DPAGT1 myasthenia and myopathy

Genetic, phenotypic, and expression studies

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ABSTRACT

Objective: To investigate patients with DPAGT1 (UDP-*N*-acetylglucosamine-dolichyl-phosphate *N*-acetylglucosaminephosphotransferase 1)-associated myasthenic syndrome.

Methods: We performed exome and Sanger sequencing, determined glycoprotein expression in patient muscles, assessed pathogenicity of the mutant proteins by examining their expression and enzymatic activity in transfected cells, evaluated structural changes in muscle and the neuromuscular junction, and examined electrophysiologic aspects of neuromuscular transmission in vitro.

Results: Patients 1 and 2, 16 and 14 years of age, had progressive fatigable weakness since infancy and are intellectually disabled. Patient 3, a less severely affected brother of patient 1, also has autistic features. Each patient harbors 2 novel heteroallelic mutations in *DPAGT1*, an enzyme subserving protein *N*-glycosylation. Patients 1 and 3 harbor Met1Leu, which reduces protein expression, and His375Tyr, which decreases enzyme activity. Patient 2 carries Val264Met, which abolishes enzyme activity, and a synonymous Leu120Leu mutation that markedly augments exon skipping, resulting in some skipped and infrequent nonskipped alleles. Therefore, the nonskipped allele rescues the phenotype. Intracellular microelectrode studies indicate combined pre- and postsynaptic defects of neuromuscular transmission with evidence for somatic mosaicism in patient 2. Structural studies reveal hypoplastic endplates, fiber-type disproportion, tubular aggregates, and degeneration of muscle fiber organelles resulting in autophagocytosis.

Conclusions: DPAGT1 myasthenia affects multiple parameters of neuromuscular transmission, causes fiber-type disproportion and an autophagic myopathy, and can be associated with intellectual disability. We speculate that hypoglycosylation of synapse-specific proteins causes defects in central as well as motor synapses. *Neurology*® 2014;82:1822-1830

GLOSSARY

AChE = acetylcholinesterase; **AChR** = acetylcholine receptor; **α -bgt** = α -bungarotoxin; **cDNA** = complementary DNA; **CMS** = congenital myasthenic syndrome; **3,4-DAP** = 3,4-diaminopyridine; **DPAGT1** = UDP-*N*-acetylglucosamine-dolichyl-phosphate *N*-acetylglucosaminephosphotransferase 1; **EP** = endplate; **MEPC** = miniature endplate current; **MEPP** = miniature endplate potential; **STIM1** = stromal interaction molecule 1.

Congenital myasthenic syndromes (CMS) are heterogeneous disorders in which the safety margin of neuromuscular transmission is compromised by one or more specific mechanisms. Most CMS are caused by defects in endplate (EP)-specific proteins.¹ Recently, however, it became apparent that proteins distributed in many tissues, namely the intermediate filament linker plectin² and enzymes subserving glycosylation of nascent peptides such as GFPT1,^{3,4} DPAGT1,⁵ ALG2, and ALG14,⁶ are also CMS targets. GFPT1 is the initial and rate-limiting enzyme in the biosynthesis of *N*-acetylglucosamine, an essential substrate for *O*- and *N*-glycosylation.⁷ DPAGT1 catalyzes the first step, ALG14 in concert with ALG13 catalyzes the second step, and ALG2 catalyzes the fourth step of the *N*-linked-committed glycosylation pathway.⁸ A limb-girdle distribution of the weakness, tubular aggregates in type 2 fibers, and responsiveness to pyridostigmine are considered common features of these disorders.

Supplemental data
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Mutations in *DPAGT1* myasthenia were reported in 4 kinships in 2012,⁵ their clinical features were further documented,⁹ and in 2013 a homozygous *DPAGT1* mutation was identified in a consanguineous Iranian kinship.¹⁰ The neuromuscular transmission defect was attributed to decreased cellular synthesis and export of the hypoglycosylated acetylcholine receptor (AChR) to the postsynaptic membrane.⁵ The mean α -bungarotoxin (α -bgt) binding per EP was 11.2 atomoles in 4 patients compared with 13 to 30 atomoles in controls.⁹ These studies did not examine neuromuscular transmission at patient EPs in vitro, details of EP ultrastructure, or effects of the mutations on *DPAGT1* expression and enzyme activity. Except for tubular aggregates in muscle, no myopathic features were documented. Herein, we report our findings in 3 patients in 2 kinships with *DPAGT1* myasthenia. Using whole-exome and Sanger sequencing, we identified 4 novel mutations, determined enzyme activities and expression levels of the mutant proteins, investigated parameters of neuromuscular transmission at patient EPs in vitro, and quantitatively analyzed the ultrastructure of 62 EP regions.

METHODS **Standard protocol approvals, registrations, and patient consents.** All human studies were approved by the Institutional Review Board of the Mayo Clinic, and the parents gave informed consent to participate in the study.

Genetic analysis. Exome sequencing of genomic DNA isolated from the blood of patient 1 and her brother, and from patient 2 and her parents was performed at the Mayo Clinic. In both patients and their family members, variants were filtered by dbSNP130 and SeattleSeq annotation. We then excluded variants at intergenic and intronic sites, genes not expressed in skeletal muscle and spinal cord based on the GEO database (<http://www.ncbi.nlm.nih.gov/geo/>), and variants not consistent with recessive inheritance. Variants not present in the brother of patient 1 or in parents of patient 2 were also excluded. Because a recently identified limb-girdle CMS with tubular aggregates in muscle was caused by a defect in protein glycosylation,^{3,4} we focused on genes subserving glycosylation pathways. All identified mutations were confirmed by Sanger sequencing. Nucleotides of *DPAGT1* complementary DNA (cDNA) were according to GeneBank accession number NM_001382.3.

Immunoblot studies. Extracts of frozen muscle of patients and controls were prepared for immunoblotting as previously described.¹¹ After transfer to nitrocellulose or polyvinylidene fluoride membrane (Life Technologies, Carlsbad, CA), the blots were probed with rabbit polyclonal RL2 antibody (Abcam, Cambridge, MA), mouse monoclonal O-GlcNAc antibody CTD110.6 (Covance, Berkeley, CA), and antibody to detect stromal interaction molecule 1 (STIM1) (Cell Signaling, Danvers, MA). The bands

were detected using the alkaline phosphatase method with appropriate secondary antibodies as described.¹²

Extracts of COS-7 cells transfected with plasmids containing C-terminal *myc*-labeled Met1Leu, His375Tyr, Val264Met, and wild-type *DPAGT1* were immunoblotted on nitrocellulose membranes and probed with *myc* antibody (Cell Signaling). Wells were loaded with equal amount of protein with the endoplasmic reticulum ERp72 protein (Cell Signaling) serving as the loading control. Immunoblots were quantitatively analyzed using NIH Image 1.61 software.

Real-time quantitative PCR and RNA splicing studies. These were done by standard methods and are detailed in appendix e-1 on the *Neurology*[®] Web site at Neurology.org.

Assay of *DPAGT1* enzyme activity in transfected COS cells. Details of the assay are described in appendix e-2.

Structural studies. Intercostal muscle specimens were obtained from origin to insertion from patients 1 and 2 and from patients without muscle disease undergoing thoracic surgery serving as controls. Appendix e-3 describes EP localizations of AChR and acetylcholinesterase (AChE)^{13,14} as well as ultrastructural analysis^{15,16} and AChR content of EPs.^{17,18}

In vitro electrophysiologic studies. The amplitude of the miniature EP potential (MEPP) and miniature EP current (MEPC), the quantal content of the EPP (m), estimates of the probability of quantal release (p), and quanta available for release (n) were determined as previously described.^{19–22}

Isoelectric focusing and capillary zone electrophoresis of serum-transferrin. These tests were performed at the Metabolic Center of the University of Leuven, Belgium, as described.²³

RESULTS **Clinical features.** Patient 1 is one of 8 siblings. She was hypomotile in utero but appeared normal at birth. At age 5 months, she had poor head control and was hypotonic. She took a few steps by age 3 years but became wheelchair-dependent by age 5 and had difficulty swallowing since age 9. At age 11, she was diagnosed with a myasthenic syndrome and was treated with pyridostigmine but responded poorly. The patient is intellectually disabled and is in second instead of seventh grade in school. At age 13 (figure 1A), she had mild scoliosis, moderately severe to severe fatigable weakness of facial, axial, proximal limb, and intrinsic hand muscles, and mild weakness of distal leg muscles. The tendon reflexes were hypoactive. An ECG showed a long QTc interval of 462 milliseconds. EMG studies revealed a 16% to 41% decrement of the evoked compound muscle action potential in different muscles. The patient's strength and EMG decrement were markedly improved 1 hour after combined administration of test doses of 3,4-diaminopyridine (3,4-DAP) and pyridostigmine. She was advised to take 240 mg pyridostigmine and 30 mg 3,4-DAP per day in divided doses. One year later, she still had moderately severe weakness, her scoliosis worsened, and she underwent scoliosis surgery. The patient's brother (patient 3) is similarly but less severely affected (figure 1B) and was previously diagnosed

Figure 1 Patients, mutations, and immunoblot studies

with an autistic spectrum disorder and a borderline prolonged QTc interval. The only other full sibling is healthy.

Patient 2 was born of normal pregnancy and delivery. She appeared normal at birth but had delayed motor development; she stood at age 1 year and started to walk at age 18 months but could not keep up with her peers in physical activities. After age 11, she became weaker and had difficulty walking, chewing, and swallowing. She is intellectually disabled with major learning and social adjustment problems. At age 14 (figure 1C), she had a lordotic stance, cubitus valgus, and an intoeing footdrop gait. The arm elevation time was only 5 seconds. She had trace limitation of lateral gaze, slight facial and moderately severe proximal-predominant limb muscle weakness, and severe weakness of the finger extensor muscles. EMG studies revealed a 20% decrement of the evoked compound muscle action potential in the peroneal muscles not improved by 2 mg IV edrophonium or 10 mg 3,4-DAP. Because the patient had become stronger when taking a cough syrup containing ephedrine, we treated her with 2 mg albuterol sulfate 3 times daily and 90 mg pyridostigmine 4 times daily. Eighteen months later, she could walk two-thirds of a mile with brief periods of rest, climb stairs without holding the railings, and throw a softball 50 feet. Both patients had negative AChR and MuSK antibody tests, normal serum creatine kinase levels, and normal brain MRI.

Genetic analysis. Exome sequencing confirmed by Sanger sequencing identified mutations in *DPAGT1* in each patient (see figure 1, D and E). Patient 1 and her brother harbor c.1A>C predicting p.Met1Leu, and c.1123C>T predicting p.His375Tyr. The Met1Leu mutation is followed by an alternative start site at codon 9, which truncates the signal peptide and slightly decreases the molecular weight of the mutant protein (figure 1H). Their mother carries p.Met1Leu, and an unaffected sister carries no mutations. Paternal DNA was unavailable. Patient 2 harbors c.360G>C in exon 3, predicting p.Leu120Leu, and c.790G>A predicting p.Val264Met. Each parent carries one mutation, and the unaffected brother carries the synonymous variant (figure 1E). None of the observed variants is present in NHLBI GO Exome Variant Server (NHLBI GO Exome Sequencing Project, Seattle, WA, 10/2013; URL: <http://evs.gs.washington.edu/EVS>), and all are conserved among vertebrate species.

To further characterize the synonymous Leu120 mutation in patient 2, we sequenced *DPAGT1* from cDNA isolated from the patient's muscle and from 2 normal control muscles. This revealed an exon 2 and 3 skipped transcript (T-2,3) in both patient and

control cDNAs, but the T-2,3 transcript was present at a 10-fold-higher level in patient 2 than in the controls. We also cloned whole cDNA from patient 2; this revealed that 4 of 41 clones harbored the unskipped synonymous Leu120 transcript. To prove that Leu120 variant augments exon skipping, we cloned genomic DNA containing exons 2 to 4 with flanking intronic regions into Exontrap vector pET and analyzed cDNA extracted from transfected COS-7 cells. cDNA from the wild-type allele displayed a strong band corresponding to the unskipped segment and a weak band representing T-2,3, whereas cDNA from the mutant allele harbored the T-2,3 transcript exclusively.

Wild-type and mutant DPAGT1 expression in COS-7 cells. Densitometric evaluation of immunoblots of cell extracts in 3 experiments showed that, compared with wild-type, the Met1Leu mutant was expressed at $39\% \pm 9\%$ (mean $\pm$ standard error, $p = 0.025$), whereas expression of the His375Tyr and Val264Met mutants were not significantly different from wild-type (figure 1H).

Enzyme activities of Val264Met and His375Tyr mutants in COS-7 cells. To further investigate the pathogenicity of the Val264Met and His375Tyr mutants, we cloned these mutants and wild-type DPAGT1 into COS-7 cells and compared enzyme activities in extracts of control and mutant cells by the paired t test. In 3 separate assays, enzyme activity of the His375Tyr mutant was reduced to 18.5 ± 0.43 (mean $\pm$ standard error) compared with wild-type of 32.4 ± 0.60 ($p < 0.002$); in 2 separate assays, enzyme activity of the Val264Met mutant was barely detectable at 0.41 ± 1.69 compared with wild-type of 31.44 ± 0.60 ($p = 0.022$). Enzyme activity of the Met1Leu mutant was not significantly different from that of wild-type.

Expression of glycosylated proteins in muscle extracts. Muscle extracts of patients 1 and 2 and of 2 controls were immunoblotted with anti-RL2 and anti-O-GlcNac antibodies that recognize approximately 70-kDa *N*- and *O*-glycosylated proteins²⁴ (figure 1F). With both antibodies, control muscles displayed a major band around 70 kD. This band was essentially absent in patient 1 but was normally expressed in patient 2. We also immunoblotted muscle extracts for STIM1, a glycosylated sarcoplasmic reticulum-associated Ca^{2+} sensor protein.²⁵ Normal control muscle had a single band of STIM1 protein at the expected approximately 80 kD, whereas immunoblots of both patients displayed double bands with the lower than expected band likely representing a hypoglycosylated protein (figure 1G).

In vitro electrophysiology and counts of AChRs per EP. The MEPP and MEPC amplitudes and the quantal

content of the EPP (m) fell between 40% and 60% of the corresponding control values (table 1). The MEPC decay time was normal, consistent with normal AChR channel kinetics. It is of note that, in patient 2, the values for the quantal content of the EPP (m) and for quanta available for release (n) were not normally distributed but fell into a very low and a normal group. The probability of quantal release (p) was normal in both patients. The number of α -bgt binding sites per EP was 36% and 38% of the control mean.

Histochemical observations. In both patients, the mean diameter of the type 1 fibers was smaller than that of the type 2 fibers (26 vs 46 μ m in patient 1 and 41 vs 70 μ m in patient 2), consistent with fiber-type disproportion²⁶ (figure 2I). Small NADH (nicotinamide adenine dinucleotide) dehydrogenase–reactive tubular aggregates were present in all muscle specimens (figure 2H). Both patients harbored sparse fibers with large acid phosphatase–positive autophagic vacuoles (figure 2G), but numerous autophagic vacuoles were detected in a previous thigh muscle specimen of patient 2. Rare necrotic and regenerating muscle fibers were present in patient 2.

In frozen sections, the reaction for AChE and AChR was less intense at patient (figure 2, A and C) than at control EPs (figure 2, B and D). Face-on views of EPs on AChE-reacted, fixed, teased muscle fibers showed small EP regions arranged in irregularly ovoid arrays (figure 2F). Few fibers had multiple EPs (figure 2E). No EPs had a normal pretzel shape.

EP ultrastructure. Simple inspection of 62 EP regions of both patients revealed pre- and postsynaptic

abnormalities: 58% of the nerve terminals were abnormally small (figure 2, K and L), 14% were completely surrounded by Schwann cells, and 10% were degenerating (figure 2J); 76% of the postsynaptic regions were abnormally simple (figure 2, J–M), and 7% were degenerating (figure 2M). Numerous EPs were associated with abundant junctional sarcoplasm (figures 2K and e-1). Quantitative analysis of 38 EP regions in patient 1 and of 24 EP regions in patient 2 revealed abnormally small nerve terminal and postsynaptic areas, and reduced postsynaptic membrane density (table 2). Postsynaptic AChR expression, visualized with peroxidase-labeled α -bgt, was focally attenuated at most EPs (figure 2, K and L) but was strongly expressed at some EPs of patient 2 (figure e-1).

Muscle fiber ultrastructure. Electron microscopy revealed more extensive abnormalities than detected by light microscopy. A fair number of fibers displayed degenerating mitochondria (figure 2N), lipofuscin bodies (figure 2O), dilated profiles of the sarcoplasmic reticulum, and myofibrillar rarefaction (figure 2O). Some fibers contained apoptotic nuclei, numerous lipid droplets, or focal Z-disk streaming associated with mitochondrial depletion, and small autophagic vacuoles. Fibers with advanced degenerative change harbored electron-dense bodies and large autophagic vacuoles filled with myeloid structures and debris (figure 2P).

Isoelectric focusing of serum transferrin. Isoelectric focusing and capillary zone electrophoresis of serum transferrin was performed in all patients. The transferrin glycosylation pattern was normal in patient 1,

Table 1 Microelectrode studies of neuromuscular transmission and EP AChR content

	Controls	Patient 1 (no. of EPs), p^a	Patient 2 (no. of EPs), p^a
MEPP, mV ^b	1.00 $\pm$ 0.03 (165)	0.47 $\pm$ 0.06 (10), <0.001	0.59 $\pm$ 0.06 (12), <0.001
MEPC, nA ^c	3.92 $\pm$ 0.10 (79)	1.68 $\pm$ 0.24 (6), <0.001	—
MEPC decay time constant, ms ^c	3.34 $\pm$ 0.08 (58)	3.71 $\pm$ 0.26 (6), NS	—
Quantal content of EPP 1 Hz, m^d	30 $\pm$ 2.0 (15)	12 $\pm$ 1.4 (10), <0.001	Low values: 12.12 $\pm$ 2.27 (6), <0.001 High values: 37.1 $\pm$ 4.72 (6), NS
Quanta available for release, n	230 $\pm$ 14 (12)	70 $\pm$ 10 (13), <0.005	Low values: 93 $\pm$ 19 (6), <0.001 Normal values: 307 $\pm$ 49 (6), NS
Probability of release, p	0.15 $\pm$ 0.012 (12)	0.17 $\pm$ 0.018 (13), NS	0.14 $\pm$ 0.017 (12), NS
EP AChR content, [¹²⁵ I] α -bgt binding sites	12.8 $\pm$ 0.79 (13)	4.56 E6	4.79 E6

Abbreviations: AChR = acetylcholine receptor; α -bgt = α -bungarotoxin; EP = endplate; EPP = EP potential; MEPC = miniature EP current; MEPP = miniature EP potential; NS = nonsignificant.

Values indicate mean $\pm$ standard error. Numbers in parentheses indicate number of EPs except for control values of [¹²⁵I] α -bgt binding sites and of m and n , where they indicate number of patients.

^aThe p value based on comparison of patient and control means by t test.

^bCorrected for membrane potential of -80 mV and fiber diameter of 50 μ m; 30°C.

^c -80 mV, 22°C.

^dCorrected for membrane potential of -80 mV, nonlinear summation, and non-Poisson release; 30°C; EPPs recorded in absence of curare.

(A-D) Paired fluorescence localization of acetylcholine receptor (AChR) (red color) and acetylcholinesterase (AChE) (green color) at patient (A and C) and control (B and D) endplates (EPs). Note attenuated reactivity for AChR and AChE at patient EPs. (E, F) Face-on views of AChE reactive EPs. In panel E, single muscle fiber displays 3 ovoid-shaped EPs. EP in panel F is composed of multiple small round regions. (G) Muscle fiber harboring acid phosphatase-positive autophagic vacuoles. (H) Type 2 muscle fibers display punctate NADH (nicotinamide adenine dinucleotide) dehydrogenase-reactive tubular aggregates. (I) Fiber-type disproportion (adenosine triphosphatase reaction, pH 4.3). Examination of EP ultrastructure reveals degenerating nerve terminal (NT) in (J), degenerating Schwann cell (SC) in (K), degenerating junctional folds (asterisk in M), and highly simplified postsynaptic regions in J through L. Localization of AChR with peroxidase-labeled α -bungarotoxin reveals patchy and focally attenuated expression of AChR on the postsynaptic membrane (K, L). Examination of muscle fiber ultrastructure reveals degenerating organelles (N, O), loss of myofibrils (O), and large autophagic vacuoles and degraded myofibrils (P). Bars: 50 μ m for A-D and in G; 25 μ m in E and H; 15 μ m in F; 100 μ m in I; 1 μ m in J; 0.5 μ m in K-M; and 2 μ m in N-P.

Table 2 Endplate morphometry

	Patient 1 (38 regions), <i>p</i>	Patient 2 (24 regions), <i>p</i>	13 Controls (45–61 regions)
Nerve terminal area, μm^2	2.7 ± 0.4 , <0.005	2.1 ± 0.2 , <0.001	3.88 ± 0.39
Postsynaptic area, μm^2	3.6 ± 0.4 , <0.001	2.7 ± 0.6 , <0.001	9.89 ± 0.81
Postsynaptic membrane length, μm	9.8 ± 1.2 , <0.001	12.5 ± 1.5 , <0.001	52.74 ± 5.16
Postsynaptic membrane density, $\mu\text{m}/\mu\text{m}^2$	2.8 ± 0.2 , <0.001	4.4 ± 0.4 , <0.01	5.67 ± 0.23

Values indicate mean $\pm$ standard error. More than one region can be present at a given endplate.

but patient 3 displayed an asialotransferrin band, and both patients 2 and 3 showed more disialo- than trisialotransferrin (figure e-2).

DISCUSSION Congenital disorders of glycosylation can cause abnormalities in nearly all organ systems.^{8,27,28} DPAGT1 localizes to the endoplasmic reticulum membrane, traversing it 10 times⁸ (figure 1D). It is essential for embryogenesis, is present in all tissues except some endocrine glands, and is highly expressed in muscle and brain.²⁹ In addition to patients with identified myasthenia,^{5,10} other patients with DPAGT1 congenital disorders of glycosylation were described with hypotonia and feeding difficulties since infancy, refractory seizures, multiple dysmorphic features, microcephaly, and intellectual disability,^{30–34} but were not tested for myasthenia or investigated for a myopathy.

Genotype-phenotype correlations are hindered in patients harboring biallelic mutations. In patients 1 and 3, the Met1Leu mutation reduces protein expression to 39% and eliminates the N-terminal region of the signal peptide, which likely affects incorporation of the mutant protein into the endoplasmic reticulum. That enzyme activity of the Met1Leu mutant was not reduced could be attributable to the assay being done on whole homogenate and not on the microsomal fraction of COS-7 cells, and thus may not reflect *N*-glycosylation in vivo. The His375-Tyr mutation reduced enzyme activity to 57% of wild-type. That combined effects of the 2 mutations impeded glycosylation is shown by markedly reduced glycosylation of the 70-kDa protein in muscle (see figure 1F). Patient 3 is less severely affected than patient 1, indicating intrafamily phenotypic heterogeneity. The transferrin glycosylation pattern was abnormal in patient 3 but not in patient 1 because of normalization of the pattern with age.³⁵

In patient 2, the Val264Met mutation abolishes effective enzyme activity and the synonymous Leu120-Leu mutation causes excessive exon skipping, predicting premature termination of translation. Of note, she retains a low level of unskipped transcript, which partially rescues her phenotype, and on immunoblotting, she

shows normal glycosylation of a 70-kDa protein (figure 1F) but hypoglycosylation of STIM1 (figure 1G) and abnormal glycosylation of serum transferrin (figure e-2). A likely key to the conundrum is that the 2 commercially available antibodies to glycosylated proteins recognize only an approximately 70-kDa protein band, yet myriad glycosylated proteins have different molecular weights, and that different mutant genotypes may result in different patterns of altered glycosylation. We infer that glycosylation of the 70-kDa protein is not relevant to the clinical presentation of patient 2. Microelectrode studies point to mosaicism in *m* and *n*, which segregate into abnormally low and normal values (see table 1). Similarly, at some of her EPs, AChR is normally expressed (figure e-1). Of note, patient 2 illustrates that synonymous mutations can be deleterious and should not be indiscriminately filtered after exome sequencing.

Muscle specimens of our patients displayed fiber-type disproportion, small tubular aggregates, degenerating mitochondria, and destruction of the muscle fiber organelles associated with autophagy. We attribute these abnormalities to hypoglycosylation of multiple muscle fiber proteins. Fiber-type disproportion is caused by defects in structural proteins of the muscle fiber.²⁶ When associated with myasthenic symptoms, it can be a clue to a disorder of glycosylation. Tubular aggregates are a nonspecific finding in diverse myopathies. A recent study identifies mutations in *STIM1* as the cause of a tubular aggregate myopathy.²⁵ STIM1 is a glycosylated protein associated with the sarcoplasmic reticulum and regulates Ca^{2+} uptake by that organelle. We consider STIM1 hypoglycosylation (see figure 1G) a likely cause of the tubular aggregates in patients harboring mutations in *DPAGT1*, *GFPT1*,⁴ and *ALG2*.⁶

We ascribe decreased MEPP amplitude to the AChR deficiency and the simplified postsynaptic region, which reduce postsynaptic input resistance and hence the MEPP amplitude. Because the probability of quantal release was normal, the decreased quantal content of the EPP is attributed to a reduced number of quanta available for release (table 1). This could result from small nerve terminals,³⁶ paucity of synaptic vesicles,³⁷ impaired synaptic vesicle transport to the presynaptic membrane, or a defect in the

molecular machinery governing exocytosis.³⁸ Nerve terminal size was 70% and 54% of normal in patients 1 and 2. This probably contributes to the decrease of n, but hypoglycosylation of one or more proteins of exocytotic machinery may also contribute.

Our findings emphasize that neuromuscular transmission should be evaluated in patients with congenital defects of glycosylation. Finally, we speculate that hypoglycosylation of synapse-specific proteins causes defects in central as well as motor synapses.

AUTHOR CONTRIBUTIONS

Dr. Selcen contributed to patient evaluation, study concept and design, and data acquisition, interpretation, and analysis, and manuscript preparation. Dr. Shen contributed to study data acquisition and interpretation. Joan Brengman contributed to data acquisition. Dr. Li contributed to data analysis and interpretation. Dr. Stans contributed to patient evaluation and tissue acquisition. Dr. Wieben contributed to data analysis and interpretation. Dr. Engel contributed to patient evaluation, study concept and design, data acquisition, interpretation and analysis, and manuscript preparation.

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DISCLOSURE

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