Published in final edited form as:

Neuromuscul Disord. 2011 June ; 21(6): 387–395. doi:10.1016/j.nmd.2011.03.002.

Endplate Structure and Parameters of Neuromuscular Transmission in Sporadic Centronuclear Myopathy Associated with Myasthenia

Teerin Liewluck^a, Xin-Ming Shen^a, Margherita Milone, and Andrew G. Engel^{*}

Department of Neurology and Neuromuscular Research Laboratory, Mayo Clinic College of Medicine, Rochester, MN

Abstract

Centronuclear myopathy is a pathologically diagnosed congenital myopathy. The disease genes encode proteins with membrane modulating properties (*MTM1*, *DNM2*, and *BINI*) or alter excitation-contraction coupling (*RYR1*). Some patients also have myasthenic symptoms but electrodiagnostic and endplate studies in these are limited. A sporadic patient had fatigable weakness and a decremental EMG response. Analysis of centronuclear myopathy disease- and candidate- genes identified no mutations. Quantitative endplate structure and in vitro microelectrode studies revealed simplified postsynaptic regions, endplate remodeling with normal nerve terminal size, normal synaptic vesicle density, and mild acetylcholine receptor deficiency. The amplitude of the miniature endplate potential was decreased to 60% of normal. Quantal release by nerve impulse was reduced to 40% of normal due to a decreased number of releasable quanta. The safety margin of neuromuscular transmission is compromised by decreased quantal release by nerve impulse and by a reduced postsynaptic response to the released quanta.

Keywords

Centronuclear myopathy; neuromuscular junction; myasthenic syndrome; synaptic vesicle cycle

1. Introduction

Centronuclear myopathies (CNM) represent a group of clinically and genetically heterogeneous congenital myopathies sharing a common pathology of centronucleated muscle fibers without a significant number of necrotic or regenerating fibers. To date, mutations in genes encoding myotubularin (*MTM1*), dynamin 2 (*DNM2*), amphiphysin 2 (*BINI*) and skeletal muscle ryanodine receptor (*RYR1*) have been identified in nearly 70% of CNM patients [1].

Myotubularin is a phosphoinositide phosphatase important for endosomal trafficking [2]. Non-quantitative EM studies find disorganization of the T-tubules in zebrafish model of

© 2011 Elsevier B.V. All rights reserved.

^{*}Corresponding author: Andrew G. Engel, MD, Department of Neurology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA. Tel.: +1 507-284-5102; fax: +1-507-284-5831; age@mayo.edu.

^aDrs. Liewluck and Shen contributed equally to this work.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

myotubularin-CNM [3]. Dynamin 2 is a large GTPase involved in the release of nascent vesicles during endocytosis and intracellular membrane trafficking. Dynamin 2 induces membrane curvature in both clathrin-dependent and clathrin-independent endocytosis and associates with amphiphysin on phagosomes [4]. Amphiphysin 2 is involved in membrane remodeling, is important for organization of the T-tubules, and is regulated by phosphoinositides [5]. T-tubules have been described as sometimes slightly dilated in amphiphysin 2-CNM [6]. At the neuromuscular junction, amphiphysin promotes assembly of neuronal dynamin 1 at the neck of clathrin coated synaptic vesicles undergoing endocytosis [7]. Dynamin 1 then binds to clathrin and adaptor protein 2 (AP-2) and acts as a molecular scissor to effect fission of the coated vesicle from the presynaptic membrane [8]. RYR1 is the calcium release channel of the sarcoplasmic reticulum (SR) essential for excitation-contraction coupling [9,10].

The age of onset and severity of weakness in CNM patients vary widely depending on the causative gene. X-linked CNM due to mutations of *MTM1* causes severe neonatal-onset and often lethal weakness. *DNM2* mutations typically give rise to a childhood-onset, autosomal dominant myopathy with mild weakness [11,12]. *RYR1* and less commonly *BINI* mutations underlie an autosomal recessive CNM with an intermediate phenotype between *MTM1*- and *DNM2*-CNMs [10,13]. Four intermediate phenotype CNM patients with different dominant *RYR1* mutations were also reported [9,10]. Recently, a heterozygous mutation in *MTMR14*, the gene encoding myotubularin-related protein 14, was observed in two Brazilian patients with an intermediate phenotype. One of these patients also carried a known *DNM2* mutation and the other has an asymptomatic parent carrying the same *MTMR14* mutation [14], suggesting that *MTMR14* is a modifying rather than a causative gene, and this was recently confirmed by a study in a zebrafish model of *MTMR14*-CNM [15].

Apart from limb weakness, ptosis and ophthalmoparesis are common manifestations in different CNM subtypes [6,16–24]. Some CNM patients also experience abnormal fatigability [6,16,25–27]. The constellation of these symptoms suggests an associated defect of neuromuscular transmission but a decremental EMG response, abnormal SFEMG, or changes in EP morphology were documented in only few CNM patients [6,16,18,19,21,22] (Table 1). Moreover, some previously reported ultrastructural changes represent preparatory artifacts. Most of these studies were done in the pre-genetic era but a recently reported CNM patient with fatigable weakness and a decremental EMG response is homozygous for a missense mutation in *BINI* [6]. Detailed analysis of endplate ultrastructure and neuromuscular transmission have not been available to date.

We here report a CNM patient with fatigable weakness and a decremental EMG response. We analyze the structure of intercostal muscle endplates by quantitative electron microscopy and the parameters of neuromuscular transmission by in vitro microelectrode studies. Mutation analysis of currently identified and candidate CNM genes revealed no mutations.

Methods

All human studies were in accord the regulations of the Mayo Institutional Review Board.

Endplate studies

Acetylcholinesterase (AChE) and the acetylcholine receptor (AChR) were localized in frozen sections with a monoclonal anti-AChE antibody and rhodamine-labeled α -bungarotoxin (α -bgt) [28]. Endplates were localized for electron microscopy and quantitatively analyzed by established methods [29]. Peroxidase-labeled α -bgt was used for the ultrastructural localization of AChR [30]. The number of AChRs per endplate (EP) was measured with [125 I] α -bgt, as described previously [31]. The amplitude of miniature EP

potential (MEPP), and EP potential (EPP), estimates of the number of quanta released by nerve impulse (m), the number of releasable quanta (n), and the probability of quantal release (p) were determined as previously described [32–34].

Mutation analysis

We directly sequenced all exons and flanking untranslated regions of all known causative genes of CNM (*MTM1*, *DNM2*, *BIN1*, *RYR1* and *MTMR14*) and candidate genes including genes encoding amphiphysin 1 (*AMPH*), dynamin 1 (*DNM1*), and Sorting Nexin 9 (*SNX9*). Genomic sequences of all genes were obtained from GenBank. *DNM1* encodes a neuron-specific dynamin and *AMPH* codes for a neuron-specific amphiphysin isoform and both proteins play an important role in synaptic vesicle endocytosis [7,35]. *SNX9* is a ubiquitously expressed protein that recruits dynamin-2 to sites of endocytosis and stimulates its GTPase activity. *SNX9* also contains a BAR domain that promotes membrane curvature like amphiphysin [36].

3. Results

Clinical data

A 39-year-old man was the product of a normal pregnancy. There were no perinatal complications. His early motor development was normal but he never ran well and fell easily while running. At age 13 years, he noted difficulty raising his arms overhead, combing his hair, and climbing stairs. He had had mild ptosis when tired but no other symptoms referable to cranial muscles. His symptoms progressed slowly until the age of 25 years but then became stable or even slightly improved, but he also experienced episodes of increased fatigability lasting 3–4 weeks and low grade myalgias sometimes related to overexertion, alcohol consumption, or aphthous stomatitis. The serum CK level was intermittently elevated to 3 to 10 times above the upper limit of normal. He had no episodes of pigmenturia. The parents are fourth degree cousins. There are no other similarly affected family members.

Physical examination revealed high-arched feet and hammertoes. There was hypertrophy of gastrocnemius and atrophy of thigh, upper arm, and pectoral muscles. He rose from the floor with a Gowers maneuver (Fig. 1). The arm elevation time was close to a minute, but he struggled to keep his arms in the horizontal position. The leg elevation time was only 25 seconds. He rose from a low-sitting position ten times but with increasing difficulty. Repeated shoulder abductions against resistance caused increasing weakness of the deltoid muscles. There was no weakness of the cranial muscles, and the pupillary light reflex was normal. There was no ptosis or fatigability of eyelid elevators. Detailed manual muscle testing showed mild to moderately severe weakness of pelvic and pectoral girdle muscles with selectively severe involvement of the gluteus maximus and toe extensor muscles and mild weakness of the distal limb muscles. The tendon reflexes were hypoactive to absent in the arms but normally active at the knees and ankles. The rest of the neurologic examination was unremarkable.

Tests for acetylcholine receptor and MuSK antibodies were negative. The serum CK level was 878 U/L (normal, 52–336 U/L).

Standard nerve conduction studies gave normal results. Repetitive nerve stimulation at 2-Hz of the musculocutaneous and spinal accessory nerves revealed a 19% decrement in the biceps and a 35% decrement in the trapezius muscle of the fourth compared to the first evoked compound muscle action potential. Edrophonium decreased the decremental response in the trapezius to 23%, and 3,4-diaminopyridine (3,4-DAP) diminished it to 13%,

of the initial value. Needle EMG examination of limb muscles showed unstable, short-duration, low-amplitude motor unit potentials.

Based on results of the EMG studies, we treated the patient with 60 mg pyridostigmine twice daily and 10 mg 3,4-DAP three times daily. Two days later he could climb 60 steps without having to rest whereas previously he could climb only 20 steps due to fatigue and myalgias. There was now only subtle weakness of the gluteus maximus and tibialis anterior muscles and slight fatigable weakness of the deltoid muscles. A few weeks later, however, he experienced myalgias after taking both medications and now uses only 60 mg pyridostigmine when he is more active than usual.

Histochemistry

Biopsy specimens of intercostal, serratus anterior and latissimus dorsi muscles showed similar findings. The muscle fibers varied from 30 to 120 μm in diameter except serratus anterior contained few atrophic fibers consisting of clumps of nuclei surrounded by a thin rim of cytoplasm (Fig. 2). Nearly all intercostal and serratus anterior and 80% of the latissimus dorsi fibers displayed one or more internal nuclei. Rare fibers were necrotic and replaced by macrophages or were regenerating. There was a moderate increase in perimysial fibrous and fatty connective tissue. In NADH dehydrogenase-reacted section, some fibers displayed small irregular attenuations of enzyme activity or aberrant or coiled myofibrils. Rare fibers showed a radial arrangement of the myofibrils. Tubular aggregates were absent. As adjudged by ATPase-reacted sections, most fibers in serratus anterior and latissimus dorsi and all fibers in the intercostal muscle were histochemically type 1. In latissimus dorsi, type 1 fibers had a smaller mean diameter than type 2 fibers.

Endplate studies: Light microscopy

Patient EPs observed in transversely sectioned frozen sections expressed AChE normally but the expression of AChR was mildly attenuated compared to control EPs (Fig. 3). In AChE reacted teased muscle fibers, 28 of 48 fibers displayed multiple synaptic contacts consisting of 2 to 4 ovoid EPs each about 10 μm in diameter (Fig. 3E) or multiple small pleomorphic EP regions, with the smallest regions reacting for AChE with reduced intensity, arrayed over an extended length of the fiber (Fig. 3F). The latter finding also occurs in other myasthenic disorders associated with ongoing destruction and remodeling of the postsynaptic region [37,38].

Endplate ultrastructure

Fifty-five patient and 162 control EP regions were inspected for changes of EP conformation. Forty-one of 55 patient EP regions displayed one or more of the following abnormalities: simplified (Fig. 4A and C) or absent (Figs. 4B and 5) junctional folds, partial occupancy of the postsynaptic region by nerve terminal, abundant junctional sarcoplasm harboring numerous dilated vesicles ranging from 200 to 900 nm in diameter some of which resembled dilated components of the sarcoplasmic reticulum (Fig. 5), and occasional Schwann cell encasement of the nerve terminals (Table 2).

Extrajunctional muscle fiber regions showed streaming of few Z-disks or focal dilations of the sarcoplasmic reticulum, but no clearly identifiable T-tubule abnormalities.

Morphometric analysis of 40 to 48 EP regions showed the nerve terminal area decreased to 75% of the control value but this did not reach statistical significance. The synaptic vesicle density (number/ μm^2 of nerve terminal area) was not significantly different from that at control EP regions. In contrast, the postsynaptic area of folds and clefts was reduced to 40%,

and the postsynaptic membrane density was decreased to 76%, of the control value (Table 3).

In vitro electrophysiology and counts of acetylcholine receptors per endplate

Indirect stimulation of muscle elicited no contractions. Therefore all measurements were obtained in the absence of curare (Table 4). The MEPP amplitude was reduced to 59% of normal and its decay time was prolonged 1.5-fold. The quantal content of the EPP was reduced to 42% of normal. The probability of quantal release (p) was normal but the number of releasable quanta (n) was only half-normal. The number of α -bungarotoxin binding sites per EP was mildly reduced.

Depolarization of the nerve terminal by raising the $[K^+]$ from 5 to 20 mM in the bath increased the MEPP frequency 65-fold in patient and 41-fold in control fibers, indicating the decreased quantal content of the patient EPP was not likely due to reduced Ca^{2+} ingress into the depolarized nerve terminal.

Mutation analysis

Direct sequencing of the known CNM genes *MTM1*, *DNM2*, *BIN1*, *RYR1*, of the modifier gene *MTMR14*, and of candidate genes *AMPH*, *DNM1* and *SNX9* revealed no mutations.

Discussion

We here present the first detailed structural and electrophysiologic analysis of the neuromuscular junction in a CNM associated with a myasthenic syndrome. The myasthenic symptoms were mitigated by cholinergic agonists but their use was limited by myalgias. Although it is now known that a myasthenic component can be a facet of CNMs, it is not known whether it is present in all CNMs. Therefore it will be important to carefully search for a defect of neuromuscular transmission in all CNM patients.

Endplate pathology

Intercostal muscle studies show formation of new EP regions on individual fibers and simplified or absent junctional folds at some EPs (Table 2). At individual EP regions the postsynaptic area of folds and cleft is reduced to 40% and the postsynaptic membrane density is decreased to 65% of the corresponding control value. These alterations can at least in part be attributed to immaturity of newly formed EP regions. In contrast, the nerve terminal area and the density of the synaptic vesicles in the nerve terminal were not significantly different from normal.

The numerous dilated vesicles in the junctional sarcoplasm of many EPs were of special interest. Some of these vesicles were empty but others had a fine granular content similar to that present in lateral vesicles of the sarcoplasmic reticulum, and some of these vesicles appeared in a triadic configuration. The functional significance of this finding remains unknown.

Endplate electrophysiology

The in vitro electrophysiologic studies reveal reduction of the quantal content of the EPP (m) to ~40% of normal and of the MEPP amplitude to ~60% of normal. In addition, the decay time of the MEPP is 1.5 times longer than normal (Table 4). Because m is a function of the probability of quantal release (p) and the number of releasable quanta (n) according to the relationship $m = n \times p$ [39], and because p is normal, the decrease in m is attributed to the decrease in n . The parameters affecting n include the nerve terminal volume [40], synaptic vesicle density [41], and integrity of the synaptic vesicle cycle. The total nerve

terminal volume per EP was not determined but synaptic vesicle density in individual nerve terminals was not significantly altered. A defect in the synaptic vesicle cycle could be due to impaired exocytosis or endocytotic retrieval of the vesicles. Impaired vesicle exocytosis is unlikely to reduce the synaptic vesicle density but decreased endocytotic retrieval could reduce it in the wake of physiologic activity; this, however, would not be reflected by our analysis because the EPs for EM studies were fixed in the resting state. Future quantitative EM studies comparing the synaptic vesicle density before and after tetanic stimulation or fluorescent dye studies to monitor vesicle traffic in patient and control muscles will be required to distinguish between these possibilities.

The decrease of the MEPP amplitude can be attributed to a combination of factors. (1) Simplification of the postsynaptic regions reduces the input resistance of the EP and hence the amplitude of the MEPP [42]. (2) The number of AChRs per EP and the AChR index were decreased to 78% and 76% of normal, respectively. (3) The immature EP regions may express immature AChRs containing the γ instead of the ϵ subunit, and opening events of the γ -AChR are of lower amplitude and of longer duration than those of the adult ϵ -AChR. Presence of γ -AChR at some EPs would also account for the 1.5-fold prolonged decay time of the MEPP.

Our genetic studies exclude with reasonable certainty mutations in currently recognized disease and candidate genes for CNM. This implies that other CNM disease genes likely exist and await discovery. That the physiologic abnormality in our patient involves the synaptic vesicle cycle predicts that the disease gene, like previously identified CNM disease genes, has membrane modulating properties.

Acknowledgments

This work was supported by National Institutes of Health R01 Grant NS6277 and by a Research Grant from the Muscular Dystrophy Association.

Reference List

1. Romero NB. Centronuclear myopathies: a widening concept. *Neuromuscul Disord.* 2010; 20:223–228. [PubMed: 20181480]
2. Tsujita K, Itoh T, Ijuin Y, et al. Myotubularin regulates the function of the late endosome through the GRAM domain-phosphatidylinositol 3,5-bisphosphate interaction. *J Biol Chem.* 2004; 279:13817–13824. [PubMed: 14722070]
3. Dowling JJ, Vreede AP, Low SE, et al. Loss of myotubularin function results in T-tubule disorganization in zebrafish and human myotubular myopathy. *PLoS Genet.* 2009:e31000372. Epub 2009 Feb 6.
4. Lundmark R, Carlsson SV. Driving membrane curvature in clathrin-dependent and clathrin-independent endocytosis. *Semin Cell Dev Biol.* 2010; 21:363–370. [PubMed: 19931628]
5. Lee E, Marcucci M, Daniell L, et al. Amphiphysin 2 (Bin1) and T-tubule biogenesis in muscle. *Science.* 2002; 297:1193–1196. [PubMed: 12183633]
6. Claeys KG, Maisonobe T, Bohm J, Laporte J, Hezode M, Romero NB. Phenotype of a patient with recessive centronuclear myopathy and a novel BIN1 mutation. *Neurology.* 2010; 74:519–521. [PubMed: 20142620]
7. Wigge P, McMahon HT. The amphiphysin family of proteins and their role in endocytosis at the synapse. *Trends Neurosci.* 1998; 21:339–344. [PubMed: 9720601]
8. DAvid C, McPherson PS, Mundigl O, De Camilli P. A role of amphiphysin in synaptic vesicle endocytosis suggested by its binding to dynamin in nerve terminals. *Proc Natl Acad Sci USA.* 1996; 93:331–335. [PubMed: 8552632]

9. Jungbluth H, Zhou H, Sewry CA, et al. Centronuclear myopathy due to a de novo dominant mutation in the skeletal muscle ryanodine receptor (RYR1) gene. *Neuromuscul Disord.* 2007; 17:338–345. [PubMed: 17376685]
10. Wilmhurst JM, Lillis S, Zhou H, et al. RYR1 mutations are a common cause of congenital myopathies with central nuclei. *Ann Neurol.* 2010; 68:717–726. [PubMed: 20839240]
11. Jungbluth H, Wallgren-Pettersson C, Laporte J. Centronuclear (myotubular) myopathy. *Orphanet J Rare Dis.* 2008; 3:26. [PubMed: 18817572]
12. Bitoun M, Maugren S, Jeanette PY, et al. Mutations in dynamin 2 cause dominant centronuclear myopathy. *Nat Genet.* 2005; 37:1207–1209. [PubMed: 16227997]
13. Nicot AS, Toussaint A, Tosch V, et al. Mutations in amphiphysin 2 (BIN1) disrupt interaction with dynamin 2 and cause autosomal recessive centronuclear myopathy. *Nat Genet.* 2007; 39:1134–1139. [PubMed: 17676042]
14. Tosch V, Rhode HM, Tronchere H, et al. A novel PtdIns3P and PtdIns(3,5)P2 phosphatase with an inactivating variant in centronuclear myopathy. *Hum Mol Genet.* 2006; 15:3098–3106. [PubMed: 17008356]
15. Dowling JJ, Low SE, Busta AS, Feldman EL. Zebrafish MTMR14 is required for excitation-contraction coupling, developmental motor function and the regulation of autophagy. *Hum Mol Genet.* 2010; 19:2668–2681. [PubMed: 20400459]
16. Sher JH, Rimalowski AB, Athassiades TJ, Aronson SM. Familial centronuclear myopathy: a clinical and pathological study. *Neurology.* 1967; 17:727–742. [PubMed: 15088533]
17. Sarnat HB. Myotubular myopathy: Arrest of morphogenesis of myofibers associated with persistence of fetal vimentin and desmin. *Can J Neurol Sci.* 1990; 17:109–123. [PubMed: 2357647]
18. Elder GB, Dean D, McComas AJ, DeSa D. Infantile centronuclear myopathy. Evidence suggesting incomplete innervation. *J Neurol Sci.* 1983; 60:79–88. [PubMed: 6875615]
19. Fidzianska A, Goebel HH. Aberrant arrested in maturation neuromuscular junctions in centronuclear myopathy. *J Neurol Sci.* 1994; 124:83–88. [PubMed: 7931426]
20. Baradello A, Vita G, Girlanda P, Roberto ML, Carozza G. Adult-onset centronuclear myopathy: evidence against a neurogenic pathology. *Acta Neurol Scand.* 1989; 80:162–166. [PubMed: 2816279]
21. Ambler MW, Neave C, Singer DB. X-linked recessive myotubular myopathy: II. Muscle morphology and human myogenesis. *Hum Pathol.* 1984; 15:1107–1120. [PubMed: 6542063]
22. Harriman DG, Haleem MA. Centronuclear myopathy in old age. *J Pathol.* 1972; 108:237–247. [PubMed: 4119091]
23. Bergen BJ, Carry MP, Wilson WB, Barden MT, Ringel SP. Centronuclear myopathy: extraocular- and limb-muscle findings in an adult. *Muscle Nerve.* 1977; 3:165–171. [PubMed: 6445039]
24. Bender AN, Bender MB. Muscle fiber hypotrophy with intact neuromuscular junctions. A study of a patient with congenital neuromuscular disease and ophthalmoplegia. *Neurology.* 1977; 27:206–212. [PubMed: 557754]
25. Sugie H, Hanson R, Rasmussen G, Verity MA. Congenital neuromuscular disease with type I fibre hypotrophy, ophthalmoplegia and myofibril degeneration. *J Neurol Neurosurg Psychiatr.* 1982; 45:507–512. [PubMed: 7119813]
26. Zanoteli E, Oliveira AS, Schmidt B, Gabai AA. Centronuclear myopathy. Histopathological aspects in ten patients with childhood onset. *J Neurol Sci.* 1998; 158:76–82. [PubMed: 9667782]
27. Bradley WG, Price DL, Watanabe CK. Familial centronuclear myopathy. *J Neurol Neurosurg Psychiatr.* 1970; 33:687–93. [PubMed: 5478951]
28. Fambrough DM, Engel AG, Rosenberry TL. Acetylcholinesterase of human erythrocytes and neuromuscular junctions: Homologies revealed by monoclonal antibodies. *Proc Natl Acad Sci USA.* 1982; 79:1078–1082. [PubMed: 6175961]
29. Engel, AG. Quantitative morphological studies of muscle. In: Engel, AG.; Franzini-Armstrong, C., editors. *Myology*. 2. New York: McGraw-Hill; 1994. p. 1018-1045.
30. Engel AG, Lindstrom JM, Lambert EH, Lennon VA. Ultrastructural localization of the acetylcholine receptor in myasthenia gravis and in its experimental autoimmune model. *Neurology.* 1977; 27:307–315. [PubMed: 557772]

31. Engel AG. The investigation of congenital myasthenic syndromes. *Ann N Y Acad Sci.* 1993; 681:425–434. [PubMed: 7689310]
32. Elmqvist D, Quastel DMJ. A quantitative study of end-plate potentials in isolated human muscle. *J Physiol (Lond).* 1965; 178:505–529. [PubMed: 5827910]
33. Engel AG, Nagel A, Walls TJ, Harper CM, Waisburg HA. Congenital myasthenic syndromes. I. Deficiency and short open-time of the acetylcholine receptor. *Muscle Nerve.* 1993; 16:1284–1292. [PubMed: 8232383]
34. Uchitel O, Engel AG, Walls TJ, Nagel A, Atassi ZM, Bril V. Congenital myasthenic syndromes. II. A syndrome attributed to abnormal interaction of acetylcholine with its receptor. *Muscle Nerve.* 1993; 16:1293–1301. [PubMed: 8232384]
35. Smillie KJ, Cousin MA. Dynamin I phosphorylation and the control of synaptic vesicle endocytosis. *Biochem Soc Symp.* 2005; 72:87–97. [PubMed: 15649133]
36. Lundmark SR, Carlsson SR. SNX9 - a prelude to vesicle release. *J Cell Sci.* 2009; 122:5–11. [PubMed: 19092055]
37. Engel AG, Santa T. Histometric analysis of the ultrastructure of the neuromuscular junction in myasthenia gravis and in the myasthenic syndrome. *Ann N Y Acad Sci.* 1971; 183:46–63. [PubMed: 5287834]
38. Engel AG, Lambert EH, Mulder DM, et al. A newly recognized congenital myasthenic syndrome attributed to a prolonged open time of the acetylcholine-induced ion channel. *Ann Neurol.* 1982; 11:553–569. [PubMed: 6287911]
39. Katz, B. *Nerve, Muscle, and Synapse.* New York: McGraw-Hill; 1966.
40. Engel AG, Lambert EH, Gomez MR. A new myasthenic syndrome with end-plate acetylcholinesterase deficiency, small nerve terminals, and reduced acetylcholine release. *Ann Neurol.* 1977; 1:315–330. [PubMed: 214017]
41. Walls TJ, Engel AG, Nagel AS, Harper CM, Trastek VF. Congenital myasthenic syndrome associated with paucity of synaptic vesicles and reduced quantal release. *Ann N Y Acad Sci.* 1993; 681:461–468. [PubMed: 8395161]
42. Martin AR. Amplification of neuromuscular transmission by postjunctional folds. *Proc R Soc Lond B.* 1994; 258:321–326.

Fig. 1. Patient at age 39 years. Upper panels: note decreased bulk of pectoralis major, upper arm and thigh muscles, asymmetric enlargement of calf muscles, and increased lumbar lordosis.. Lower panel shows patient rising from the floor.

Fig. 2.
Serratus anterior muscle, H&E stain. Note internalized nuclei and three highly atrophic fibers.

Fig. 3. Endplate cytochemistry. (A–D): Transverse sections showing paired fluorescence localization of AChE (green signal) and AChR (red signal) at control (A and B) and patient (C and D) EPs. AChR expression is mildly attenuated at patient EPs. (E) and (F): Synaptic contacts visualized with reaction for AChE on teased patient fibers. Note double EP in (E) and multiple small EP regions (F). Bar = 20 μ m for each panel.

Fig. 4. Endplate ultrastructure. In panel (A) the imaged region shows poorly developed junctional folds with only a single secondary cleft opening into the primary synaptic cleft. In panel (B) the postsynaptic membrane is highly simplified and lacks junctional folds. Empty mitochondrion represents fixation artifact. Panel (C) is an unstained section showing localization of AChR with peroxidase labeled α -bungarotoxin. The EP region has few secondary clefts and AChR expression is mostly on tops of the junctional folds. Bars = 0.5 μ m.

Fig. 5. Dilated vesicles in junctional sarcoplasm. The vesicles are either empty or contain fine, floccular material and resemble dilated SR vesicles. One dilated vesicle (x) abuts on a tubule next to a smaller vesicle suggesting a triad. Bar = 0.5 μ m.

Table 1

Reported CNM patients with findings suggesting neuromuscular transmission defect

Gene or mode of inheritance	Sex/onset	Clinical presentations			Tensilon test	RNS or SFEMG	Endplate morphology	Refs.
		Prosis	Oculo-paresis	Fatigue				
<i>BN/1</i>	Male/early childhood	+	+	+	–	14–30% decrement	N/A	[6]
X-linked	Male/neonate	N/A	N/A	N/A	N/A	N/A	Absent junctional folds at some EPs	[19]
X-linked	Male/neonate	N/A	N/A	N/A	–	N/A	Absent junctional folds at single EP	[21]
Sporadic	Male/neonate	+	+	N/A	–	N/A	Variable shape and size of cholinesterase reactive EPs	[18]
Sporadic	Female/Adult-onset	–	–	N/A	N/A	N/A	Sprouting axons; elongated EPs with methylene blue stain	[22]
Autosomal recessive	Female/infancy	+ in 2 patients	+ in 1 patient	+ in 2 patients	N/A	N/A	Elongated cholinesterase reactive EPs	[16]

+, present; –, absent or negative; N/A, not available; RNS, repetitive nerve stimulation; SFEMG, single fiber EMG

Table 2Frequencies of conformational changes at patient and controls endplates^a

	Patient EP regions (%)	Control EP regions (%)	<i>P</i> ^b
Degenerating junctional folds ^c	3.6	4	NS
Partially occupied postsynaptic region	18.2	0	<0.001
Denuded postsynaptic region	7.2	9.3	NS
Absent or highly simplified junctional folds	40	8.6	<0.001
Dilated vesicles in junctional sarcoplasm	47	0	<0.001
Schwann cell encasement of nerve terminals	5.5	0	<0.02

^aFifty-five patient and 162 control EP regions were analyzed. An EP region is defined as a nerve terminal and the associated postsynaptic region. More than one region can occur at an EP, and more than one conformational change can occur at an EP region.

^b*P* values based on *z* test

^cDegeneration of junctional folds also simplifies the postsynaptic region.

Table 3**Morphometric Analysis of Endplate Regions**

	Patient (No. of Regions)	13 Controls (No. of Regions)	<i>P</i>^a
Nerve terminal area (μm^2)	3.05 ± 0.34 (45)	3.88 ± 0.39 (63)	NS
Synaptic vesicles/ μm^2	47.5 ± 4.3 (35)	50.3 ± 3.6 (47)	NS
Postsynaptic area (μm^2)	4.611 ± 0.31 (42)	10.60 ± 0.79 (59)	<0.001
Postsynaptic membrane density ($\mu\text{m}/\mu\text{m}^2$)	3.75 ± 0.18 (40)	5.83 ± 0.25 (47)	<0.001
AChR index ^b	2.3 ± 0.27 (14)	3.01 ± 0.09 (85)	0.005

Values indicate mean $\pm$ SE. More than one region can occur at an EP.

^a *P* values are based on *t*-test except for the synaptic vesicle density which is based on the rank-sum test.

^b AChR-reactive postsynaptic membrane length normalized for the length of the primary synaptic cleft.

Table 4Microelectrode studies of neuromuscular transmission and [125 I] α -bungarotoxin binding sites per endplate

	Patient	Controls	<i>P</i>
MEPP amplitude (MEPP _A), mV ^a	0.59 $\pm$ 0.045 (16)	1.00 $\pm$ 0.025 (164)	>0.001
MEPP decay time, ms	5.47 $\pm$ 0.43 (12)	3.75 $\pm$ 0.14 (46)	<0.001
Quantal content of EPP (<i>m</i>) at 1 Hz ^b	13 $\pm$ 2.97 (14)	31 $\pm$ 1.0 (190)	<0.001
Probability of quantal release (<i>p</i>)	0.15 $\pm$ 0.029 (4)	0.15 $\pm$ 0.012 (12)	NS
No. of quanta available for release (<i>n</i>)	115 $\pm$ 51 (4)	230 $\pm$ 14 (12)	<0.01
[125 I] α -bgt binding sites/EP	9.92 E6	12.82 $\pm$ 0.79 E6 (13)	

Values represent mean $\pm$ SE. T = 22 $\pm$ 0.5°C. Numbers in parenthesis indicate number of fibers except for [125 I] α -bgt binding studies where they indicate number of control subjects.

^a MEPP amplitude corrected for a resting membrane potential of -80 mV and a muscle fiber diameter of 55 μ m.

^b Quantal content of EP potential (EPP) at 1 Hz stimulation corrected for resting membrane potential of -80 mV, nonlinear summation, and non-Poisson release.