except for laryngeal stridor in infants[211] but was present in a patient who carries a readthrough mutation in the last codon of *DOK7*.[15]

The disease may present with hypomotility in utero, at birth, or later in infancy. In 16 patients observed by us, the age at onset ranged from birth to 5 years (mean, 1.6 years, median, 1 year).[15] The clinical course varied from mild static weakness limited to the limb-girdle muscles to severe generalized progressive disease with conspicuous muscle atrophy. All had short-term fatigability on exertion. Ten patients experienced intermittent worsenings lasting from days to weeks, as also observed by others.[206,207] Seven patients had significant respiratory embarrassment. The overall course was progressive in 12 of the 16 patients.

Different therapeutic regimens were explored by different investigators.[15,205-210,212,213] Most studies found that pyridostigmine worsened the disease either immediately or gradually. Some patients required admission to intensive care units due to respiratory distress after only a few days of treatment. Treatment with 3,4-DAP is better tolerated but not very effective.[208] In contrast, treatment with ephedrine is beneficial in Dok-7-myasthenia.[15,205,209,210,212,213] The therapeutic doses range from 0.5 to 1 mg/kg/day[212] and from 37.5 to100 mg/day.[213] Because ephedrine is no longer available in the US, we use albuterol sulfate instead with good and sometimes strikingly beneficial results in doses of up to 4 mg three times daily in adults, 2 mg three to four times daily in children 6 to 14 years of age, and 0.1 mg/kg in children 2 to 6 years of age. Some patients respond better to the extended release than the short acting preparation of the drug.

MORPHOLOGY

Type 1 fiber preponderance and type 2 fiber atrophy are common findings. Sparse necrotic and regenerating fibers, pleomorphic decreases in oxidative enzyme activity and target fibers suggesting denervation appear in some patients. The synaptic contacts are small relative to fiber size and are single or multiple on a given fiber. Most EPs lack the normal pretzel shape indicating impaired differentiation of the postsynaptic region (Fig. 8-30). The expression of Dok-7 at the EP is normal or reduced and does not consistently correlate with the clinical state; moreover, Dok-7 expression is attenuated in patients whose CMS is due to low-expressor expressor mutations in AChR.[15]

Electron microscopy analysis reveals ongoing destruction of existing endplates and attempts to form new endplates. Some EPs are normal (Fig. 8-31A) but many display one or more of the following abnormalities: degeneration of junctional folds, frequently severe (Figs. 8-31B and 32A); partial occupancy by nerve terminal (Fig. 8-32A) or absence of nerve terminal (Fig. 8-32B); highly simplified junctional folds (Fig. 8-31B); and degeneration of subsynaptic organelles (Fig. 8-32B). Some nerve terminals are partly or completely encased by Schwann-cell, and few are degenerating. Nerve sprouts appear near degenerating or simplified EPs (Fig. 8-32B). Interestingly, the density and distribution of AChR on nondegenerate junctional folds is normal. Taken together, the light and electron microscopy findings indicate that Dok-7 is required not only for the normal development of the EP but also for maintaining its structural integrity throughout life.

ELECTROPHYSIOLOGY

EMG studies reveal a decremental response of the CMAP but not in all muscles. In our experience the decrement is most consistently detected on stimulation of the facial and trapezius muscles.[15]

In vitro microelectrode studies of neuromuscular transmission of intercostal muscle EPs in 14 patients observed by us showed the mean of the MEPP and MEPC amplitudes were

reduced to approximately two thirds of normal, and there was marked reduction of the quantal content of the EPP (m) in some patients. The predicted amplitude of the EPP, calculated from MEPP amplitude x m, was significantly reduced. That multiple parameters of neuromuscular transmission are affected is likely related to both pre- and postsynaptic structural defects at the junction. However, there was no correlation between the altered parameters of neuromuscular transmission and the clinical state.[15]

MOLECULAR STUDIES

Numerous pathogenic mutations in *DOK7* have been identified since 2006[15,207-209,214,215]; those detected in our laboratory by 2008 are shown in Fig. 8-28B. Nearly all patients carry a common 1124_1127dupTGCC mutation in exon 7. This and other mutations upstream of the C-terminal phosphorylation sites abrogate the ability of Dok-7 to associate with Crk1/Crk1L and hence its activation.[196,199] Mutations disrupting or eliminating the PH and PTB domains of Dok-7 prevent dimerization and association of Dok-7 with MuSK.[198] Mutations resulting in skipping of exons 1 to 6 causing intron inclusion are often intronic and require analysis of cDNA or cloned cDNA (Fig 28B). A recent review lists all Dok-7 mutations reported since 2006.[209]

CMS Caused by Defect in the Hexosamine Biosynthetic Pathway

This CMS was reported in 2011 by Muller and co-workers.[216] It is caused by mutations in *GFPT1* coding for glutamine-fructose-6-phosphate transaminase 1. The enzyme controls the flux of glucose into the hexosamine pathway, and thus the formation of hexosamine products and the availability of precursors for N- and O-linked glycosylation of proteins. The disease gene was discovered by linkage and homozygosity analysis studies of multiplex kinships with a limb-girdle CMS often associated with tubular aggregates in skeletal muscle. The affected patients harbored no mutations in Dok-7, and unlike patients with Dok-7 myasthenia, responded favorably to anticholinesterase medications.

Among the 13 reported patients, most presented in the first decade, about one-fourth had elevated serum CK levels, some had distal as well as proximal weakness, but very few had ptosis or respiratory muscle involvement. Immunoblots of muscle of affected patients revealed decreased expression of O-N-acetylglucosamine residues on numerous muscle proteins. One patient was shown to have a decreased number of EP AChRs. EP fine structure and how the enzyme defect affects parameters of neuromuscular transmission were not determined.[216]

Sodium Channel Myasthenia

Only one patient with this syndrome has been observed to date.[24] A 20-year-old normokalemic woman had abrupt attacks of respiratory and bulbar paralysis since birth lasting 3 to 30 minutes recurring one to three times per month. The apneic attacks were similar to those due to ChAT deficiency. She survived only because she has been on an apnea monitor and received ventilatory support during apneic attacks. She had limited ocular ductions, diffuse fatigable weakness, a high-arched palate, adduction deformity of the knees and ankles, and increased lumbar lordosis (Fig. 8-33). She was mentally retarded and had mild cerebral atrophy attributed to previous hypoxic episodes. Tests for anti-AChR antibodies were negative. She had no similarly affected family members. EMG studies revealed a decremental response of the CMAP at 2 Hz only after a conditioning train of subtetanic or tetanic stimulation. The CMAP declined abnormally during subtetanic stimulation but recovered within 2-3 minutes.

An intercostal muscle specimen showed mild type 1 fiber atrophy. The configuration of cholinesterase reacted EPs and the number of AChRs per EP, were normal. EP ultrastructure and the density and distribution of AChR on the junctional folds was normal. Sodium channel

immunolocalization over the surface membrane and at the EP gave normal results.

Patch-clamp recordings from intercostal muscle EPs revealed no kinetic abnormality of the AChR channel. In vitro microelectrode recordings showed normal amplitude MEPPs and EPPs and normal evoked quantal release but EPPs of the order of 40 mV that depolarized the membrane potential to -40 mV or more failed to trigger action potentials. Taken together, the findings pointed to defect in the muscle sodium channel.

Mutation analysis of *SCN4A* that encodes the muscle sodium channel $Na_v1.4$ revealed two heteroallelic missense mutations: S246L in the cytoplasmic link between the S4 and S5 segments of domain I, and V1442E in the extracellular link between the S3 and S4 segments of domain IV (Fig. 8-34A). Both S246 and V1442 are conserved across $Na_v1.4$ channels of different species.

Expression studies in HEK cells revealed normal expression of the mutant channels. The salient finding was of a hyperpolarizing shift in the voltage dependence of fast inactivation predicting that nearly all V1442E channels at the EP are fast-inactivated and inexcitable at a normal resting membrane potential of -80 mV (Fig. 8-34B). Moreover, a 50 Hz train of 3 ms pulses revealed a precipitous drop of 30% of the peak current amplitude during the first few pulses for the V1442E channels but only a 5% depression for the wild-type or S246L channels (Fig. 8-34C). This indicates V1442E channels are trapped in the fast-inactivated state and accounts for the progressive impairment of neuromuscular transmission at physiologic rates of stimulation. The findings also imply that S246L is a benign mutation.

The phenotype in this CMS differs from that of periodic paralyses caused by other mutations of *SCN4A*. In sodium channel myasthenia the onset is neonatal, the disorder is normokalemic, the attacks selectively involve bulbar and respiratory muscles, physiologic rates of stimulation decrement the CMAP abnormally, and the muscle fiber membrane potential is normal when action potential generation fails. Periodic paralyses due to mutations in *SCN4A* present later in life, the attacks typically spare cranial, bulbar, and respiratory muscles, the serum potassium level increases or declines during attacks, mild exercise for brief periods does not decrement the CMAP, and the resting membrane potential of the muscle fiber is decreased when action potential generation fails.[217,218] In contrast to sodium channel myasthenia, *SCN4A* mutations in periodic paralysis cause a depolarizing shift of the voltage dependence of fast inactivation and exert a gain-of-function effect on sodium channel activation.

After the defect in $Na_v1.4$ was established, the patient was treated with pyridostigmine which improved her endurance, and with acetazolamide which prevented further attacks of respiratory and bulbar weakness.

CMS Caused by Plectin Deficiency

Plectin, encoded by *PLEC*, is a highly conserved and ubiquitously expressed intermediate filament-linking protein concentrated at sites of mechanical stress, such as the postsynaptic membrane of the EP, the sarcolemma, Z-disks in skeletal muscle, hemidesmosomes in skin, and intercalated disks in cardiac muscle. Pathogenic mutations in plectin result in epidermolysis bullosa simplex, a progressive myopathy and, in some patients, a myasthenic syndrome.[219,220] The pathogenesis of the myasthenic syndrome was recently elucidated in two unrelated African American patients suffering from epidermolysis bullosa simplex, progressive myopathy, and abnormal fatigability involving of ocular, facial and limb muscles. Both had a decremental EMG response, no anti-AChR antibodies, and were refractory to pyridostigmine and 3,4-DAP. The first patient can barely take a few steps at age 31 years. The second patient became respirator dependent at age 26 years; eventually he became motionless and died at age 42 years. In both patients, plectin expression was markedly reduced to absent in muscle and severely decreased in

skin. Microelectrode studies of intercostal muscle EPs showed a reduced amplitude of the MEPP amplitude in both patients.

Morphologic studies revealed a wide spectrum of structural abnormalities: clusters of eccentrically positioned large nuclei (Fig. 8-35A and B) with an abnormal patches of chromatin deposits (Fig. 8-36A), focal intrafiber calcium deposits (Fig. 8-35C) attributed to small sarcolemmal defects (Fig. 8-36E), dislocation of the fiber organelles with myofibrillar disarray (Fig. 8-36C, D) rare apoptotic nuclei, cytoplasmic and intranuclear nemaline rods, vacuolar change, and pathologic alterations in membranous organelles. Importantly, there was extensive degeneration and loss of the junctional folds (Fig. 8-37) and signs of EP remodeling (Fig. 8-35D).

The dystrophic changes in muscle are attributed to dislocation of the fiber organelles no longer anchored by the cytoskeletal intermediate filaments. Misalignment and displacement of the myofibrils weakens contractile strength. Separation of mitochondria from myofibrils renders energy delivery to contracting myofibrils inefficient. The eccentrically positioned and structurally abnormal nuclei may be inefficient in their translational activities and in nuclear-cytoplasmic trafficking when not adjacent to fiber domains they subserve. The myasthenic syndrome is attributed destruction of the junctional folds lacking adequate cytoskeletal support.

In the first patient, analysis of *PLEC* revealed a nonsense mutation (p.Gln2057X) and a frameshifting duplication (c.12043dupG/p.Glu4015GlyfsX69). The second patient was heterozygous for a previously reported nonsense mutation at nt 6955 in exon 31 which generates a stop codon (c.6955C>T/p.Arg2319X)[221] and the same duplication mutation detected in P1.

Recently, another patient with EBS, muscular dystrophy and myasthenia was reported.[222] This was the case of an 8-year-old-boy heterozygous for a 3 nucleotide deletion (c.1087delTGC), and a splice site mutation (IVS11+2 T>G) in *PLEC*. Plectin deficiency was detected in skin. Muscle fiber and EP ultrastructure were not examined and the mechanism of the neuromuscular transmission defect was not investigated.

Myasthenic Syndrome Associated with Centronuclear Myopathy (CNM)

Centronuclear myopathies (CNM) are clinically and genetically heterogenous congenital myopathies in which the predominant pathologic alteration is centralization of the muscle fiber nuclei. The implicated disease proteins/genes are myotubularin (*MTM1*), dynamin 2 (*DNM2*), amphiphysin 2 (*BIN1*), and the ryanodine receptor (*RYR1*).[223] Features suggesting a myasthenic disorder, ptosis, ophthalmoparesis,[224-227] abnormal fatigability,[228-230] decremental EMG response[224] or abnormally increased jitter[231] have been observed in clinically and genetically different CNM patients but the mechanism of the putative myasthenic disorder has not been determined.

We investigated a 39-year-old man with centronuclear myopathy and a myasthenic syndrome[232] (Fig. 8-38A and B). He had normal early motor development but could never run, had mild ptosis when tired, showed moderately severe limb-girdle weakness, and fatigued abnormally since his early teens, responded partially to Mestinon, and had a 19-35% EMG decrement in different muscles. Serologic tests for AChR and MuSK antibodies were negative. No mutations were detected in *MTM1, DNM2, BIN1*, and *RYR1*.

Intercostal muscle EP studies demonstrated formation of new EP regions on individual fibers. (Fig. 8-38G and H). AChE expression was similar at patient and control EPs (Fig. 8-38C and E), but AChR expression was mildly attenuated at patient EPs compared to control EPs (Fig. 8-38D and F). Quantitative electron microscopy revealed simplified postsynaptic regions (Fig. 8-39 A-C), with normal nerve terminal size, normal synaptic vesicle density, and mild AChR deficiency (Fig. 8-39C). The MEPP amplitude was decreased to 60% of normal. Quantal release by nerve impulse (m) was reduced to 40% of normal due to a decreased the number of quanta

available for release (*n*). The probability of quantal release (*p*) was normal. Parameters affecting *n* include the size of the nerve terminals, the density of synaptic vesicles in the nerve terminals, and the integrity of the synaptic vesicle cycle. Because nerve terminal size and synaptic vesicle density were normal the decrease in *n* is attributed to a defect in the synaptic vesicle cycle. This, in turn, could be due to a defect in vesicle exocytosis or in retrieval of the exocytosed vesicles from the presynaptic membrane. In summary, the safety margin of neuromuscular transmission in this patients is impaired by decreased quantal release by nerve impulse and, to a lesser extent, by simplification of the postsynaptic regions and the mild AChR deficiency.[232]

Table 1. Classification of Congenital Myasthenic Syndromes Based on 306 Index Patients Observed at the Mayo Clinic*

Defect site	Index cases	Relative frequency (%)
Presynaptic		
Choline acetyltransferase	17	5.5
Paucity of synaptic vesicles**	1	0.3
Congenital Lambert-Eaton-like syndrome**	1	0.3
Synaptic Basal Lamina		
Endplate AChE deficiency	43	14
Postsynaptic Defects		
Primary AChR deficiency with/without kinetic abnormality	109	35.4
Primary kinetic abnormality with/without AChR deficiency	53	17.2
Rapsyn deficiency	48	15.6
Dok-7 myasthenia	31	10
Glutamine-fructose-6-phosphate transaminase deficiency	2	0.6
β2-laminin deficiency	1	0.3
Na-channel myasthenia	1	0.3
Plectin deficiency	1	0.3
Myasthenic syndrome associated with centronuclear myopathy	1	0.3
Total	308	100

* Mutations in MuSK,[201] agrin,[200] and β2-laminin,[121] have been identified in few kinships at other medical centers; **No gene defect identified

Table 2. INVESTIGATION OF MYASTHENIC SYNDROMES

CLINICAL OBSERVATIONS
  History, examination, response to AChE inhibitor
  EMG: conventional needle EMG, repetitive stimulation, SFEMG
  Serologic tests (AChR antibodies, tests for botulism)
MORPHOLOGIC STUDIES
  Routine histochemical studies
  Cytochemical and immunocytochemical localization at EP of AChE, ChAT, AChE, agrin, β_2-
    laminin, AChR, AChR subunits, rapsyn, MuSK, Dok-7, plectin, utrophin, IgG, C3, C9, MAC
  Estimate of the size, shape, and configuration of AChE-reactive EPs or EP regions on teased
    muscle fibers
  Quantitative electron microscopy and electron cytochemistry
ENDPLATE -SPECIFIC ^{125}I-α-BGT BINDING SITES
IN VITRO ELECTROPHYSIOLOGY STUDIES
  Conventional microelectrode studies: MEPP, MEPC, evoked quantal release *(m, n, p)*
  Single-channel patch-clamp recordings: channel types and kinetics
MOLECULAR GENETIC STUDIES
  Mutation analysis (begin with candidate gene analysis; if none identified, analysis is
  initiated according to the relative frequency of mutations in known disease genes)
  Linkage analysis (if no candidate gene or protein recognized)
  Whole genome sequencing
  Microarrays specifically designed for screening multiple candidate genes
EXPRESSION STUDIES IF MUTATION IDENTIFIED

Abbreviations: AChE = acetylcholinesterase; AChR = acetylcholine receptor; α-bgt = α-bungarotoxin; EP = endplate; EMG = electromyography; MAC = C5b-9 complement membrane attack complex; MEEP = miniature endplate potential; MEPC = miniature endplate current; m = number of ACh quanta released by nerve impulse; n = number of ACh quanta available for release; p = probability of quantal release; SFEMG = single fiber EMG.

Table 3 THE DIFFERENTIAL DIAGNOSIS OF CMS

NEONATAL PERIOD, INFANCY, CHILDHOOD
   Spinal muscular atrophy
   Morphologically distinct congenital myopathies (central core disease, nemaline myopathy,
      myotubular myopathy)
   Congenital muscular dystrophies
   Infantile myotonic dystrophy
   Mitochondrial myopathy
   Brain stem anomaly
   Möbius syndrome
   Infantile botulism
   Seropositive and seronegative forms autoimmune myasthenia gravis[a]

OLDER PATIENTS
   Motor neuron disease
   Radial nerve palsy[b]
   Peripheral neuropathy[b]
   Syringomyelia[b]
   Limb girdle or facioscapulohumeral dystrophy
   Mitochondrial myopathy
   Chronic fatigue syndrome
   Seropositive and seronegative forms of autoimmune myasthenia gravis

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[a]Not reported in the first year of life.
[b]This diagnosis has been made in some cases of the slow-channel CMS

LEGENDS

Figure 8- 8- 1. CMS caused by a defect in ChAT. This 5-year-old boy has had numerous apneic episodes since birth and has mild to moderately severe myasthenic symptoms between these episodes. Note, ptosis, esophoria, facial diplegia, tracheostomy and percutaneous gastrostomy.

Figure 8- 2. Effects of subtetanic stimulation. on the EPP in ChAT-CMS. Indirect 10-Hz stimulation over 5 minutes reduces of the EPP to 13% of its initial value. In control muscle strips stimulated at 10 Hz, the EPP falls with a half-decay time >5 min.[5,6] Restricting quantal release by low-calcium, high-magnesium levels in the bath prevents the abnormal decrease of the EPP whereas enhancing quantal release with 3,4-DAP accelerates it. CMAP = compound muscle fiber action potential; EPP = endplate potential. (Reproduced from Engel AG, Ohno K, and Sine SM, Muscle Nerve27:4-25, 2003, by permission.)

Figure 8- 3. Structural model of human ChAT indicating recently identified ChAT mutations (upper panel) and comparison of kinetic landscapes of wild-type and mutant ChATs (lower panels). Asterisk in structural model marks the active site tunnel. Histidine 442 is at the center of the catalytic site. The M202R mutation near the histidine 442 abrogates enzyme activity. The T553N mutation near the active site tunnel markedly increases the Michaelis-Menten constants for both AcCoA and choline. The S572W mutation abuts on the AcCoA binding site and reduces the catalytic efficiency of the enzyme to ~3% of wild-type. The structural model of human ChAT is based on PDB 2FY2.

Figure 8- 4. A 23-year-old women with CMS caused by paucity of synaptic vesicles and reduced quantal release. The patient is attempting to look up. Note ptosis, ophthalmoparesis, and facial weakness. (Reproduced from Engel AG, Ohno K, and Sine SM, Muscle Nerve 27:4-25, 2003, by permission.)

Figure 8- 5. CMS with a paucity of synaptic vesicles and reduced quantal release. The nerve terminal contains only a few synaptic vesicles near the presynaptic membrane. x26,000.

Figure 8- 6. EP region in CMS resembling the Lambert Eaton syndrome. The pre- and postsynaptic regions are structurally intact. The nerve terminal harbors abundant synaptic vesicles (compare with Fig. 8-2). x25,500.

Figure 8- 7. Eleven-year-old girl with EP AChE deficiency. Note ptosis, hyperactive frontalis muscle, ophthalmoparesis, scoliosis, cubitus valgus, right heel contracture, small muscle bulk, and tracheostomy. The patient can only stand with support.

Figure 8- 8. EP AChE deficiency. **A.** Decremental EMG response and repetitive CMAP recorded from thenar muscle of patient during 2 Hz stimulation of the median nerve. At this rate of stimulation the second response decrements more rapidly than the first and appears only once. **B.** Representative MEPCs from a patient with EP AChE deficiency and a control subject. The best-fit exponential curve is superimposed on the decay phase of each current. Arrows indicate decay time constants. The MEPC is smaller and decays more slowly in the patient than in the control. MEPC = miniature endplate current. (From Hutchinson DO et al, Brain 116:633-653, 1993, with permission.)

Figure 8- 9. Electron cytochemical localization of AChE at a control EP incubated for 30 minutes (**A**) and at a patient EP incubated for 45 minutes (**B**). At the control EP, heavy reaction product fills the synaptic space and extends into the adjacent regions. At the patient EP there is no reaction product for AChE in the synaptic space. Sparse dark granules over the nerve terminal and junctional folds represent background staining. **A**, x9,200; **B**, x16,200. (From Hutchinson DO et al, Brain 116:633-653, 1993, with permission.)

Figure 8- 10. EP region in patient with EP AChE deficiency. The nerve terminal (N) is applied against only a fraction of the postsynaptic region and is encased by Schwann cell processes except for the segment indicated by arrows. Many junctional folds are degenerating and globular residues of the degenerate folds accumulate in the widened synaptic space (asterisk). x17,600. (From Hutchinson DO et al, Brain 116:633-653, 1993, with permission.)

Figure 8- 11. EP regions in patient with EP AChE deficiency. In **A**, note myriad pinocytotic vesicles and labyrinthine membranous networks in the junctional folds, degenerating fold (arrow), and an apoptotic nucleus in the junctional sarcoplasm (N). In **B**, AChR is visualized with peroxidase-labeled α-bgt. AChR reactive debris is shed from degenerating junctional folds. In both **A** and **B**, the nerve terminals (n) are small relative to the size of the postsynaptic region. **A**, 15,400x ; **B**, x 28,900.

Figure 8- 12. (A) Schematic diagram showing domains of a ColQ strand. (B) Schematic diagram showing the A12 species of asymmetric AChE with 24 identified ColQ mutations. (C-H) Density gradient profiles of AChE extracted from COS cells transfected with wild-type $ACHE_T$ and different types of $COLQ$ mutants. (I-K) Schematic diagrams of the abnormal species of AChE formed in transfected HEK cells. In (E) and (I) note that disruption of the PRAD domain produces a sedimentation profile identical with that obtained after transfection with $ACHE_T$ alone. Thus, $ACHE_T$ fails to attach to ColQ and no asymmetric AChE is formed. In (F) and (J), note that the asymmetric A_4, A_8, or A_{12} moieties are absent and there is a prominent -10.5 S mutant (M) peak, representing a G_4 tetramer of the catalytic subunit linked to the truncated ColQ peptide. In (G) and the left diagram in (K), note presence of a small M peak but absence of peaks corresponding to triple-stranded asymmetric enzymes. In (H) and the right diagram in (K), note that both an M peak and asymmetric AChE are present. PRAD, proline-rich attachment domain; HSPBD, heparan sulfate proteoglycan binding domain. (Reproduced from Engel AG et al. Congenital myasthenic syndromes.

Figure 8- 13. Slow-channel syndrome patients showing fatiguable weakness. Patient on the left can only keep her mouth closed by supporting her chin with her hand. On the right patient is attempting to extend her wrists and fingers as shown by examiner (with sleeve). Note atrophy of patient's forearm muscles. The patients carries the εL269F slow-channel mutation in the M2 transmembrane domain of AChR.

Figure 8- 14. Physiology and mutations in the slow-channel syndrome. **A.** Repetitive compound muscle action potential evoked from a limb muscle by a single nerve stimulus. The second and subsequent responses are triggered by the prolonged EPP that outlasts the absolute refractory period of the muscle fiber. **B.** MEPCs and channel events recorded from a control EP and an EP in a SCCMS patient caused by αV249F. Upper traces, MEPCs; lower traces, channel events; left, control EP; right, patient EP. Note markedly prolonged MEPC and some highly prolonged channel events at the patient EP (openings are upward deflections).The MEPC decay in the

patient is best fitted by two exponentials. Vertical arrows indicate decay time constants. **C.** AChRs expressed in HEK cells. The two traces on the left show opening bursts of wild-type and αV249F-AChR channels elicited by 10 nM ACh; two traces on the right show spontaneous openings of the αV249F-AChR channel at low and high resolution. **D.** Schematic diagram of slow-channel mutations identified in our patients. The mutations appear in different subunits of AChR and in different functional domains of the subunits. (Panels **B** and **C** are reproduced from Milone M et al, J Neurosci 17:5651-5665, 1977, with permission.)

Figure 8- 15. Slow-channel syndrome caused the εL269F mutation. **A.** The cholinesterase stain reveals many small EP region linked by ultraterminal nerve sprouts on a single fiber. x300. **B.** Electron microscopy of an EP region shows extensive degeneration of the junctional folds; the synaptic space is widened and contains myriad globular residues of the degenerated folds and remnants of the basal lamina that had invested the preexisting folds (asterisk). Widening of the synaptic space decreases the concentration of ACh reaching the postsynaptic membrane by dilution, and by permitting increased destruction of ACh by AChE. Degeneration of the junctional folds causes loss of AChR. The combination of these factors decreases the efficiency of ACh quanta. Also note focal myofibrillar degeneration beginning at the Z disk (x) in the nearby fiber region. x17,600.

Figure 8- 16. Endplate pathology in a patient with the slow-channel syndrome. **A.** Many junctional folds are honeycombed by membranous networks. This is a common ultrastructural reaction of the EP in states of cholinergic overactivity. **B.** Degeneration of the junctional folds leaves globular debris in the widened synaptic space (asterisk). **C.** The junctional sarcoplasm at the left is filled with degenerating organelles; star indicates remnants of degenerated junctional folds. **D.** Localization of EP AChR with peroxidase labeled α-bgt. Note loss of AChR from degenerating junctional folds (arrowhead). **E.** The junctional sarcoplasm harbors nuclei in early (x) and advanced (X) stages of apoptosis. Star indicates remnants of degenerated junctional folds. **A,** x16,000; **B,** x19,000; **C,** x15,500; **D,** x21,000; **E,** x13,100. (From Milone M et al, J Neurosci 17:5651-5665, 1977, with permission.)

Figure 8- 17. The effects quinidine (**A** and **B**) and fluoxetine (**C** and **D**) on opening bursts of genetically engineered slow-channel AChRs expressed in HEK cells. Panels **A** and **C** show effects of increasing concentrations of the drugs on the longest bursts open duration of 5 slow channel mutants (solid circles) and wild-type AChR (open circles). Vertical lines indicate SD. Note that 5 μM quinidine and 10 μM fluoxetine normalize the length of the mutant bursts. Panels **B** and **D** show examples of mutant channel openings in the absence and presence of indicated concentrations of quinidine and fluoxetine, respectively. (From Ref. 113 and 114, by permission.)

Figure 8- 18. (A) Fast-channel mutations result in endplate currents that decay abnormally fast due to abnormally brief channel opening events. Arrows point to the MEPC decay time constants. (B). Schematic diagram of fast-channel mutations identified in our patients. The mutations appear in different subunits of AChR and in different functional domains of the subunits. (Reproduced from Engel AG, Franzini-Armstrong C, editors. Myology, 3rd ed. New York: McGraw-Hill, 2004:1755-90, by permission.)

Figure 8- 19. A 4-year-old patient harboring the V132L fast-channel mutation in the signature

Cys-loop and a nonsense mutation in the AChR α subunit. Note eyelid ptosis, facial diplegia, open mouth, gastrostomy and lack of head control. (Upper panel reproduced from Ref. 47, by permission).

Figure 8- 20. A 4-year-old girl with severe EP AChR deficiency due to low-expressor mutations in the β subunit of AChR. Patient is using her fingers to elevate her eyelids. Also note open mouth and tracheostomy.

Figure 8- 21. Patient with EP AChR deficiency caused by recessive mutations in the AChR ε subunit gene. Note asymmetric ptosis, facial paresis, and mild esotropia. This patient is homozygous for ε553del7 that truncates the ε subunit in its extracellular domain. Single channel recordings revealed only γ-AChR at the patient's EPs.

Figure 8- 22. AChR deficiency caused by low-expressor or null mutations in the AChR ε subunit gene. **A** and **B**. Cholinesterase reactive EP regions in a patient with ε127ins5 and εP245L mutations (**A**) and in a control subject (**B**). Note dispersion of patient's EP regions over an extended length of the muscle fiber. **C** and **D**. Ultrastructural localization of AChR with peroxidase-labeled α-bgt at an EP from a patient harboring ε553del7 and εR311W mutations (**C**) and at a control EP (**D**). The control EP shows heavy reaction for AChR on the terminal expansions of the junctional folds. At the patient EP, the junctional folds are simplified, the reaction for AChR is attenuated (arrows), and the length of the postsynaptic membrane reacting for AChR is reduced. **A** and **B**, x310; **C**, x21,900; **D**, x6,500. (Reproduced from Ohno K et al, Hum Mol Genet 6:753-766, 1997, with permission.)

Figure 8- 23. Single channel currents recorded from a control EP and from EP of patient harboring two null mutations in the AChR ε subunit. Currents at patient EP have a lower amplitude and longer duration than currents at the control EP, signaling the presence of fetal AChR harboring the γ instead of the ε subunit. The dominant component of the burst duration histogram is shifted to the right for the patient. (Reproduced from Engel AG et al. Congenital myasthenic syndromes. In: Engel AG, Franzini-Armstrong C, editors. Myology, 3rd ed. New York: McGraw-Hill, 2004:1755-90, by permission.)

Figure 8- 24. Schematic diagram of low-expressor and null mutations reported in the α, β, δ, and ε subunits of AChR. Note that most mutations appear in the ε subunit and especially in the long cytoplasmic loop between M3 and M4. Square indicates a chromosomal microdeletion; hexagons are promoter mutations; open circles are missense mutations; closed circles are nonsense mutations; shaded circles are frameshifting mutations; dotted circles are splice-site mutations. The most likely consequence of a splice-site mutation is skipping of a flanking exon; therefore the splice-site mutations point to N-terminal codons of the predicted skipped exons. (Reproduced from Engel AG, et al, Muscle Nerve 2003; 27:4-25, by permission).

Figure 8- 25. Schematic diagram showing structure of the *RAPSN* gene (A), rapsyn domains (B), and identified mutations. Seven tetratricopetide repeats (TPRs) are required for rapsyn self-association; the coiled-coil domain binds to the long cytoplasmic loop of AChR subunits; the RING-H2 domain links rapsyn to β-dystroglycan. Shaded areas in (A) indicate untranslated regions in *RAPSN*. E, E-box.

Figure 8- 26. A six-year-old boy and a 27-year-old woman are homozygous for the N88K rapsyn mutation. The six-year-old boy can only stand with support; he has small stature, short neck, low set ears, and elbow, knee and ankle contractures. The 27-year-old woman shows only mild eyelid ptosis and mild fatigable weakness on exertion. (Left panel reprinted from Ref. 23 and right reprinted from Ref. 182 by permission.)

Figure 8- 27. Structural features of rapsyn deficient EPs. (A) Small cholinesterase reactive EP regions are dispersed over an extended length of the muscle fiber. (B) and (C) Multiple small nerve terminals are apposed against a highly simplified postsynaptic regions with no (B) or few (C) junctional folds. In (B), the distribution of AChR on the postsynaptic membrane, visualized with peroxidase-labeled α-bungarotoxin, is patchy. Bars 50 μm in (A) and 1 μm in (B) and (C). (Reproduced from Ref. 181 by permission).

Figure 8- 28. A. Genomic structure of *DOK7*. **B.** Identified rearrangements in 16 patients. Inset: Intron 1 retention, indicated by thick horizontal line.

Figure 8- 29. Phenotypic variability of Dok-7 myasthenia. Left panel: This patient has mild weakness and atrophy of limb girdle muscles, and mild eyelid ptosis. Right panels: This patient has severe diffuse weakness and atrophy of limb and axial muscles.

Figure 8- 30. Synaptic contact areas visualized with the cholinesterase reaction. Single small (B), multiple small (D-F), and perforated (A and C) contact areas are present. Nerve sprouts are recognizable (asterisk in D) as faint brown lines connecting contact areas. Bar = 50 μm.

Figure 8- 31. Electron micrographs of normal (A) and degenerating (B) EP in same patient. In (B) most junctional folds are replaced by globular debris (asterisk) causing widening of the synaptic space. This predicts a decreased synaptic response to ACh due to loss of AChR from tips of the destroyed folds, loss of ACh by diffusion from the widened synaptic space, and decreased input resistance of the remaining simple folds.

Figure 8- 32. EPs with pre- and postsynaptic abnormalities. **A.** EP region in shows marked degeneration of its junctional folds (asterisks). Schwann cell process (SC) is present amidst relics of the folds. A nerve sprout appears near the top. **B.** A highly abnormal EP region devoid of nerve terminal. Some junctional folds are degenerating (asterisk). The subsynaptic sarcoplasm harbors large myeloid structures. A nerve sprouts surrounded by Schwann cell (SC) appears above the junction.

Figure 8- 33. Patient with myasthenic syndrome caused by mutation of the $Na_v1.4$ sodium channel. Note asymmetric ptosis, strabismus, lumbar lordosis, and adduction deformities of knees and ankles. (Reproduced from Engel AG et al. Congenital myasthenic syndromes. In: Engel AG, Franzini-Armstrong C, editors. Myology, 3rd ed. New York: McGraw-Hill, 2004:1755-90, by permission.)

Figure 8- 34. A. Scheme of skeletal muscle sodium channel $Na_v1.4$ encoded by *SCN4A* and the identified mutations. **B.** Gating behavior of mutant and wild-type sodium channels expressed in HEK cells. Channel availability (*left*) after a 300 ms prepulse is left-shifted –33 mV for the

V1442E channel, and –7 mV for the S246L channel, whereas activation (*right*) is unchanged. **C.** Relative Na current during a 50-Hz train of 3 ms depolarizations to -10 mV from a holding potential of -100 mV. Every 200th response is shown after the break at the 10th pulse.

Figure 8- 35. Myopathy and CMS caused by plectin deficiency. **A** and **B**. Note marked variation in fiber size, regenerating fiber elements (asterisks), endomysial fibrosis in (B), and clusters of large nuclei at periphery of several fibers. (C) Alizarin red stain reveals focal calcium deposits in two fibers. (D) Multiple small cholinesterase reactive EP regions arrayed over an extended length of the fiber.

Figure 8- 36. Ultrastructural findings in abnormal muscle fibers (A) Note subsarcolemmal rows of large nuclei harboring multiple prominent chromatin bodies. (B) and (C) Subsarcolemmal and intrafiber clusters of mitochondria surrounded by fiber regions devoid of mitochondria. (D) Aberrant and disrupted myofibrils surrounded by clusters of mitochondria intermingled with glycogen, ribosomes, and dilated vesicles (x). Note pre-apoptotic nucleus at upper right. (E) Focal sarcolemma defects due to gaps in the plasma membrane. Where the plasma membrane is absent, the overlying basal lamina is thickened (x). Small vesicles underlie the thickened basal lamina. Asterisks indicate segments of the preserved plasma membrane. Bars = 4 μm in (A), 3 μm (B), (C), 1.4 μm in (D), 1 μm in (E). (Reprinted by permission from Ref. 219).

Figure 8- 37. Abnormal EP regions in plectinopathy. On the right, the junctional folds have disappeared and the nerve terminal is absent (asterisks). Grey streaks indicate basal lamina remnants that had invested preexisting folds. At the center a nerve sprout (ns) and a nerve terminal are separated by a Schwann cell process from the underlying degenerate folds On the left, the postsynaptic region is only partially occupied by the nerve terminal (nt). Bar= 1 μm.

Figure 8- 38. CMS associated with centronuclear myopathy. **A.** Patient at age 39 years. In upper panels, note decreased bulk of pectoralis major, proximal arm and thigh muscles, asymmetric enlargement of calf muscles, and increased lumbar lordosis. In lower panel shows patient rising from the floor with Gowers maneuver. **B.** Serratus anterior muscle, H&E stain. Most fibers contain internal nuclei. Bar = 50 μm. **C-E.** Paired fluorescence localization of AChE (green signal) and AChR (red signal) at control (**C** and **D**) and patient (**E** and **F**) EPs. AChR expression is mildly attenuated at patient EPs. **G** and **H.** Synaptic contacts visualized with reaction for AChE on teased patient fibers. Note double EP in (E) and multiple small EP regions (F). Bar = 20 μm. (Reprinted from Ref. 231, by permission.)

Figure 8- 39. Endplate ultrastructure. **A.** EP region shows poorly developed junctional folds with only a single secondary cleft opening into the primary synaptic cleft. **B.** At this EP region the postsynaptic membrane the highly simplified postsynaptic region lacks junctional folds. Empty mitochondrion represents fixation artifact. **C.** Unstained section showing localization of AChR with peroxidase labeled α-bungarotoxin. The EP region has only few folds and clefts and AChR appears mostly on tops of the folds. Bars = 0.5 μm.

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