O-benzyl is substantially lower than in the control sample. Application of the POMGnT1 assay conditions that were previously used for analysis in patients with MEB (1 mmol/L of UDP-GlcNAc and 62.5 mmol/L of Manα1-O-benzyl) to our patient with limb-girdle muscular dystrophy yields a POMGnT1 rate that is significantly higher than the val-
ceived equally to the manuscript.

This mutation had no effect on the subcellular localization or expression of a recombinant form of POMGnT1 when overexpressed in C2C12 myotubes (data not shown). However, the p.Asp556Asn mutation introduces a potential N-glycosylation site (Asn-X-Ser) into the protein, which is predicted to have no other such sites. The presence of an N-glycan at this position may cause defective enzyme folding that results in an active but inefficient enzyme.

CONCLUSION

This article substantially expands the spectrum of disorders associated with POMGnT1 mutations, and our patient has the mildest POMGnT1 deficiency described. The finding of normal POMGnT1 activity at conventional enzyme assay but the identification of altered kinetic properties of the mutant enzyme highlight the importance of careful interpretation of functional data, especially when studying atypical clinical findings.

This finding reinforces the presence of glycosyltransferase mutations among the limb-girdle muscular dystrophies. We propose to name this new phenotype LGMD2M.

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