vesicles and labyrinthine membranous networks (Figs. 8-16A). At more severely affected EPs, the junctional folds are degenerating, causing a widening of the synaptic space, accumulation of electron-dense debris (Figs. 8-15B, 16B and D), and loss of AChR from the junctional folds (Fig. 8-16D). Some of the highly abnormal postsynaptic regions are denuded of their nerve terminals. Unmyelinated nerve sprouts appear near some EPs. The intramuscular nerves are normal. Degenerative changes also occur in the junctional sarcoplasm and in nearby fiber regions. These consist of the accumulation of membrane-bound vesicles (Fig. 8-16C), apoptotic nuclei (Fig. 8-16E), focal myofibrillar degeneration, and appearance of large membrane-bound vacuoles. Morphometric reconstruction of individual EP regions shows a significant decrease of nerve terminal size. The postsynaptic membrane length and density are reduced due to degeneration of the junctional folds.

PATHOPHYSIOLOGY

The prolonged EPPs, MEPPs, and MEPCs all stem from prolonged opening episodes of the AChR channel. As in congenital EP AChE deficiency, the repetitive CMAP can be explained by the prolonged EPP.

The prolonged opening episodes (Fig. 8-14B and 14C left) and spontaneous openings of the AChR channel (see Fig. 8-14C, right) result in abnormal ingress of cations into the junctional folds and nearby muscle fiber regions. For the normal adult human AChR, 7% of the synaptic current is carried by Ca^{2+}; this is higher than for human fetal AChR or for muscle AChR of other species, and predisposes to postsynaptic Ca^{2+} overloading when the synaptic current is prolonged. Slow-channel mutations in the α subunit do not augment the already high Ca^{2+} permeability of the receptor, but slow-channel mutations in the ε subunit do and thereby potentiate the deleterious effects of the prolonged synaptic currents and the intrinsically high Ca^{2+} permeability of the human receptor.[123,124] The focal Ca^{2+} excess exerts a deleterious effect on cellular proteins and membranes through activation of proteases such as the calpains, by promoting free radical production by activation of lipases or nitric oxide synthase,[125] and promotes apoptosis through activation of caspases and endonucleases.[126-128] This readily explains degeneration of the junctional folds, nuclear apoptosis, and other features of the endplate myopathy. The morphologic findings at slow-channel EPs resemble those at mouse muscle EPs exposed to carbachol, a cholinergic agonist, and the carbachol induced changes can be prevented by exclusion of calcium from the extracellular fluid.[129]

Multiple mechanisms compromise the safety margin of neuromuscular transmission: (1) Widening of the synaptic space causes diffusional loss of ACh and increases the chance of destruction of ACh by AChE. (2) Degeneration of the junctional folds results in loss of AChR. (3) Apoptosis of a proportion of junctional nuclei may compromise transcriptional regulation at the EP. (4) The marked tendency of some SCCMS mutants to desensitize (e.g., αV249F) predicts that an appreciable fraction of AChR is desensitized even in the resting state, further decreasing the number of receptors that can be activated. (5) The markedly prolonged decay of the EPPs (often longer than 40 ms) predicts their staircase summation and a depolarization block of transmission during even normal physiologic activity. (6) The spontaneous openings, or leakiness, of the mutant channels may partially depolarize the perijunctional Na^{+} channels, producing a depolarization block even at rest, and contribute to the cationic overloading of the postsynaptic region.

The structural and mechanistic features of the mutant slow-channel AChRs are detailed in Chapter 9.

MOLECULAR GENETIC SUDIES

The abnormal kinetic properties of AChR predicted that the slow-channel syndrome stemmed

from mutations in AChR subunits. Since 1995, numerous slow-channel mutations have been discovered.[26,45,46,49,122,130-138] The different mutations occur in different AChR subunits and in different functional domains of the subunits (Fig. 8-14D). Interestingly, a patient suffering from autoimmune myasthenia gravis had an acquired slow-channel syndrome attributed to an unusual kinetic effect of an anti-AChR antibody.[139]

Mutations in the channel domain have more severe phenotypic consequences than those at the ACh binding site. For example, a patient with the εT264P mutation in the second transmembrane domain (M2) of the receptor has been wheelchair dependent since her teens; a patient with the αN217K mutation in M1 is less severely affected but can only walk about 100 yards before having to rest; and patients with the αG153S mutation in the extracellular domain of AChR can still ski and play tennis in their 60s. However, there are also variations in phenotypic expressivity between and within kinships harboring the same mutation.[140] Thus, the mutation site is not a consistently reliable predictor of phenotypic severity.

DIAGNOSIS

The clinical diagnosis is supported by dominant inheritance, selective distribution of the weakness and fatigability, and a decremental and repetitive CMAP. A repetitive CMAP can also occur with EP AChE deficiency but here the repetitive response is typically single and unaffected by edrophonium whereas in the SCCMS it is often multiple and edrophonium increases the amplitude and number repetitive CMAPs. This and normal reactivity for AChE at the EP establishes the diagnosis of SCCMS. In vitro electrophysiological studies confirm the diagnosis by demonstrating abnormally slowly and biexponentially decaying MEPCs and abnormally prolonged opening events of single AChR channels. Misdiagnoses of SCCMS patients have included Möbius syndrome, peripheral neuropathy, radial nerve palsy, motor neuron disease, syringomyelia, mitochondrial myopathy, limb-girdle dystrophy, facioscapulohumeral dystrophy, and myotonic dystrophy. Careful assessment of the clinical and EMG features can exclude these entities.

THERAPY

Anticholinesterase drugs can provide temporary improvement but are ineffective or harmful in the long run. By further increasing the number of normal and abnormal receptors activated by ACh, AChE inhibitors enhance cationic overloading of the endplate and likely accelerate the progression of the endplate myopathy.

Long-lived open-channel blockers of AChR shorten the openings of the AChR channel and are thus ideally suited to treat the slow-channel syndrome. Quinidine proved to be such an agent [141] and attainable levels of the drug normalized prolonged opening episodes of slow-channel mutants expressed in human embryonic kidney (HEK) cells[113] (Fig. 8-17A and B). Based on this clue, Harper and Engel [114] treated slow-channel patients with 200 mg quinidine sulfate three to four times daily, producing serum levels of 0.7-2.5 μg/ml (2.1- 7.7 μM/L), and found that the patients improved gradually by clinical and EMG criteria. The discovery that fluoxetine blocks neuronal AChR channels,[142] prompted examination of its effects on opening episodes of slow-channel mutants expressed in HEK cells. This revealed that fluoxetine was another a long-lived open-channel blocker of muscle AChRs at clinically attainable levels (Fig. 8-17C and D) and pointed the way to successful therapy of SCCMS patients with 60 to 80 mg fluoxetine per day.[143] The safe use of both quinidine and fluoxetine requires monitoring the serum level and close observation of the patient for possible side effects. Fluoxetine has been reported to increase the risk of suicide-related behaviors in depressed children and adolescents.[144,145] Therefore caution is required when the medication is used in this age group, and it should not be used in patients with signs of depression. Because quinidine is now difficult to obtain commercially and

because it is prone to cause allergic reactions, the authors use fluoxetine to treat the SCCMS.

The structural and mechanistic features of the mutant slow-channel AChRs are detailed in Chapter 9.

Fast-Channel Syndromes

The fast-channel syndromes are caused by recessive loss-of function mutations that decrease affinity for ACh, or reduce gating efficiency, or destabilize channel kinetics, or act by a combination of these mechanisms. Each of these derangements results in abnormally brief channel opening events that are reflected by an abnormally fast decay of the synaptic response (Fig. 8-18A). A fast-channel mutation dominates the clinical phenotype when the second allele harbors a null mutation or if occurs at homozygosity. The fast channel mutations identified to date are shown in (Fig. 8-18B)

CLINICAL ASPECTS

The symptoms resemble those of autoimmune myasthenia gravis. They can be mild when the main effect is on gating efficiency,[48,146] moderately severe when channel kinetics are unstable,[22,147] and severe (Fig. 8-19) when affinity for ACh, or both affinity and gating efficiency, are impaired[28,47,148,149]

ELECTROPHYSIOLOGY FEATURES

The common electrophysiologic features of the fast-channel CMS are rapidly decaying low-amplitude endplate currents and abnormally brief channel activation episodes (Fig. 8-18A) The amplitude of the synaptic response is reduced by decreased agonist affinity, decreased gating efficiency, impaired gating fidelity, or a combination of these factors.[22,28,47,146,150]

The structural and mechanistic features of the mutant fast-channel AChRs are detailed in Chapter 9.

MORPHOLOGY

The low-affinity fast-channel syndromes caused by εP121L near the ACh binding site[28] and αV132L in the Cys-loop of the receptor[47] leave no anatomic footprint; the structural integrity of the EP is maintained, and there is no EP AChR deficiency (New Fig. 8-19). Those syndromes caused by the εN182Y or the εD175N mutation in the extracellular domain,[150] αV285I in the M3 domain,[146] and ε1254ins18 in the long cytoplasmic loop of the ε subunit,[22] are associated with variable decrease of AChR expression. These patients display multiple small EP regions dispersed over an extended length of the fiber surface, and some of the postsynaptic regions are simplified.

DIAGNOSIS

The specific diagnosis of a fast-channel syndrome requires in vitro microelectrode studies to show abnormally rapidly decaying MEPCs at voltage-clamped EPs, or the recording of abnormally brief channel openings from EP AChRs or from mutant AChRs engineered into HEK cells.

THERAPY

An attenuated postsynaptic response to ACh is common to all fast-channel mutations. Increasing the postsynaptic response is therefore the logical therapy. Indeed, most patients with fast-channel

CMS generally respond well to combined therapy with 3,4-diaminopyridine (3,4-DAP) which increases the number of quanta released by nerve impulse, and anticholinesterase drugs which increase the number of receptors activated by each quantum. Patients with a normal density of AChR on the junctional folds respond best, for a decreased density of receptors on the folds entails a proportionate reduction in the number of receptors that can be saturated by any given quantum. However, neither increasing the release of ACh quanta nor prolonging the lifetime of ACh in the synaptic space mitigates the deleterious effects of mutations at the ACh binding site This was observed in an 8-year-old girl with severe weakness of all voluntary muscles since birth and three similarly affected siblings who died in infancy. She carries a homozygous ε-subunit mutation that substitutes a positively charged arginine for an anionic tryptophan at codon 55 (εW55R). The mutated tryptophan is one of the aromatic residues that contributes pi-electrons to the anionic agonist binding site at the α/ε subunit interface. Compared to wild-type AChR, the mutation reduces agonist affinity 670-fold, decreases the channel opening probability to 1%, and shortens the channel burst open duration to 9%.

Combined therapy with pyridostigmine and 3,4-DAP was also of limited benefit in the case of a 4-year-old with life-threatening myasthenic symptoms since birth requiring frequent ventilatory support (Fig. 8-19). She carries an αV132L mutation in the highly conserved Cys-loop of the receptor[47] and a null mutation in the second allele of the α-subunit.

AChR Deficiency Caused by Recessive Mutations in AChR Subunits

CLINICAL FEATURES

The clinical phenotypes of patients with low expressor mutations in AChR subunit genes vary from mild to severe. Patients with recessive mutations in the ε subunit are generally less affected than those with mutations in other subunits, because expression of the fetal γ-subunit can compensate at least in part for the defect in the ε subunit. Low expressor or null mutations in both alleles of non-ε subunits cause very severe disease and often are lethal in embryonic or early life. The most severely affected patients have marked ocular, bulbar, and respiratory muscle weakness from birth and survive only with respiratory support and gavage feeding. They may be weaned from a respirator and begin to tolerate oral feedings during the first year of life, but they will have bouts of aspiration pneumonia and may need intermittent respiratory support during childhood and adult life. Motor milestones are severely delayed; they can seldom learn climb steps and can walk for only a short distance. Older patients close their mouth by supporting the jaw with their hand and elevate their eyelids with their fingers (Fig. 8-20). Facial deformities, prognathism, malocclusion, and scoliosis or kyphoscoliosis become noticeable during the second decade. Muscle bulk is reduced. The tendon reflexes are normal or hypoactive.

The least affected patients pass their motor milestones with slight or no delay and only show mild ptosis and limited ocular ductions. They are clumsy in sports, fatigue easily, and cannot run well, climb rope, or do pushups. In some instances, a myasthenic disorder is suspected only when the patient develops prolonged respiratory arrest on exposure to a curariform drug during a surgical procedure.

Patients with intermediate clinical phenotypes experience moderate physical handicaps from early childhood. Ocular palsies and ptosis of the lids become apparent during the first year of life. They fatigue easily and cannot keep up with their peers in sports, they walk and negotiate stairs with difficulty, but they can perform most activities of daily living (Fig. 8-21).

ENDPLATE STUDIES

Morphologic studies show an increased number of EP regions distributed over an increased span of the muscle fiber (Fig. 8-22A and B). The integrity of the junctional folds is preserved but

some EP regions are simplified and smaller than normal (Fig. 8-22C). The distribution of AChR on the junctional folds is patchy and the density of the reaction for AChR is attenuated compared to normal (Fig. 8-22C and D). Conventional microelectrode studies show a decreased amplitude of the miniature EP potentials and currents and frequently high or higher than normal quantal release by nerve impulse. Single channel recordings at the EP[19,151] or immunocytochemical studies[21] often reveal the presence of fetal γ-AChR (Fig 23).

MOLECULAR PATHOGENESIS

CMS with severe EP AChR deficiency result from different types homozygous or, more frequently, heterozygous recessive mutations in AChR subunit genes. The mutations are concentrated in the ε subunit (Fig. 8-24, lower panel). There are two likely reasons for this: (1) Expression of the fetal type γ subunit, although at a low level, partially compensates for absence of the ε subunit,[19,21,22] whereas patients harboring null mutations in non-ε subunits (Fig. 8-24, upper panel) often die early for lack of a substituting subunit. (2) The gene encoding the ε subunit, and especially exons coding for the long cytoplasmic loop, have a high GC content that predispose to DNA rearrangements.

GENETIC HETEROGENEITY

Different types of recessive mutations causing severe endplate AChR deficiency have been identified. Some mutations cause premature termination of the translational chain. These mutations are frameshifting,[19,21,47,51,152-155] occur at a splice site,[51,153] or produce a stop codon directly.[19] An important mutation in this group is the 1369delG in the ε subunit that results in loss of a C-terminal cysteine, C470, crucial to both maturation and surface expression of the adult receptor.[156] Thus any mutation that truncates the ε subunit upstream of C470 is predicted to inhibit ε expression.

Three recessive point mutations were identified in the Ets binding site, or N-box, of the promoter region of the ε subunit gene: ε-154G>A,[157], ε-155G>A,[158] and ε-156C>T.[159] The N-box represents the end point of a signaling cascade driven by neuregulin through ErbB receptors. ErbB receptors phosphorylate mitogen-activated protein (MAP) kinases. Phosphorylated MAP kinases phosphorylate GABPα and GABPβ (members of the Ets family of transcription factors), which then bind to the N box.[160-162] That these mutations impair AChR expression is direct evidence that the neuregulin signaling pathway participates in regulation of synapse-specific transcription at the human EP.

There are also missense mutations in a signal peptide region (εG-8R[28] and εV-13D[153]), and missense mutations involving residues essential for assembly of the pentameric receptor. Mutations of the latter type were observed in the ε subunit at an N-glycosylation site (εS143L)[28], in Cys 128 (εC128S) --a residue that is an essential part of the C128-C142 disulfide loop in the extracellular domain,[22] in Arg 147 (εR147L), which is part of a short extracellular span of residues that contributes to subunit assembly[19], in Thr 51 (εT51P)[153], and in the long cytoplasmic loop of the β subunit causing the deletion of three codons.[163] Another important missense mutation is δE381K in the long cytoplasmic loop of the δ subunit that causes clinical symptoms typical of rapsyn deficiency. Cotransfection of the δE381K-AChR with wild-type rapsyn showed reduced coclustering of the mutant receptor with rapsyn compared to wild type indicating the importance of δGlu381 as an AChR binding partner for rapsyn.[164]

Finally, it is important to note that some ε subunit mutations occurring at homozygosity are endemic in Mediterranean or other Near Eastern countries.[153,165] The frameshifting ε1267delG mutation occurring at homozygosity is endemic in Gypsy families[51,152,154] where it

derives from a common founder.[152]

THERAPY

Most patients respond favorably but incompletely to anticholinesterase medications. The additional use of 3,4-DAP (1 mg per kg per day given in divided doses every 3 to 5 hours) results in further improvement but the ocular ductions are often refractory to 3,4-DAP.[166] Perioral and distal paresthesias are common at the beginning of therapy. Convulsions are a rare but important complication of 3,4-DAP treatment; therefore, a potential or actual epileptiform abnormality on the electroencephalogram or a history of seizures contraindicate the use of the drug. 3,4-DAP can also prolong the QT interval; therefore its use is contraindicated in patients whose electrocardiogram shows a borderline prolonged or prolonged QT interval.

Escobar Syndrome

This is a prenatal myasthenic syndrome caused by recessive, nonsense, frameshift, splice site, or missense mutations in the fetal γ-subunit of AChR. In humans, γ-AChR appears on myotubes around the ninth developmental week and becomes concentrated at nascent nerve-muscle junctions around the sixteenth developmental week. Subsequently, the γ subunit is replaced by the adult ε subunit and is no longer present at fetal EPs after the thirty-first developmental week.[167] Thus pathogenic mutations of the γ-subunit result in hypomotility in utero mostly during the sixteenth and thirty-first developmental week. The clinical consequences at birth are multiple joint contractures, small muscle bulk, multiple pterygia (webbing of the neck, axilla, elbows, fingers, or popliteal fossa), fixed flexion contractures of the fingers (campodactyly), rocker-bottom feet with prominent heels, and characteristic faces with mild ptosis and a small mouth with downturned corners. Myasthenic symptoms are absent after birth because by then the normal adult ε subunit is expressed at the EPs.[167,168]

CMS Caused by Defects in Rapsyn

Rapsyn (receptor associated protein of the synapse), under the influence of agrin, LRP4, MuSK and Dok-7 concentrates AChR in the postsynaptic membrane and links it to the subsynaptic cytoskeleton through dystroglycan.[169-172] In myotubes, agrin, MuSK, and Dok-7, and possibly other myotube specific mechanisms, regulate rapsyn aggregation,[173] but rapsyn expressed in heterologous systems self-aggregates and can then recruit AChRs, dystroglycan, and MuSK.

The structural domains of rapsyn include an N-terminal a myristoylation signal required for membrane association;[174] seven tetratrico peptide repeats (TPRs; codons 6 to 279) that subserve rapsyn self-association;[174,175] a coiled-coil domain (codons 298 to 331) the hydrophobic surface of which can bind to determinants within the long cytoplasmic loop of each AChR subunit;[176] a Cys-rich RING-H2 domain (codons 363-402) that binds to the cytoplasmic domains of β-dystroglycan [177]and mediates the MuSK induced phosphorylation of AChR;[178]and a serine phosphorylation site at codon 406 (Fig. 8-25). Transcription of rapsyn in muscle is under the control of helix-loop-helix myogenic determination factors that bind to the *cis*-acting E-box sequence in the *RAPSN* promoter.[179]

CLINICAL FEATURES

In most patient, myasthenic symptoms present at birth or infancy; in a few it presents in the second or third decade.[180] Arthrogryposis at birth and other the congenital malformations occurs in nearly a third of the patients[23,180,181] but are not associated with specific mutations (Fig. 8-26).

Motor milestones are typically delayed and fatigable weakness persists during life. Respiratory infections or other intercurrent febrile illnesses precipitate increased weakness and respiratory crises and can result in anoxic encephalopathy.[23,180,182,183] Mutations in the open reading frame of *RAPSN* result in clinical features that resemble those of autoimmune myasthenia except for involvement of the extraocular muscles. Most patients have ptosis of varying severity that can be asymmetric, which is uncommon in other types of CMS.[184] Ophthalmoparesis is thought to be uncommon[180] but 9 of 39 patients in our series have had constant or episodic ophthalmoparesis.[185] Therefore absence of ophthalmoparesis is not a reliable criterion for distinguishing rapsyn-CMS from CMS caused by mutations in the AChR subunits or from autoimmune myasthenia gravis. Facial and bulbar weakness are common, often associated with neck muscle weakness. Proximal muscle weakness is more severe than distal weakness. Out-of-proportion weakness of the foot dorsiflexors was reported a feature of the late-onset phenotype[180] and was not detected in our series of early onset patients.[185]

Near-Eastern Jewish patients who carry an E-box mutation (-38A>G) in *RAPSN* have facial deformities associated with prognathism and malocclusion. They have mild to severe weakness of the masticatory muscles, moderate to severe eyelid ptosis without ophthalmoparesis, facial weakness, and slurred or hypernasal speech. Cervical, trunkal and limb muscles are usually spared.[179]

ELECTROPHYSIOLOGY

A decremental EMG response is present in some but not all patients. The decremental response on 2-Hz stimulation can appear only after subtetanic stimulation for 5 min or SFEMG is required to uncover the defect of neuromuscular transmission.[23] Similar EMG findings were reported in the Near-Eastern Jewish patients with facial malformations.[186,187]

In vitro electrophysiologic studies show a higher than normal quantal release in some patients. Consistent with the endplate AChR deficiency, the MEPP and MEPC amplitudes are reduced. Single-channel patch-clamp recordings show no kinetic abnormality of the AChR channel.[23]

MORPHOLOGY

The morphologic alterations resemble those in patients with low-expressor mutation of the AChR. At the light microscopic level, multiple small synaptic contacts are dispersed over an extended length of the muscle fiber (Fig. 8-27A). Immunostains of the EPs show reduced expression of rapsyn and a proportionately of AChR. The decrease in the number of AChRs per endplate is less marked than in patients with low-expressor mutation of the AChR.[23,185] Ultrastuctural studies show patchy expression of AChR on the shallow postsynaptic folds, few secondary clefts, and smaller than normal nerve terminals and postsynaptic regions, but the structural integrity of the pre- and postsynaptic regions is preserved[23,179] (Fig. 8-27B and C).

MOLECULAR FEATURES

Mutations have now been detected in the entre open reading frame and promoter region of *RAPSN*[23,179,180,188-192] (Fig. 8-25). Importantly, however, nearly all Indo-Europeans harbor a common N88K mutation.[190] Expression studies in different cell lines reveal that different rapsyn mutations hinder rapsyn colocalization with AChR, prevent formation of agrin-induced AChR clusters, impede rapsyn self-association, or reduce rapsyn expression.[191] Despite these differences, there are no consistent genotype-phenotype correlations except for that associated with homozygous -38A>G mutations.[185] For example, among two patients homozygous for the same N88K mutation, one had severe myasthenic symptoms and joint contractures at age 6 years

but the other had only mild weakness at age 27 years (Fig. 8-26). One patient heterozygous for N88K and L14P was as severely affected as another patient homozygous for N88K; and one patient who harbors N88K and 553ins5 and was born with arthrogryposis, but has only mild weakness at age 11.That identical mutations can have different phenotypic effects in different patients is likely due to polymorphisms in functionally related genes that can mitigate or worsen the effects of the mutations.

DIAGNOSIS

The diagnosis can be suspected on clinical grounds in presence congenital joint contractures or other malformations, worsening of symptoms and respiratory crises precipitated by febrile illness, and mild or no limitation of the ocular ductions. The definitive diagnosis depends on mutation analysis of *RAPSN*. In Indo-European patients this begins by screening for the N88K mutation but few Indo-Europeans do not carry this mutation; therefore, if the clinical history warrants, entire gene needs to be sequenced.[193,194] If this also fails to reveal a mutation, one needs to search for mutations in the long cytoplasmic loop of the δ subunit the consequences of which can mimic those of mutations in rapsyn.[164]

THERAPY

Most patients respond well to anticholinesterase medications; some derive additional benefit from the use of 3,4-DAP.[182,185] Some patients observed by the author benefited form the added use of ephedrine[182] or albuterol.

DEFECTS IN MECHANISMS GOVERNING ENDPLATE DEVELOPMENT AND MAINTENANCE

Since 2006, novel signaling and adapter molecules other than agrin and MuSK and novel pathways governing the development and maintenance of the EP have been identified. The newly identified molecules include Dok-7 (docking protein -7),[171] LRP4 (low-density lipoprotein receptor-related protein 4),[172,195] Crk and Crk-L (v-crk avian sarcoma virus CT10 oncogene homolog, and like Crk),[196] and Tid1 (a mammalian homolog of Drosophila tumorous imaginal discs).[197]

According to current understanding, Lrp4 is a coreceptor for agrin that mediates activation of MuSK by agrin.[172,195] MuSK activity is also regulated by the muscle-intrinsic protein Dok-7.[171] Tid1 is required for Dok7 to bind to MuSK.[197] Once recruited to MuSK, Dok-7 is phosphorylated by MuSK and activates MuSK via dimerization enhancing MuSK phosphorylation and MuSK kinase activity.[198] Agrin signaling also causes phosphorylation of two tyrosine residues in the C-terminal region of Dok-7; this leads to recruitment of the adapter proteins Crk and Crk-L that serve as downstream activators of Dok-7.[196,199] Each of the above proteins is a potential CMS target. Defects in MuSK, agrin, and Dok-7 are now known to cause CMS.

CMS caused by Defect in Agrin***

A homozygous G1709R mutation was identified in a 42-year-old woman with right lid ptosis since birth, no oculoparesis, and mild weakness of facial, hip-girdle and anterior tibial muscles, and refractoriness to pyridostigmine or 3,4-DAP.[200] The mutation is in the laminin G-like 2 domain, upstream of the *y* and *z* inserts of neural agrin required for MuSK activation and neuromuscular junction formation. AChR and agrin expression at the EP were normal. Structural studies showed EPs with misshaped synaptic gutters partially filled by nerve endings and

formation of new EP regions. The postsynaptic regions were preserved. Expression studies in myotubes using a mini-agrin construct revealed the mutation did not affect MuSK activation of agrin or agrin binding to α-dystroglycan. Forced expression of the mutant mini-agrin gene in mouse soleus muscle showed changes similar to those at patient EPs. Thus, the observed mutation perturbs the maintenance of the EP without altering the canonical function of agrin to induce development of the postsynaptic compartment.[200]

CMS Caused by Defects in MuSK

MuSK (a muscle specific receptor tyrosine kinase) under the influence of agrin, LRP4, Dok-7, Crk/Crk-L, and Tid1 regulates the development and maintenance of the EP and acts on rapsyn to concentrate AChR in the postsynaptic membrane.

Three reports document CMS caused by mutations in MuSK. The first report describes heteroallelic frameshift (220insC) and a missense (V790M) mutations in a patient with respiratory distress in early life, mild ptosis, decreased upward gaze, and fatigable weakness of the cervical and proximal more than distal muscles. The symptoms were worsened by pregnancy. Treatment with pyridostigmine and 3,4-DAP was ineffective.[201] The frameshift mutation prevents MuSK expression; the missense mutation decreases MuSK expression and impairs its interaction with Dok-7.[171] Forced expression of the mutant protein in mouse muscle decreased AChR expression at the EP and caused aberrant axonal outgrowth.[201] Interestingly, mice homozygous for MuSK V789M (which corresponds to the human MuSK V790M) are normal but mice hemizygous for V789M are severely affected; this suggests that MuSK V790M in humans is haploinsufficient only when accompanied by a null mutation.[202]

A second report describes heteroallelic M605I and A727V mutations in MuSK in a patient with severe myasthenic symptoms since early life that improved after puberty but worsened after menstrual periods. The MEPP and MEPC amplitudes in anconeus muscle were reduced to about 30% of normal and the EPP quantal content was half-normal. Synaptic contacts were small and electron microscopy showed simplified postsynaptic regions with too few secondary synaptic clefts. The patient failed to respond to pyridostigmine, ephedrine or 3,4-DAP but responded partially to albuterol.[203]

A third report describes a homozygous P31L mutation in the extracellular domain of MuSK in 5 patients in a consanguineous Sudanese kinship. The findings included ptosis from an early age, partial ophthalmoparesis, and weakness of torso and limb girdle muscles. Pyridostigmine therapy gave only slight benefit.[204]

CMS Caused by Defects in Dok-7

After the discovery in 2006 of Dok-7 as a muscle-intrinsic activator of MuSK,[171] numerous CMS-related mutations were identified in *DOK7* (see section below on Molecular Studies) and Dok-7 myasthenia is now recognized as a common cause of CMS.

Dok-7 is strongly expressed at the postsynaptic region of skeletal muscle and in heart. It harbors an N-terminal pleckstrin homology domain (PH) important for membrane association, a phosphotyrosine-binding (PTB) domain, and C-terminal sites for phosphorylation (Fig. 8-28A). The PTB and PH domains are required for association with and phosphorylation of MuSK. Phosphorylation of two of the C terminal residues is a requisite for Dok-7 activation by Crk and Crk-L.[196]

CLINICAL FEATURES AND RESPONSE TO THERAPY

The weakness in Dok-7 myasthenia typically has limb-girdle distribution but mild ptosis and facial weakness are not infrequent[15,205-210] (Fig. 8-29). Severe bulbar symptoms are uncommon

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