INVITED REVIEW

ABSTRACT: Congenital myasthenic syndromes (CMS) stem from defects in presynaptic, synaptic basal lamina, and postsynaptic proteins. The presynaptic CMS are associated with defects that curtail the evoked release of acetylcholine (ACh) quanta or ACh resynthesis. Defects in ACh resynthesis have now been traced to mutations in choline acetyltransferase. A basal lamina CMS is caused by mutations in the collagenic tail subunit (ColQ) of the endplate species of acetylcholinesterase that prevent the tail subunit from associating with catalytic subunits or from becoming inserted into the synaptic basal lamina. Most postsynaptic CMS are caused by mutations in subunits of the acetylcholine receptor (AChR) that alter the kinetic properties or decrease the expression of AChR. The kinetic mutations increase or decrease the synaptic response to ACh and result in slow- and fast-channel syndromes, respectively. Most low-expressor mutations reside in the AChR ϵ subunit and are partially compensated by residual expression of the fetal type γ subunit. In a subset of CMS patients, endplate AChR deficiency is caused by mutations in rapsyn, a molecule that plays a critical role in concentrating AChR in the postsynaptic membrane.

Muscle Nerve **27:** 4–25, 2003

CONGENITAL MYASTHENIC SYNDROMES: PROGRESS OVER THE PAST DECADE

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Accepted 8 August 2002

The past decade saw rapid advances in identifying different types of congenital myasthenic syndromes (CMS), in unraveling their pathogenesis at the molecular level, and in using mammalian expression systems to decipher how identified mutations in endplate-associated proteins derange neuromuscular transmission. The investigation of all identified CMS began with careful clinical studies. Clinical observations were often followed by in vitro electrophysiological analysis of neuromuscular transmission, patch-clamp recordings of currents passing through single acetylcholine receptor (AChR) channels, and detailed cytochemical and ultrastructural examination of the endplate. In many instances, the preceding investigations pointed to a candidate gene or protein. For example, a kinetic abnormality of the AChR detected at the single-channel level predicted a kinetic mutation in an AChR subunit; endplate AChR deficiency suggested mutations residing in an AChR subunit or in rapsyn; absence of acetylcholinesterase (AChE) from the endplate foretold mutations in the catalytic or collagenic tail subunit of AChE; and a history of abrupt episodes of apnea associated with a stimulation-dependent decrease of endplate potentials and currents implicated proteins concerned with acetylcholine (ACh) resynthesis or vesicular filling. The expression studies that followed in the wake of mutation analysis not only afforded proof of pathogenicity but also provided clues for rational therapy, led to precise structure–function correlations, and highlighted functionally significant molecular domains that previous systematic mutagenesis studies had failed to detect. In this review, we summarize progress in the CMS over the past

Abbreviations: 3,4-DAP, 3.4-diaminopyridine; ACh, acetylcholine; AChBP, ACh binding protein; AChE, acetylcholinesterase; $AChE_T$, T isoform of catalytic subunit; AChR, acetylcholine receptor; *CACNA1A*, gene encoding pore-forming subunit of P/Q type calcium channel; cDNA, complementary DNA; ChAT, choline acetyltransferase; *CHAT*, gene encoding ChAT; CMAP, compound muscle action potential; CMS, congenital myasthenic syndrome; ColQ, collagenic tail subunit of AChE; *COLQ*, gene encoding ColQ; CMS-EA, CMS associated with episodic apnea; EPP, endplate potential; HEK, human embryonic kidney; HSPBD, heparan sulfate proteoglycan binding domain; LEMS, Lambert–Eaton myasthenic syndrome; *m*, number of quanta released by nerve impulse; MEPP, miniature endplate potential; MEPC, miniature endplate current; MWC, Monod–Wyman–Changeux; *n*, number of quanta available for release; *p*, probability of quantal release; PRAD, proline-rich attachment domain; SFEMG, single-fiber EMG; S-NRAP, actin-binding synaptic nebulin-related anchoring protein; TMD, transmembrane domain; TPR, tetratrico peptide repeats; VAChT, vesicular ACh transporter; *VACHT*, gene encoding VAChT

Key words: acetylcholinesterase; acetylcholine receptor; choline acetyltransferase; congenital myasthenic syndromes; neuromuscular junction; patch-clamp recordings; rapsyn

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decade and discuss the clinical and basic science aspects of the individual disorders.

DIAGNOSIS OF A CONGENITAL MYASTHENIC SYNDROME

Generic diagnosis of a CMS is often possible on the basis of myasthenic symptoms present since birth or early childhood, a typical pattern of the distribution of weakness with involvement of the cranial muscles and a high-arched palate, a history of similarly affected relatives, a decremental electromyographic (EMG) response of the compound muscle action potential (CMAP) on repetitive low-frequency (2–3 Hz) stimulation of its motor nerve, and negative tests for AChR and calcium channel antibodies. Some CMS, however, are sporadic or present in later life, a decremental EMG response may not be present in all muscles or at all times, and the weakness may be restricted in distribution and not involve cranial muscles. In some CMS, a specific diagnosis can be made by simple histological or EMG studies. In other CMS, in vitro electrophysiological, ultrastructural, and immunocytochemical investigations are needed for accurate diagnosis (see Table 1).

CLASSIFICATION OF THE CONGENITAL MYASTHENIC SYNDROMES

The currently recognized CMS arise from defects in presynaptic, synaptic basal lamina, and postsynaptic proteins, and several different types of presynaptic and postsynaptic CMS exist. Table 2 shows a site-of-defect classification of 147 CMS kinships. This classification is useful but is still tentative, because additional types of CMS likely will be discovered and because in incompletely studied disorders, such as the limb–girdle CMS[86] or the CMS associated with facial malformation in Iranian Jews,[127] the site of the defect has not been determined. Except for the slow-channel syndrome, which is caused by dominant gain-of-function mutations, all CMS are caused by recessive, loss-of-function mutations.

Table 1. Investigation of congenital myasthenic syndromes.*

Clinical data
History, examination, response to AChE inhibitor
EMG: conventional-needle EMG, repetitive stimulation, SFEMG
Serological tests (AChR antibodies, calcium channel antibodies, tests for botulism)
Morphological studies
Routine histochemical studies
Cytochemical and immunocytochemical localization of AChE, AChR, agrin, β_2-laminin, utrophin, and rapsyn at the endplate
Estimate of the size, shape, and configuration of AChE-reactive endplates or endplate regions on teased muscle fibers
Quantitative electron microscopy; electron cytochemistry
Endplate-specific ^{125}I-α-bungarotoxin–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 (if candidate gene or protein identified)
Linkage analysis (if no candidate gene or protein recognized)
Expression studies (if mutation identified)

**Not all studies need to be performed in all CMS.*

Table 2. Classification of CMS based on site of defect.*

Defect type	Index cases (*n*)
Presynaptic defects (8%)	
Choline acetyltransferase deficiency†	6
Paucity of synaptic vesicles and reduced quantal release	1
Similar to those of Lambert–Eaton syndrome	1
Other presynaptic defects	4
Synaptic basal lamina–associated defects (16%)	
Endplate AChE deficiency†	24
Postsynaptic defects (76%)	
Kinetic abnormality of AChR with/without AChR deficiency† (slow- and fast-channel syndromes)	37
AChR deficiency with/without minor kinetic abnormality†	67
Rapsyn deficiency†	6
Plectin deficiency	1
Total (100%)	147

**Classification based on cohort of CMS patients investigated at the Mayo Clinic between 1988 and 2002.*
†Genetic defects identified.

PRESYNAPTIC CONGENITAL MYASTHENIC SYNDROMES

Four presynaptic CMS have been described to date: (1) CMS associated with episodic apnea (CMS-EA) caused by defects in choline acetyltransferase (ChAT)[114]; (2) a CMS with paucity of synaptic vesicles and reduced quantal release[143]; (3) a CMS resembling the Lambert–Eaton syndrome[10,45]; and (4) a CMS with reduced quantal release due to an undefined mechanism.[84]

Congenital Myasthenic Syndrome with Episodic Apnea. In 1960 Greer and Schotland[59] described the clinical features of the disease, and in 1975 Conomy et al.[25] referred to it as "familial infantile myasthenia." Because all CMS can be familial and because most CMS present in infancy, the term "familial infantile myasthenia" has become a nonspecific term and a source of confusion.[37] Because the distinguishing clinical

feature is sudden and unexpected episodes of severe dyspnea and bulbar weakness culminating in apnea, we refer to it as CMS with episodic apnea. Previous studies of CMS-EA revealed no endplate AChR or AChE deficiency but suggested impaired resynthesis or vesicular packaging of ACh.[40,96]

Clinical Features. Some patients present at birth with hypotonia and severe bulbar and respiratory weakness requiring ventilatory support. This gradually improves but is followed by apneic attacks and bulbar paralysis in later life precipitated by infections, fever, or excitement or occurring with no apparent cause. Between these episodes, patients may have no, mild, or moderately severe myasthenic symptoms. Other patients are normal at birth and develop apneic attacks during infancy or childhood.[45] Some children, following an acute episode, experience prolonged respiratory insufficiency that may last for weeks. Phenotypic heterogeneity may occur even within a given kinship. For example, in one kinship two sibs died suddenly at 2 and 11 months of age during febrile episodes; one was asymptomatic and the other had mild ptosis prior to death. A third sibling began having abrupt episodes of dyspnea and cyanosis precipitated by fever or vaccination or without antecedent cause at age 14 months; at age 32 months, the patient developed ptosis and abnormal fatigue on exertion which led to the diagnosis of a myasthenic disorder.[21]

In clinical electrophysiology studies, a decremental response at 2-Hz stimulation and single-fiber EMG (SFEMG) abnormalities are detected only when the tested muscles are weak. Weakness and a decremental response at 2-Hz stimulation can be induced in some but not all muscles either by exercise or by a conditioning train of 10-Hz stimuli for 5–10 min.[40,61,65,96,126] This finding, however, is not specific for CMS-EA, for it can also occur in some CMS patients with endplate AChE or endplate AChR deficiency.

Endplate Studies. The number of AChRs per endplate, estimated from the number of ^{125}I-α-bungarotoxin–binding sites, and postsynaptic ultrastructure are normal, but morphometric analysis indicates that the synaptic vesicles are smaller than normal in rested muscle.[96] In vitro microelectrode studies of intercostal muscle specimens reveal that the amplitude of the miniature endplate potential (MEPP) is normal in rested muscle but decreases abnormally after 10-Hz stimulation for 5 min. The amplitude of the endplate potential (EPP) also decreases abnormally during 10-Hz stimulation and then recovers slowly over the next 10–15 min (Fig. 1A), but the quantal content of the EPP is essentially unaltered.[21,96] An abnormal decline of the EPP during 10-Hz stimulation can also occur in other CMS, but in these CMS the EPP amplitude returns to baseline in under 2 min.

Molecular Studies. That the synaptic response declines abnormally when neuronal impulse flow is increased and then recovers slowly has pointed to a defect in the resynthesis or vesicular packaging of ACh and implicated four candidate genes: the presynaptic high-affinity choline transporter,[8,118] ChAT,[100] the vesicular ACh transporter (VAChT),[46] and the vesicular proton pump[125] (Fig. 2). Mutation analysis in five CMS-EA patients uncovered no mutations in VAChT but revealed 10 recessive mutations in ChAT[114] (Fig. 1B). One mutation (523insCC) was a null mutation; three others (I305T, R420C, and E441K) markedly reduced ChAT expression in COS cells. Kinetic studies of nine bacterially expressed and purified missense mutants revealed that one (E441K) lacked catalytic activity and eight (L210P, P211A, I305T, R420C, R482G, S498L, V506L, and R560H) had significantly impaired catalytic efficiencies (e.g., see Fig. 1C).

That none of the observed CMS-EA patients had central or autonomic nervous system symptoms implies that the neuromuscular synapse is selectively vulnerable to CHAT mutations. There is no evidence that tissue-specific isoforms of ChAT explain this selective vulnerability. Although there are five alternative CHAT transcripts with at least three different promoters in humans,[39] the observed mutations are in the shared coding region of the recognized ChAT isoforms. Plausible explanations for the selective neuromuscular involvement are differences in presynaptic levels of ChAT, choline, or acetyl-CoA; rates of choline uptake; or rates of ACh release under conditions of increased neuronal impulse flow.

Finally, it is important to note that defects in the presynaptic high-affinity choline transporter,[8,118] the vesicular ACh transporter,[46] or the vesicular proton pump[125] may have similar phenotypic consequences, but no mutations in humans of these proteins have been detected to date.

Therapy. Anticholinesterase medications benefit patients with myasthenic symptoms between respiratory crises and also prevent or mitigate the crises. For this reason, prophylactic anticholinesterase therapy is advocated even for patients who are asymptomatic between crises. In a respiratory crisis, the patient should be treated with parenteral doses of neostigmine methyl sulfate or pyridostigmine bromide. Also, the parents must have an inflatable rescue bag and a fitted mask to be used in a crisis and

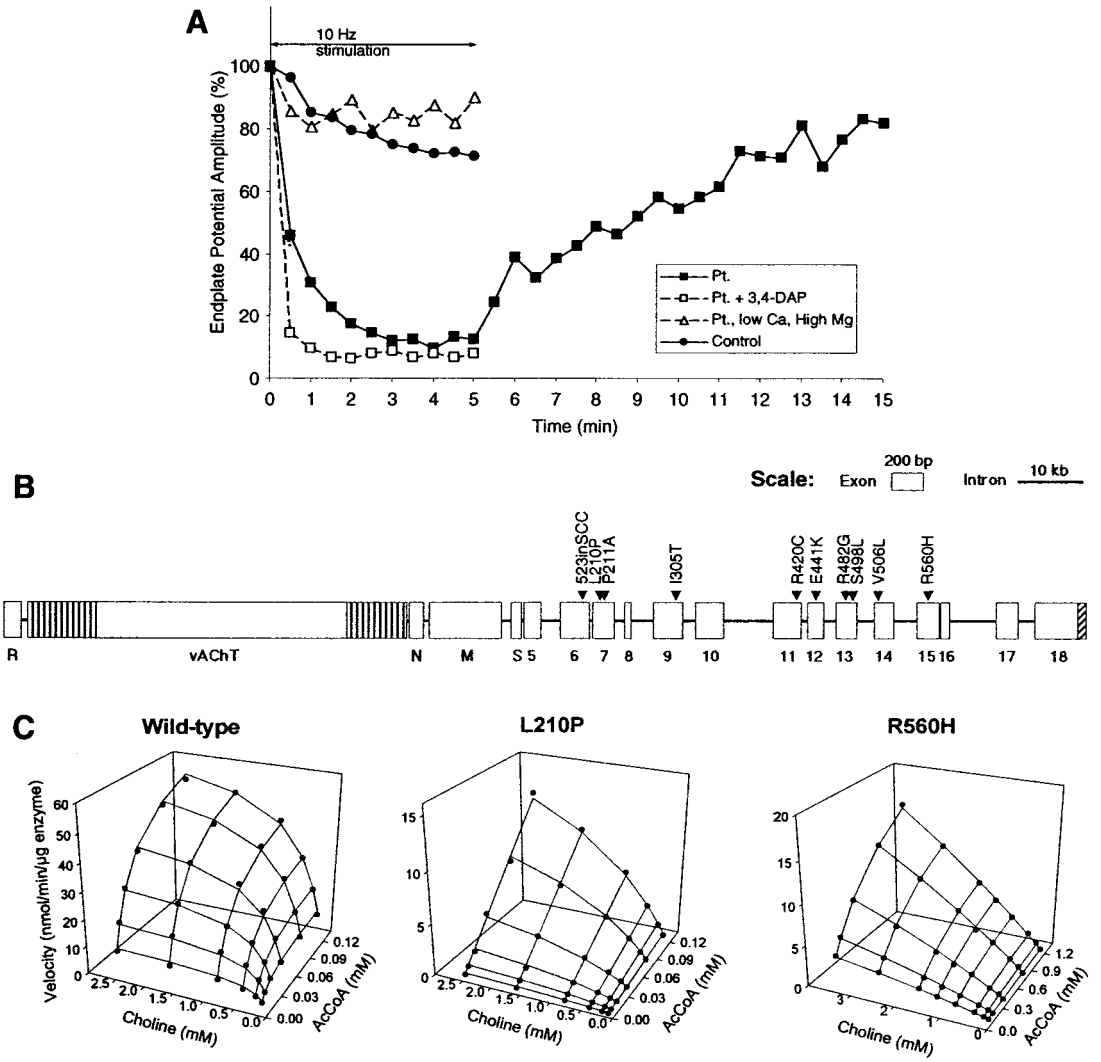


FIGURE 1. Congenital myasthenic syndrome with episodic apnea. **(A)** 10-Hz stimulation for 5 min results in a rapid abnormal decline of the EPP, which then recovers slowly over more than 10 min. 3,4-Diaminopyridine, which accelerates ACh release, enhances the defect, whereas a low Ca^{2+}–high Mg^{2+} solution, which reduces ACh release, prevents the abnormal decline of the EPP. **(B)** Genomic structure of *CHAT* and identified mutations. Note that the gene encoding the vesicular ACh transporter *VACHT* is located in the first *CHAT* intron. **(C)** Individually scaled kinetic landscapes of wild-type and of the L210P and R560H ChAT mutants. The L210P mutant shows no saturation over a practical range of acetyl-CoA (AcCoA) concentrations, indicating an extremely high K_m for AcCoA. Similarly, the R560H mutant does not saturate with increasing concentrations of choline, indicating a very high K_m for choline.

during transport to hospital. Long-term nocturnal apnea monitoring is indicated in any patient in whom CMS-EA is suspected.[21]

Paucity of Synaptic Vesicles and Reduced Quantal Release. Only one patient suffering from this disorder has been reported to date. The patient's clinical and electromyographic features were indistinguishable from those of patients with autoimmune myasthenia gravis, but the onset was at birth, anti-AChR antibodies were absent, there was no endplate AChR deficiency, and electron microscopy revealed no postsynaptic abnormality. A presynaptic defect was indicated by a decrease to ~20% of normal of the number of ACh quanta (m) released by a nerve impulse. The decrease in m was due to a decrease in the number of readily releasable quanta (n), which was associated with a decrease in the numerical density of synaptic vesicles to ~20% of normal in unstimulated nerve terminals.[143] The patient's symptoms were improved by pyridostigmine.

In this disorder the clinical consequences stem from the paucity of synaptic vesicles in the nerve terminal. Synaptic vesicle precursors associated with different sets of synaptic vesicle proteins are produced in the perikaryon of the anterior horn cell

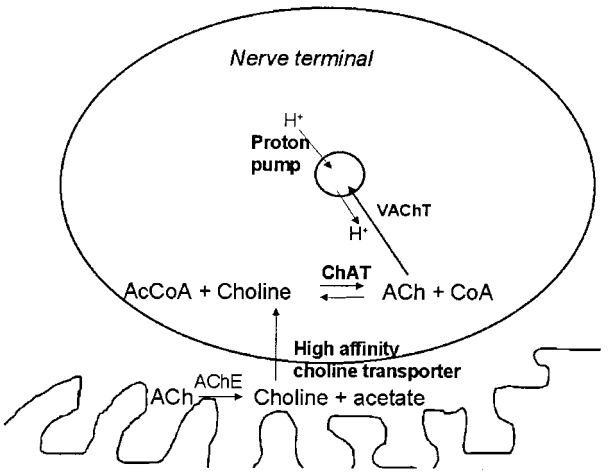


FIGURE 2. Scheme of ACh resynthesis at the endplate. After ACh is hydrolyzed by AChE in the synaptic space, choline is transported into the nerve terminal by a high-affinity choline transporter. Acetylcholine is resynthesized from choline and AcCoA by ChAT and is then transported into the synaptic vesicle by VAChT in exchange for protons delivered to the synaptic vesicle by a proton pump.

and are carried distally along motor axons to the nerve terminal by kinesin-like motors.[15,73,78,117] Mature vesicles containing a full complement of vesicular proteins are assembled in the nerve terminal[117] and are then packed with ACh. After ACh has been released by exocytosis, the vesicle membranes are recycled and then repacked with ACh.[140] In this syndrome, the reduction in synaptic vesicle density could arise from: (1) a defect in the formation of synaptic vesicle precursors in the anterior horn cell, (2) a defect in the axonal transport of one or more species of precursor vesicles, (3) impaired assembly of the mature synaptic vesicles from their precursors, or (4) impaired recycling of the synaptic vesicles in the nerve terminal. That synaptic vesicle density is reduced even in unstimulated nerve terminals argues against a defect in vesicle recycling.

Congenital Myasthenic Syndrome Resembling the Lambert–Eaton Myasthenic Syndrome. In one young child reported with this syndrome in 1987, the CMAP amplitude was abnormally small but facilitated severalfold on tetanic stimulation, and the symptoms were improved by guanidine.[10] A second patient observed at the Mayo Clinic was a 6-month-old girl with severe bulbar and limb weakness, hypotonia, areflexia, and respirator dependency since birth. The EMG showed a low-amplitude CMAP that facilitated 500% on high-frequency stimulation and decremented 40% on low-frequency stimulation. Studies of an anconeus muscle specimen revealed no endplate AChR deficiency. Electron microscopy of the endplates showed structurally intact presynaptic and postsynaptic regions and abundant synaptic vesicles in the nerve terminals. The MEPP amplitude was normal for muscle fiber size. The quantal content of the EPP, *m*, at 1-Hz stimulation was less than 10% of normal, and 40-Hz stimulation increased *m* by 300%. Thus, the in vitro electrophysiological findings were remarkably similar to those of Lambert–Eaton myasthenic syndrome (LEMS).[76] Consistent with this, the EMG abnormalities were improved by 3,4-diaminopyridine (3,4-DAP), an agent that increases the number of ACh quanta released by a nerve impulse,[79] but the patient remained weak and respirator-dependent. The molecular basis of this CMS could reside in an abnormality of the presynaptic voltage-gated calcium channel or in a component of the synaptic vesicle release complex. Mutation analysis of *CACNA1A*, the gene encoding the pore-forming α_1 subunit of the $Ca_v2.1$, or P/Q type, calcium channel expressed at the presynaptic membrane revealed no mutations (Ohno and Engel, unpublished data, 1999).

Other Presynaptic Congenital Myasthenic Syndromes Associated with Reduced Quantal Release by Nerve Impulse. Recently, Maselli and coworkers[84] reported three sporadic patients, two presenting in early infancy and one after the age of 5 years, with myasthenic symptoms that spared the external ocular muscles. All three had other neurological symptoms that included trunk or limb ataxia and, in one case, horizontal nystagmus. Unlike patients with LEMS, none had an abnormally small first-evoked CMAP, and decremental response on 2-Hz stimulation was not improved by stimulation at higher frequencies. None had endplate AChR deficiency. Electron microscopy demonstrated nerve terminals of normal size containing a normal number of synaptic vesicles and displaying small double-membrane–lined saccules filled with vesicles. In vitro microelectrode studies revealed marked decrease in the number of quanta (*m*) released by 1-Hz stimulation. In one patient, this was associated with a significantly decreased probability of quantal release (*p*); in another patient, there was <50%, but still significant, decrease in *n*. A search for mutations in selected exons of *CACNA1A* was negative. One of the three patients responded well to combined treatment with pyridostigmine and 3,4-DAP, one showed only mild improvement to combined therapy with pyridostigmine and ephedrine, and one failed to respond adequately to pyridostigmine.

SYNAPTIC BASAL LAMINA–ASSOCIATED CONGENITAL MYASTHENIC SYNDROME

Endplate Acetylcholinesterase Deficiency. This CMS is caused by the absence of AChE from the synaptic space.[41,67,105] Acetylcholinesterase is the enzyme responsible for rapid hydrolysis of ACh released at cholinergic synapses. At the normal endplate, AChE limits the number of collisions between ACh and AChR and, hence, the duration of the synaptic response.[71] Inhibition of the enzyme results in prolonged exposure of AChR to ACh, prolonged EPPs, desensitization of AChR[72] and a depolarization block at physiological rates of stimulation,[85] and an endplate myopathy with loss of AChR owing to cationic overloading of the postsynaptic region.[35,128]

Clinical Features. In most patients the disease presents in the neonatal period and is highly disabling, but in some patients it presents in childhood and becomes disabling only in the second decade[38] or later in life.[130] The following clinical clues point to the diagnosis: (1) a decremental EMG response; (2) a repetitive CMAP that is of smaller amplitude and decrements faster than the first CMAP; (3) no effect of AChE inhibitors on the decremental response, the repetitive CMAP, or the clinical state; and (4) a slow pupillary light response in some[67] but not all[130] patients. The diagnosis is confirmed by demonstrating absence of AChE from the endplate or a pathogenic mutations in *COLQ*, the gene encoding the collagenic tail subunit of endplate AChE.

Endplate Studies. Acetylcholinesterase is absent from the endplates by histochemical, immunocytochemical, and electron cytochemical criteria. Electron microscopy studies of the endplate reveal abnormally small nerve terminals, often partially or totally isolated form the postsynaptic region by Schwann cell processes that extend into the synaptic cleft. Smallness of the nerve terminals and their encasement by Schwann cells restrict the number of quanta that can be released by a nerve impulse (*n*) but tends to mitigate postsynaptic injury resulting from overstimulation by unhydrolyzed ACh. Despite this protective mechanism, many endplates display focal degeneration of the junctional folds with loss of AChR, and the junctional sarcoplasm harbors degenerating organelles, dilated vesicles, and apoptotic nuclei.[41,67]

Molecular Pathogenesis. The endplate species of AChE is a heteromeric asymmetric enzyme composed of one, two, or three homotetramers of the T isoform of globular catalytic subunits ($AChE_T$) attached to a triple-stranded collagenic tail (ColQ) (Fig. 3B). The collagenic tail subunit has an N-terminal proline-rich region attachment domain (PRAD), a collagenic central domain, and a C-terminal region enriched in charged residues and cysteines (Fig. 3A). Each ColQ strand can bind an $AChE_T$ tetramer to its PRAD, giving rise to A_4, A_8, and A_{12} species of asymmetric AChE.[14] Two groups of charged residues in the collagen domain (heparan sulfate proteoglycan binding domains, or HSPBD)[34] plus other residues in the C-terminal region[74,105] assure that the asymmetric enzyme is inserted into the synaptic basal lamina. The C-terminal region is also required for initiating the triple helical assembly of ColQ that proceeds from a C- to an N-terminal direction in a zipperlike manner.[121]

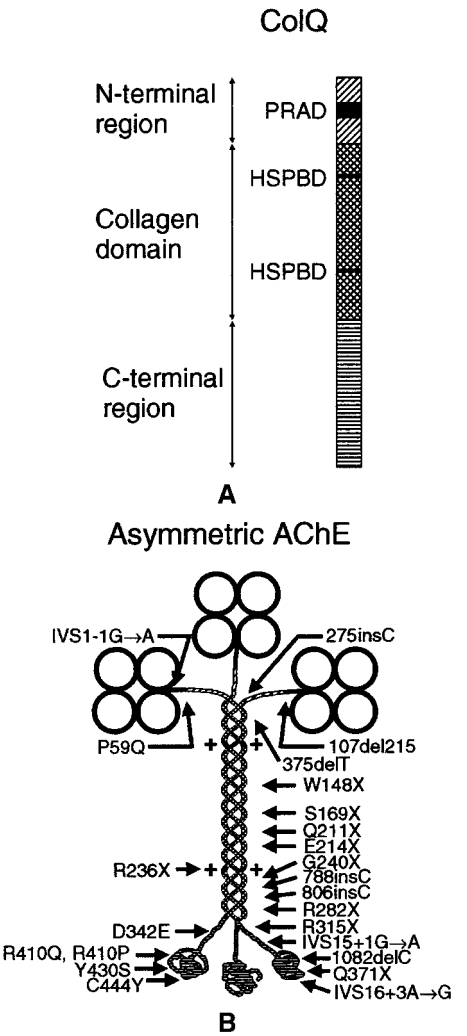


FIGURE 3. (A) Schematic diagram showing domains of a ColQ strand. **(B)** Components of the A_{12} species of asymmetric AChE and 24 identified ColQ mutations.

In 1998, human *COLQ* complementary DNA (cDNA) was cloned,[38,104] the genomic structure of *COLQ* determined,[104] and the molecular basis of

endplate AChE deficiency traced to recessive mutations in *COLQ*.[38,104] Twenty-four *COLQ* mutations in 25 kinships have been identified to date[38,68,103–105,130] (see Fig. 3B). The mutations are of three major types: (1) PRAD mutations prevent attachment of $AChE_T$ to ColQ; (2) collagen domain mutations produce a short, single-stranded ColQ that binds a single $AChE_T$ tetramer and is insertion-incompetent; (3) C-terminal mutations hinder the triple-helical assembly of the collagen domain or produce an asymmetric species of AChE that is insertion-incompetent, or both.

Therapy. At present, there is no satisfactory drug therapy for endplate AChE deficiency. Antiesterases should be avoided because they are ineffective, and AChE-deficient patients appear to have enhanced sensitivity to their muscarinic side effects. If the diagnosis of AChE deficiency is not suspected, refractoriness to an anti-AChE medication may prompt the physician to increase the dose of the medication; this, in turn, may result in excessive bronchial secretions and worsen the patient's clinical state.

POSTSYNAPTIC CONGENITAL MYASTHENIC SYNDROMES

Overview of AChR Structure. Most postsynaptic CMS stem from a deficiency or kinetic abnormality of AChR. Therefore we begin with a brief review of the structure of the muscle subtype of AChR. Muscle AChR is an integral membrane protein composed of five homologous subunits: two of α, one of β and δ, and one of ϵ in adult AChR, or one of γ instead of ϵ in fetal AChR. The genes encoding α, δ, and γ are at different loci on chromosome 2q, and those encoding β and ϵ are at different loci on chromosome 17p. The subunits are highly homologous, have similar secondary structures, fold similarly, and are organized like barrel staves around a central cation-selective channel. Each subunit has an N-terminal extracellular domain that comprises ~50% of the primary sequence, four transmembrane domains (TMD1–TMD4), a large cytoplasmic domain betweenTMD3 and TMD4, and a small C-terminal extracellular domain.

The two ACh binding sites are formed at interfaces between subunits: α–γ and α–δ in embryonic and denervated muscle, and α–ϵ and α–δ in the adult. Residues from both α and non-α subunits contribute profoundly to binding of ACh and competitive antagonists, indicating that the ACh binding sites are formed at interfaces between subunits.[27,70,120] Mutagenesis, combined with ligands that select between the two binding sites, revealed seven linearly distinct loops within each α or non-α subunits, designated loops A–G, that contribute to each site. Site-directed labeling studies physically localized all but one of the seven loops to the binding site, further demonstrating the presence of multiple loops.[23,32,51,144] The recent x-ray structure of the snail glial acetylcholine binding protein (AChBP) confirmed that residues in all seven loops are present at the ligand binding site[17] including αY93 in loop A, αW149 in loop B, αY190 and αY198 in loop C, ϵW55 in loop E, ϵL119 and ϵP121 in loop F, and ϵD175 in loop G. Each of these residues is highly conserved and may constitute minimal structures necessary for ACh binding. Additional residues at the periphery of the site have been identified by mutagenesis and confirmed by AChBP.[17] These include αG153 in loop B; αS187, αV188, and αT189 in loop C; ϵK34 in loop D; ϵD59 in loop E; ϵL109, ϵY111, ϵS115, and ϵT117 in loop F; and ϵE177 in loop G. These peripheral residues may constitute structures specialized for binding ACh at concentrations found at the motor synapse or for releasing bound ACh with sufficient speed to terminate the response.

Miyazawa and colleagues[94] recently resolved electron density in two-dimensional crystals of *Torpedo* AChR to a resolution of 4.6 Å. At the level of the membrane, the electron density reveals five rods typical of α-helices that twist upon activation to allow flow of permeant ions.[142] These rods correspond to TMD2, with possible contributions from TMD1, based on substituted cysteine accessibility mutagenesis and functional measurements. The collective studies indicate that the extracellular half of each TMD2 forms an α-helix, the middle three residues form an extended structure, and the remainder is an α helix followed by a β-strand that forms the channel gate and ion selectivity filter near the cytoplasmic limit of TMD2.[4,26,70] Electron densities corresponding to TMD3 and TMD4 have not been assigned, but the long cytoplasmic domain between them likely contributes to a fenestrated basket-like structure extending into the cytoplasm[94] that serves as an attachment site for cytoskeletal elements, bears residues that can be phosphorylated, and thus may be important for desensitization and, in the case of the ϵ subunit, stabilizes the gating mechanism.[91]

Congenital myasthenic syndrome mutations have been found in all AChR subunits and in several domains of the subunits, including the major extracellular domain contributing to the ACh binding site, TMD1–TMD3, and the long cytoplasmic domain between TMD3 and TMD4. Despite this diver-

sity of targets, CMS mutations fall into two broad categories: those that increase the response to ACh in slow-channel syndromes, and those that decrease the response in fast-channel syndromes or as a consequence of low-expressor mutations. The slow- and fast-channel syndromes stem from kinetically significant missense mutations and represent physiological opposites. Table 3 lists reciprocal features of the two syndromes. Before discussing the CMS caused by slow- and fast-channel mutations, we present fundamental kinetic tenets of receptor activation by ACh.

Overview of AChR Kinetics. Because slow- and fast-channel CMS can arise from mutations in either transmembrane domains or the ligand binding sites of AChR, a mechanistic, as opposed to a structural, framework is essential to explain the consequences of given mutations. The following molecular scheme provides such a framework:

$$A + R \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} AR + A \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} A_2R \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} A_2R^*$$

(Scheme 1)

where A is agonist, R is the resting state, and R^* is the open-channel state. Inherent in Scheme 1 and its expanded forms is the existence of stable ground states, R, AR, A_2R, and A_2R^*, which represent deep wells in the energy landscape of the receptor. The rate constants for each step provide a measure of the height of the energy barrier, or activated complex, between stable ground states. Furthermore, Scheme 1 accounts for positive cooperativity in the ACh dose–response relationship and the dependence of single-channel open and closed dwell times on ACh concentration. Thus, Scheme 1 accounts for essential features of activation for AChRs from a variety of species.[138,148] Scheme 1 is a subset of the Monod–Wyman–Changeux (MWC) allosteric description of protein function,[95]

$$\begin{array}{ccccc} R^* + A & \overset{K_1^*}{\rightleftharpoons} & AR^* + A & \overset{K_2^*}{\rightleftharpoons} & A_2R^* \\ \theta_0 \uparrow\downarrow & & \theta_1 \uparrow\downarrow & & \theta_2 \uparrow\downarrow \\ R + A & \underset{K_1}{\rightleftharpoons} & AR + A & \underset{K_2}{\rightleftharpoons} & A_2R \end{array}$$

(Scheme 2)

This contains resting (R) and active (R*) states that interconvert even in the absence of agonist and predicate tighter binding of agonist to the active than to the resting state (the equilibrium dissociation constants K* are much smaller than K). Given that ACh binds more tightly to the active state, the equilibrium constant θ between resting and active states increases progressively with increasing agonist occupancy so that $\theta_0 < \theta_1 < \theta_2$; for muscle AChR, θ_0 is approximately 10^{-6}, θ_1 is approximately 10^{-2}, and θ_2 is ~30. Thus, agonist overcomes the unfavorable equilibrium constant θ_0 by binding more tightly to the active than to the resting state.[69]

Slow-Channel Syndromes. ***Clinical Features.*** The slow-channel syndromes are caused by dominant gain-of-function mutations. The clinical phenotypes vary. Some slow-channel CMS present in early life and cause severe disability by the end of the first decade[90]; others present later in life and

Table 3. Kinetic abnormalities of AChR.

	Slow-channel syndromes	Fast-channel syndromes
Endplate currents	Slow decay	Fast decay
Channel-opening events	Prolonged	Brief
Open states	Stabilized	Destabilized
Closed states	Destabilized	Stabilized
Mechanisms*	Increased affinity	Decreased affinity
	Increased β	Decreased β
	Decreased α	Increased α
		Mode-switching kinetics
Pathology	Endplate myopathy from cationic overloading	No anatomic footprint
Genetic background	Dominant gain-of-function mutations	Recessive loss-of-function mutations
Response to therapy	Long-lived open-channel blockade of AChR with quinidine or fluoxetine	3,4-DAP and AChE inhibitors

**Different combinations of mechanisms operate in the individual slow- and fast-channel syndromes.*
Abbreviations: β, channel-opening rate; α, channel-closing rate.

progress slowly, resulting in little disability even in the sixth or seventh decade.[42,45,139] Most patients show selectively severe involvement of cervical and of wrist and finger extensor muscles. Except for the more severely affected patients, the cranial muscles tend to be spared. Progressive spinal deformities and respiratory embarrassment are common complications during the evolution of the illness.

The prolonged opening episodes of the AChR channel result in prolonged endplate currents and potentials (see Figs. 4B and 4C), which, in turn, elicit one or more repetitive CMAPs. Repetitive stimulation reveals a decremental response that is present at low stimulation frequency and increases progressively when stimulation frequency is increased. The repetitive CMAP is of lower amplitude and decrements faster than the first CMAP.

Endplate Studies. The morphological consequences stem from prolonged activation episodes of the AChR channel that cause cationic overloading of the postsynaptic region. Excessive accumulation of Ca^{2+} can be demonstrated at some endplates with alizarin red, which detects millimolar concentrations of Ca^{2+} (Figs. 5A and 5B). The endplate myopathy that develops is like that in endplate AChE deficiency but is even more severe, sometimes causing massive destruction of the junctional folds (Fig. 5C), nuclear apoptosis, and vacuolar degeneration near the endplates.[42,44,45,90]

In vitro microelectrode studies demonstrate markedly prolonged EPPs and currents that decay biexponentially.[44,109,139] Single-channel patch-clamp recordings reveal a dual population of AChR channels, one with normal and one with prolonged opening episodes, reflecting the presence of both wild-type and mutant receptors.[44,90,109] In addition, the mutant AChR channels open even in the absence of ACh,[90,109] resulting in a continuous cation leak into the postsynaptic region. The safety margin of neuromuscular transmission is compromised by the altered endplate geometry, loss of AChR from degenerating junctional folds, and a depolarization block during physiological activity owing to staircase sum-

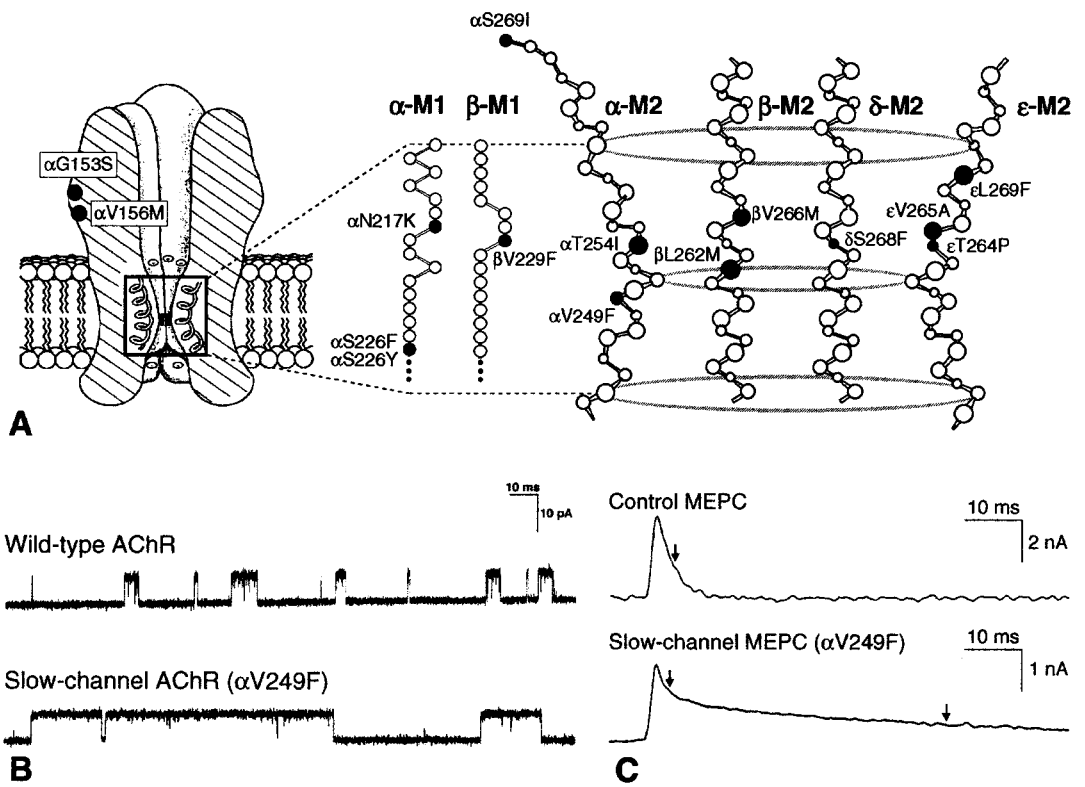


FIGURE 4. (A) Schematic diagram of slow-channel mutations. The drawing on the left shows a section through AChR, indicating approximate position of mutations that are not in TMDs of the receptor. In the drawing on the right, dotted lines delimit TMDs. Mutations appear in the TMD2 domains of the α, β, δ, and ϵ subunits and in the TMD1 domain of the α subunit. The αS269I mutation above the dotted line is in the extracellular TMD2/TMD3 linker. **(B)** Examples of single-channel currents from wild-type and slow-channel (αV249F) AChRs expressed in HEK cells. **(C)** Miniature endplate currents recorded from endplates of a control subject and a patient harboring the αV249F slow-channel mutation. The slow-channel MEPC decays biexponentially due to expression of both wild-type and mutant AChRs at the endplate, with one decay-time constant that is normal and one that is markedly prolonged.

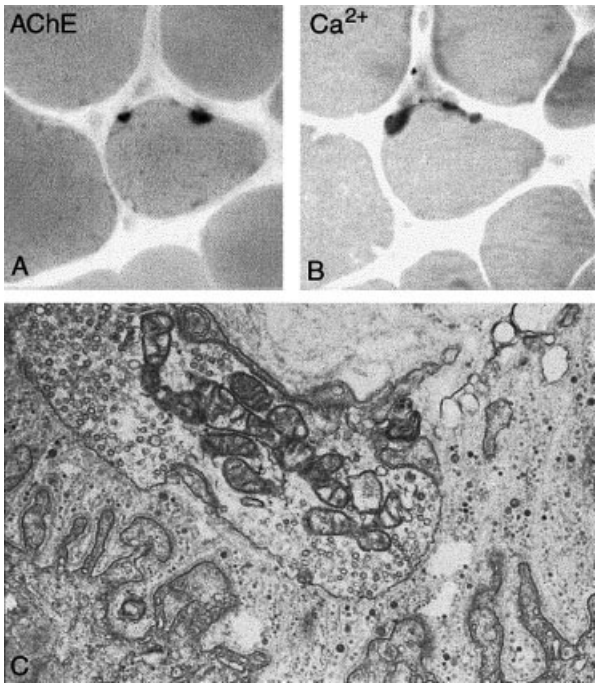


FIGURE 5. (A) and **(B)** Enzyme histochemical localization of AChE and cytochemical localization of Ca^{2+} with alizarin red at the same slow-channel endplate. (Original magnification ×470.) **(C)** Electron micrograph of a slow-channel endplate. All junctional folds are degenerating. The widened synaptic space harbors globular residues of degenerated folds that are enveloped by layers of basal lamina that had surrounded preexisting normal folds. Degeneration of the folds causes loss of AChR; widening of the synaptic space decreases the concentration of ACh reaching the postsynaptic membrane and permits increased destruction of ACh by AChE. (Original magnification ×26,500.)

mation of the markedly prolonged EPPs.

Molecular Studies. Fifteen slow-channel mutations, all dominant and causing gain-of-function, have been reported to date[28,44,55–57,90,109,110,116,139,145] (Fig. 4A). The different mutations occur in different AChR subunits and in different functional domains of the subunits. The clinical consequences vary. In general, the disease is progressive owing to structural damage to the endplate, but mutations in the TMDs have more severe clinical consequences than do those in the extracellular domain. Patch-clamp studies at the endplate, mutation analysis, and expression studies in human embryonic kidney (HEK) cells indicate that the αG153S mutation near the extracellular ACh binding site[139] (also see below) and the αN217K mutation in the N-terminal part of TMD1[145] act mainly by enhancing affinity for ACh. This slows dissociation of ACh from the binding site and results in repeated channel reopenings during receptor occupancy by agonist, which prolong the activation episode. The αS226Y as well as αS226F in TMD1 enhance both affinity and gating efficiency.[116] Mutations in TMD2 that lines the channel pore, such as βV266M, εL269F, εT264P, and αV249F, as well as αS269I in the extracellular TMD2/TMD3 linker, act mainly by enhancing gating efficiency (channel opening rate β/channel closing rate α).[44,60,90,109] Variable increases in steady-state affinity for ACh and concomitant increases in extent of desensitization are also observed with αV249F,[90] εL269F,[44] and εT264P.[109] A slow-channel mutation in TMD2 of the δ subunit, δS268F was reported to affect mainly gating.[57]

Other slow-channel mutations, namely αV156M near the ACh binding site,[28] δS268F,[56] βL262M,[55] αT254I,[28] and εV265A,[110] in TMD2, and βV229F in TMD1[56] have also been reported but without detailed analysis of their effects on the kinetics of receptor activation.

Therapy. Quinidine is a long-lived open-channel blocker of AChR,[137] and clinically attainable levels of quinidine normalize the prolonged opening episodes of mutant slow-channels expressed in HEK cells (Fig. 6).[50] On the basis of these findings, Harper and Engel[62] treated slow-channel patients with quinidine sulfate, 200 mg three to four times daily, producing serum levels of 0.7–2.5 μg/ml (2.1–7.7 μmol/L), and found that the patients improved gradually by clinical and EMG criteria. Fluoxetine, another long-lived open-channel blocker of AChR, is also therapeutically effective, but relatively high doses of the medication (about 80 mg/day in adults) are required to obtain a therapeutic effect.[64]

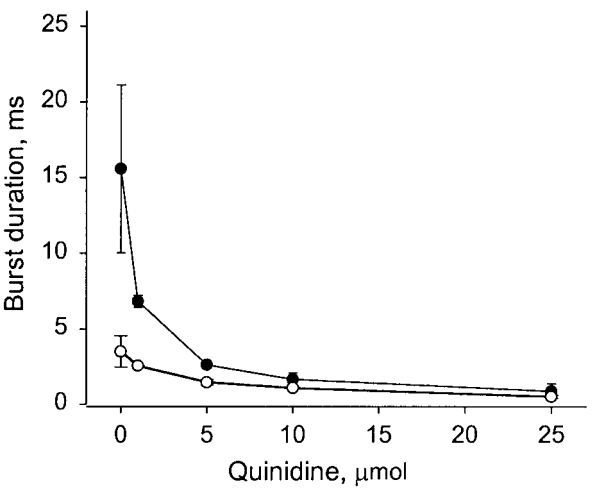


FIGURE 6. Effect of quinidine on the duration of AChR channel-opening bursts of five genetically engineered slow-channel mutants (solid circles) and wild-type AChR (open circles) expressed in HEK cells. Vertical lines indicate SD. Note that 5 μmol of quinidine normalizes the duration of the slow-channel bursts. (Reproduced from Fukudome et al.[50] by permission.)

Mechanistic Consequences of Slow-Channel Congenital Myasthenic Syndromes. The majority of slow-channel CMS result from mutations in TMD2. The most conspicuous effect of TMD2 mutations is a dramatic increase in the duration of ACh-induced openings, from ~0.5 ms for wild-type AChR up to ~50 ms for mutations in TMD2.[44,90,109] Also conspicuous is frequent opening of the AChR channel in the absence of ACh,[44,90] which occurs rarely in wild-type AChR. A complete set of activation-rate constants in Scheme 1 has not been determined for receptors with mutations in TMD2, due to the inability to assign the multiple closed-time components to particular kinetic steps.[90]

Increased spontaneous openings due to mutations in TMD2 indicate an increase of θ_0, which describes the equilibrium between resting and active states in the absence of ACh. A small value of θ is vital for minimizing cation influx at rest and for maximizing the range of the response to ACh. Increased spontaneous openings due to mutations in TMD2 may explain why the corresponding slow-channel syndromes are more severe than are those due to mutations at the ligand binding site. The ACh-independent equilibrium between resting and active states is the starting point for drawing the MWC scheme described above (Scheme 2). The first ACh binding step thus begins with a mutant receptor already prone to opening, and the enhanced affinity of ACh for the active over the resting state further promotes opening of the mono-liganded receptor according to $\theta_1 = \theta_0 K_1/K_1^*$. Analogously, the increase of θ_1 propagates to the doubly liganded AChR, increasing the corresponding opening equilibrium constant θ_2 according to $\theta_2 = \theta_1 K_2/K_2^*$. Rates of channel opening are also increased, the most obvious being the rate of opening in the absence of ACh, β_0. The doubly liganded opening rate β_2 also increases with mutations in TMD2,[90] although the extent of the increase is uncertain, because the opening rate for wild-type AChR approaches resolution limits of the patch clamp. The doubly liganded closing rate α_2 slows with mutations in TMD2, owing to stabilization of the open state relative to the transition state between open and closed states. Thus, for mutations in TMD2, channel activation is enhanced upon transient exposure to ACh operative at the synapse.

Because TMD2 is well established to form the ion channel, functional consequences of mutating this domain likely result from direct perturbation of the gating apparatus. Although the gating apparatus comprises TMDs from all five subunits, and gating comprises synchronous movement of all five, TMD2 mutations in individual subunits appear to be independent of mutations in TMD2 of the other subunits.[75] The overall results of mutating TMD2 indicate that its structure is essential for stabilizing the channel in the resting closed state relative to the active and desensitized states; closed-state stability prevents unwanted cation influx at rest while optimizing the fraction of activatable receptors. The results further show that the structure of TMD2 is tuned to establish the stability of the open relative to the transition state to provide millisecond endplate current decay times.

Slow-channel CMS also result from mutations at the ligand binding site. The CMS mutation αG153S was the first discovered at the ACh binding site and was localized to one of three key loops in the α subunit that contribute to the site.[139] Flanking the conserved aromatic residues αW149 and αY151,[33] αG153S greatly slows the rate of ACh dissociation from the doubly liganded closed state of the AChR, increasing ACh affinity for this inactive state. In the mutant AChR, the doubly liganded closed state opens repeatedly during a single ACh occupancy, because the channel opening rate β is some 46-fold greater than is the competing ACh dissociation rate k_{-2}, whereas β is only 3.5-fold greater than is k_{-2} in wild-type AChR. Mutation αG153S also stabilizes the open-channel and desensitized states, suggesting increased ACh affinity for these functional states. Increased stability of the open state is suggested by slowing of the channel closing rate α, whereas increased stability of the desensitized state is demonstrated by tighter binding of ACh to receptors desensitized by the local anesthetic proadifen. Thus, αG153S enhances ACh affinity for resting, active, and desensitized states of the AChR.

The increased channel reopenings by αG153S provide direct evidence for the long-held theory that endplate current decay depends not only on channel gating rate constants but also on binding properties of the agonist.[24] Thus, by slowing the rate of ACh dissociation k_{-2} (Scheme 1), αG153S prolongs individual AChR activation episodes, known as bursts, according to $\tau_B = \tau_o(1 + \beta/k_{-2})$, where τ_B is mean burst duration and τ_o is mean open-interval duration. Burst duration increases further, due to the slower rate of channel closing, α, which increases mean open duration according to $\tau_o = 1/\alpha$. Probability that a doubly liganded receptor will open increases according to $P = \beta/(\beta + k_{-2})$, predicting an increased peak response following instantaneous delivery of ACh. Desensitization is enhanced by αG153S, owing to tighter binding of ACh to this refractory state. Thus, by increasing residence time

of ACh at the binding site in all states of the AChR, αG153S produces a greater peak response, prolongs burst duration, and enhances desensitization.

Fast-Channel Syndromes. Fast-channel mutations have been observed in the AChR α, δ, and ϵ subunits (see Fig. 7A). Mutations in extracellular domains of subunits diminish affinity for ACh, those in TMDs impair gating efficiency, and those in the long cytoplasmic loop of the ϵ subunit destabilize channel kinetics.

Clinical Features. The clinical features resemble those of autoimmune myasthenia gravis, but symptoms are mild when the main effect is on gating efficiency,[146] moderately severe when channel kinetics are unstable,[91,147] and severe when affinity for ACh or both affinity and gating efficiency are impaired.[20,115,132,133] The δE59K mutation in the extracellular domain of the δ subunit that reduces affinity for ACh is of special interest, for it causes multiple congenital joint contractures owing to fetal hypomotility in utero.[20]

Endplate Studies. In the low-affinity fast-channel syndrome caused by ϵP121L in the extracellular domain of the ϵ subunit, endplate morphology and AChR expression are normal.[115,133] In the CMS that affects gating efficiency and is caused by αV285I in TMD3 of the α subunit,[146] as well as in the CMS with unstable channel kinetics caused by an 18 base-pair insertion (ϵ1254ins18) in the long cytoplasmic loop of the ϵ subunit,[91] AChR expression is also reduced. In these CMS, multiple small endplate regions are dispersed over an extended length of the fiber surface, some postsynaptic regions are simplified, and the expression of AChR on the junctional folds is patchy and attenuated. However, the structural integrity of the postsynaptic region is preserved.

The common electrophysiological features of the fast-channel CMS are abnormally brief channel-activation episodes (Fig. 7B) and rapidly decaying low-amplitude endplate currents (Fig. 7C). The reduced amplitude of the synaptic response is due to reduced probability of channel opening and, in the case of ϵ1254ins18 and αV285I, to reduced expression of the mutant receptor.

Molecular Studies. Eight fast-channel mutations have been identified[20,91,115,131–133,146,147] (Fig. 7A). In most cases, the mutated allele causing the kinetic abnormality is accompanied by a null mutation in the second allele so that the kinetic mutation dominates the clinical phenotype, but homozygous fast-channel mutations also exist.

Therapy. The fast-channel syndromes generally respond well to combined therapy with 3,4-DAP, which increases *m*, and cholinesterase inhibitors,

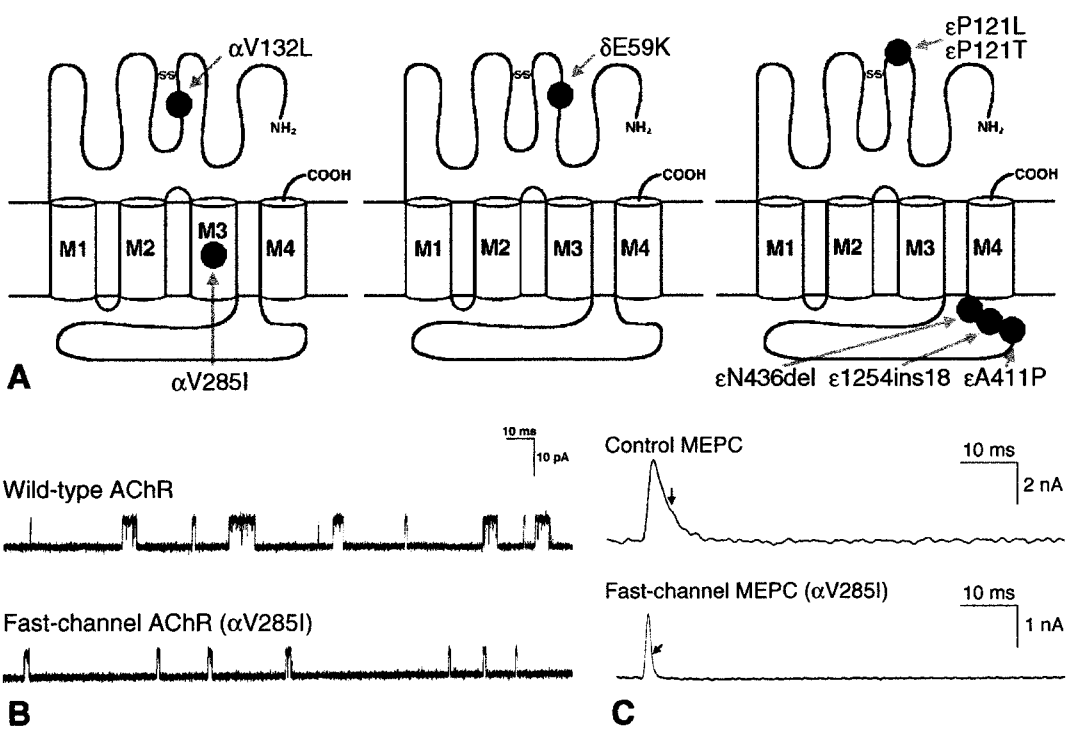


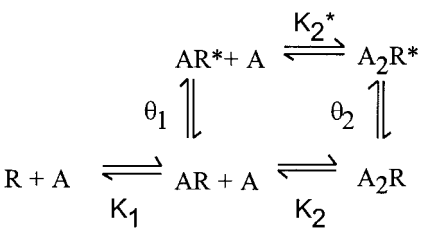
FIGURE 7. (A) Schematic diagram of fast-channel mutations in the AChR α, β, and δ subunits. **(B)** Examples of single-channel currents from wild-type and fast-channel (αV285I) AChRs expressed in HEK cells. **(C)** Miniature endplate currents recorded from endplates of a control subject and a patient harboring the αV285I fast-channel mutation. Arrows indicate decay-time constants.

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.

Kinetic Consequences of the Fast-Channel Mutations. ***Fast-Channel Mutations in the Extracellular Domain or in TMD3.*** In the fast-channel CMS resulting from mutations in the major extracellular domain (ϵP121L) or in TMD3 (αV285I), the most conspicuous effects are dramatic decreases in both the frequency and duration of bursts of ACh-induced openings.[115,146] The opening rate β_2 of the doubly occupied receptor slows by up to 500-fold, and the mean burst duration decreases from ~3 ms for wild-type AChR to <1 ms in the fast-channel mutants. Accompanying the reduced channel activity is reduced steady-state affinity for ACh.

The reduced frequency and duration of channel-opening bursts result from slowing of β_2 coupled with the rate of ACh dissociation, k_{-2}. Efficient channel opening requires that β_2 is greater than k_{-2}, because the probability that a doubly occupied receptor opens is $\beta_2/(\beta_2 + k_{-2})$. A large value of β_2 is also essential for rapid opening of the channel following instantaneous delivery of ACh, and a rapid k_{-2} allows rapid termination of the response essential for high-frequency stimulation of muscle. In the fast-channel CMS due to the mutation ϵP121L, the 500-fold slowing of β_2 might have resulted from tighter binding of ACh to the resting state, but resting state affinity was only slightly affected. Thus, ϵP121 emerges as critical in forming the transition state in the path toward the open state.

Also essential for efficient channel opening is tighter binding of ACh to the open state, which is impaired in the fast-channel CMS due to the ϵP121L mutation at the binding site. Open-state affinity was determined by detailed balancing, using the measured closed-state affinity and gating equilibria for singly and doubly liganded receptors, in the following cycle derived from Scheme 2:



(Scheme 3)

Open-state affinity, given by $K_2^* = K_2\theta_1/\theta_2$, decreased from 35 nM for wild type to 1.5 μM for ϵP121L.[115] Thus, ϵP121L hinders the fit of ACh to the binding site in the open state, which biases the channel-opening equilibrium toward the resting state. The overall results from these studies demonstrate that tighter binding of ACh to the open state is the fundamental driving force underlying agonist-induced activation of the AChR.

Fast-Channel CMS Caused by Mutations in the Long Cytoplasmic Domain between TMD3 and TMD4. Since the initial cloning of AChR subunits, the long cytoplasmic domain between TMD3 and TMD4 has drawn considerable attention. Fourier analysis of residue hydrophobicity as a function of sequence strongly suggested an amphipathic α-helical secondary structure in the cytoplasmic domain.[47] More recent secondary structural analysis, based on multiple-sequence alignments, divides the cytoplasmic domain into three predicted α-helices, designated F, G, and H and corresponding to ϵ subunit residues 320–333, 398–415, and 421–435, respectively.[77] The amphipathic composition initially suggested formation of the ion channel, but mutagenesis showed that the cytoplasmic domain could be deleted without loss of channel activity.[92] Clues about functional significance of the cytoplasmic domain emerged from studies investigating the structural basis of the fetal to adult kinetic switch, in which mean open duration decreases from about 10 ms to 1 ms, and AChRs containing the γ subunit are replaced by those containing the ϵ subunit. Multiple residues in the cytoplasmic domain of the ϵ and γ subunits were shown to account for about half the kinetic switch, with the balance due to two residues in the neighboring TMD4 domain.[16] The overall results pointed to a specific role of the ϵ subunit in tuning the kinetics of the adult AChR, with functionally significant residues located in helices G and H of the cytoplasmic domain. Two CMS mutations further pointed to the cytoplasmic domain as important in governing the kinetics of AChR activation. The first case arose from tandem duplication of six residues, STRDQE (ϵ1254ins18), originating at codons 413–418 in helix G,[91] and the second from the mutation ϵA411P, just two residues upstream from the duplication.[147]

Functional Consequences of a Six-Residue Duplication STRDQE in the ϵ Subunit. The STRDQE duplication (ϵ1254ins18)[91] causes individual receptor channels to suddenly change kinetics, also known as mode-switching. Mode-switching is quite rare in wild-type AChR,[9,97,147] but that it occurs suggests that the STRDQE mutation amplifies a normal process.

Mode switches were readily observed during activation episodes elicited by high concentrations of ACh, which appeared as clusters of events in quick succession flanked by prolonged quiescent periods. Three distinct kinetic modes could be discerned within clusters, each with reduced probability of opening due to slower rates of channel opening and faster rates of channel closing. Also, kinetic analysis of the separated modes revealed two open states at saturating concentrations of ACh, rather than the single open state observed for wild-type AChR. Thus, studies of the STRDQE insertion provided the first hint that the cytoplasmic loop governs uniformity of AChR gating kinetics.

Functional Consequences of the Mutation ϵA411P. Unlike the STRDQE duplication beginning at codon 413 of the ϵ subunit, the nearby mutation ϵA411P does not increase frequency of mode-switching within clusters but instead causes individual clusters of activation episodes to span a wide spectrum of kinetics.[147] Current pulses through most individual mutant receptors appeared kinetically uniform, but each activation episode had a unique kinetic signature, with the overall range of open probabilities greatly expanded. Hidden Markov modeling analysis of the kinetics of individual activation episodes revealed a Gaussian distribution for each rate constant in a kinetic description of receptor activation (i.e., Scheme 1). The distributions for agonist-binding rate constants were unaltered by the mutation, but those for channel opening and closing steps showed remarkable broadening. Proline mutations placed in positions flanking ϵA411 also produced a wide spectrum of kinetics similar to that produced by ϵA411P, whereas proline mutations placed in equivalent positions of β and δ subunits produced the usual narrow range of kinetics characteristic of wild-type AChR.[147] The possibility that αA411P causes folding heterogeneity of individual receptors was considered unlikely, because individual receptors could be seen to switch between modes in a small proportion of activation episodes. Thus, the ϵ subunit emerges as a structure that governs the kinetics of channel gating where residues flanking ϵA411 maintain fidelity of the opening and closing rate constants.

Mechanistic Consequences of Mutations in the Long Cytoplasmic Loop of the ϵ Subunit. Analysis of the ϵA411P mutation provides a unifying explanation for the kinetic consequences of structural alterations in the cytoplasmic loop, including the fetal to adult kinetic switch, mode-switching by the STRDQE duplication, and the wide spectrum of kinetics produced by ϵA411P. Each of these effects can be ascribed to a localized structural change in the cytoplasmic domain that affects the global energetics that govern channel gating. The observation that both wild-type and mutant AChRs shuttle among multiple stable states indicates that the energy landscape underlying gating of the AChR is corrugated.[48] Because the wild-type AChR gates in predominantly one mode, the corrugations superimpose upon a steep funnel-shaped foundation. Receptors containing the ϵA411P mutation are subject to a similar corrugated energy landscape, but the corrugations superimpose on a much shallower funnel-shaped foundation. Thus, the local region flanking ϵA411 shapes the broad foundation upon which the corrugations superimpose. In the ϵA411P mutant, barriers separating energy wells are relatively high, similar to wild type, so only rare mode switches are detected during individual activation episodes. Thus, in addition to affecting absolute rates of gating, the cytoplasmic loop of the ϵ subunit controls fidelity of the gating rate constants. The overall findings suggest that the primordial receptor opened and closed over a wide range of kinetics but that evolution introduced and fine tuned new structures, such as the cytoplasmic loop, to create the present-day receptor that gates with uniform kinetics.

AChR Deficiency Caused by Mutations in AChR Subunit Genes. ***Clinical Features.*** These vary from mild to very severe. In general, patients harboring low-expressor or even homozygous null mutations in the ϵ subunit may have mild symptoms. Conversely, patients with low-expressor mutations in non-ϵ subunits are severely affected, and no patient with null mutations in both alleles of a non-ϵ subunit has been observed to date.

Endplate Studies. Morphological studies show an increased number of endplate regions distributed over an increased span of the muscle fiber. The integrity of the junctional folds is preserved, but some endplate regions are simplified and smaller than normal. The distribution of AChR on the junctional folds is patchy, and the density of the reaction for AChR is attenuated. The immunocytochemical reaction for rapsyn, a molecule that cross-links AChRs, is decreased in proportion to the decrease of AChR expression.

The quantal response at the endplate, indicated by the amplitude of MEPPs and currents, is reduced, but quantal release by nerve impulse is frequently higher than normal. In patients with low-expressor or null mutations of the ϵ subunit, single-channel patch-clamp recordings[88,111] and immunocytochemical studies[43] reveal the presence of fetal γ-AChR at

the endplate.

Molecular Studies. Congenital myasthenic syndromes with severe endplate AChR deficiency result from different types of homozygous or, more frequently, heterozygous recessive mutations in AChR subunit genes (see Table 4 and Fig. 8). The mutations are concentrated in the ε subunit. A likely reason for this is that persistent expression of the fetal type γ subunit, although at a low level, may compensate for absence of the ε subunit,[31,43,91,111] whereas patients harboring null mutations in subunits other than ε might not survive for lack of a substituting subunit. In addition, the gene encoding the ε subunit, and especially exons encoding the long cytoplasmic loop, have a high GC content that could predispose to DNA rearrangements.

The AChR deficiency results from mutations that cause premature termination of the translational chain by frameshift, by being at a splice site, or by generating a stop codon directly; point mutations in the promoter region; chromosomal microdeletion; and missense mutations. Some missense mutations appear in the signal peptide region (εG-8R[115] and εV-13D[87]). Other missense mutations involve residues essential for assembly of the pentameric receptor; mutations of this type were observed in the ε subunit at an N-glycosylation site (εS143L),[115] in cysteine 128 (εC128S),[91] in arginine 147 (εR147L) in the extracellular domain, which lies between isoleucine 145 and threonine 150, residues that contribute to subunit assembly,[111] and in threonine 51 (εT51P)[87]; and with a three-codon deletion in the long cytoplasmic loop of the β subunit.[122] Still other missense mutations affect both AChR expression and kinetics. For example, εR311W in the long cytoplasmic loop between M3 and M4 decreases,[111] whereas εP245L in the M1 domain increases,[111] the open duration of channel events. In the case of εR311W and εP245L, the kinetic consequences are modest and are likely overshadowed by the reduced expression of the mutant gene.

Finally, it is noteworthy that the frameshifting ε1267delG mutation occurring at homozygosity is endemic in families of Gypsy or southeastern European origin[1,29,102] where it derives from a Gypsy founder.[1]

Therapy. Most patients respond moderately well to anticholinesterase drugs, and some derive additional benefit from 3,4-DAP.[63]

AChR Deficiency Caused by Mutations in Rapsyn. Endplate AChR deficiency also could stem from defects in proteins that regulate the expression or concentration of AChR at the endplate. These include

Table 4. Low expressor mutations in AChR subunits.

Subunit/effect	Mutation	Reference
α Subunit		
Frameshift	α381delC	133
	α459insG	82
Missense	αG74C	89
	αF233V	146
	αI296L	82
	αV402F	89
β Subunit		
Frameshift	β1276del9	122
	βdelEx8	122
δ Subunit		
Frameshift	δ756ins2	20
Missense	δP250Q	112
ε Subunit		
Chromosomal microdeletion	εΔ1290bp	2
Promoter point mutation	ε-156C → T	99
	ε-155G → A	101
	ε-154G → A	2
Signal peptide, missense	εV-13D	87
	εG-8R	115
Frameshift	ε59ins5	102
	ε67insG	2
	ε70insG	102
	ε127ins5	111
	ε553del7	88, 106, 111
	ε627ins2	30
	ε723delC	87
	ε734delC	18, 83
	ε760ins8	87
	ε911delT	18, 135
	ε1012del20	108
	ε1030insC	18
	ε1030delC	135
	ε1033delG	19, 30
	ε1101insT	43
	ε1197delG	2
	ε1206ins19	30, 102
	ε1208ins19	31
	ε1259del23	19
	ε1267delG	1, 2, 29, 66, 102
	ε1276delG	102
	ε1293insG	18, 31, 43, 136, 147
	ε1369delG	2
Nonsense	εR64X	111, 135
	εE154X	18
	εQ310X	18
	εQ378X	2,18
Splice-site	εIVS41G → A	135
	εIVS6-1G → C	18
	εIVS61G → T	36
	εIVS7-2A → G	2, 18
	εIVS72T → C	2, 102
	εIVS91G → T	31
	εIVS9-1G → C	106, 131
	εIVS102T → G	87
	εIVS10-9ins16	113
Missense	εR20W	132
	εT51P	87
	εC128S	2, 91
	εS143L	115
	εR147L	111
	εT159P	147
	εP245L	111
	εR311W	87, 111
	εP331L	31

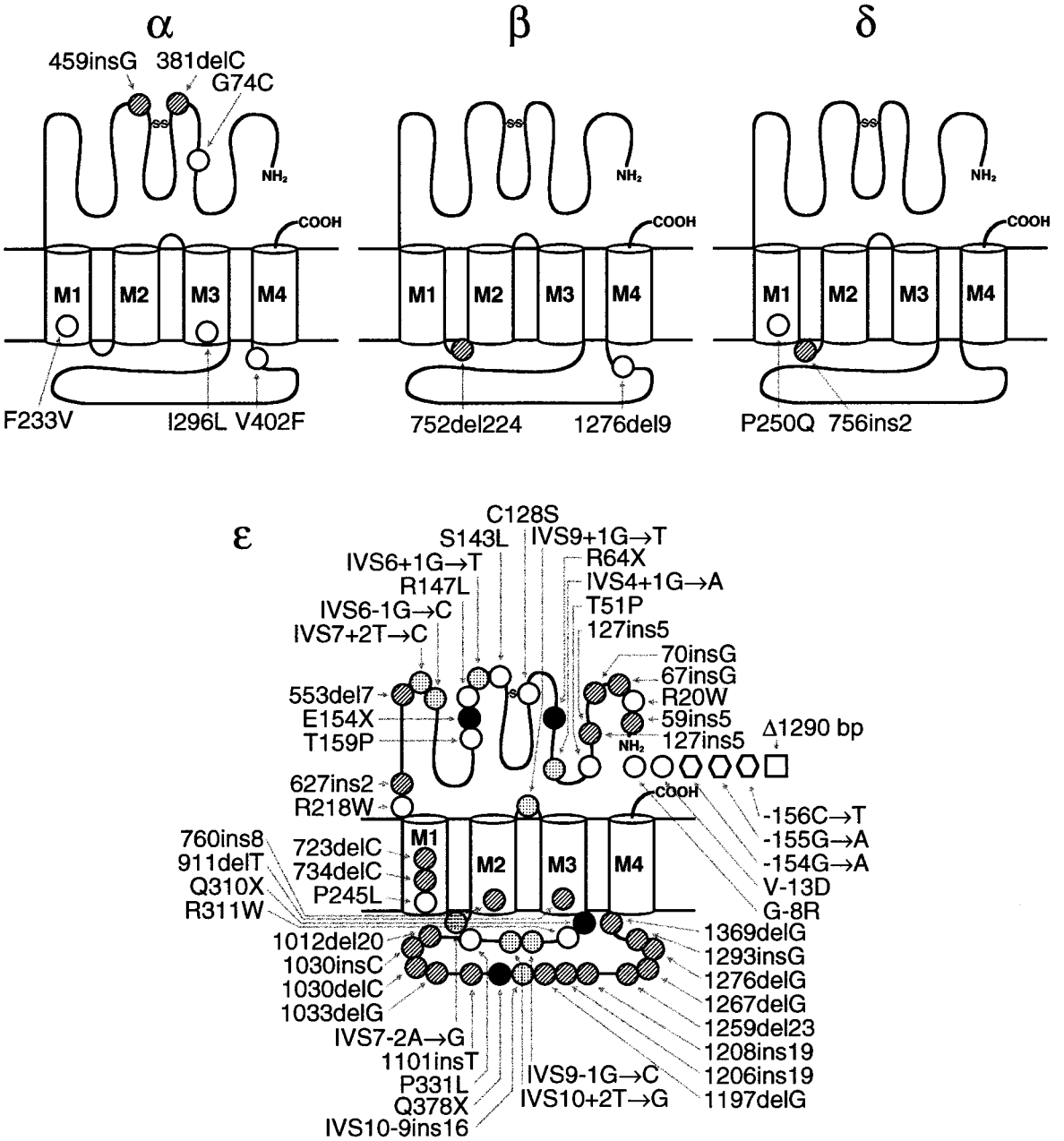


FIGURE 8. Schematic diagram of low-expressor and null mutations reported in the α, β, δ, and ϵ subunits of AChR. Square indicates a chromosomal microdeletion, hexagons are promoter mutations, open circles are missense mutations, closed circles are nonsense mutations, shaded circles are frameshifting 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. Table 4 indicates references for each mutation.

agrin[52,93] and its signaling molecules, muscle-specific kinase (MuSK)[54] and rapsyn,[6,53,124] neuregulin and its signaling molecules,[5,129,134] as well as α-dystrobrevin,[98] utrophin,[58] and α-syntrophin.[3] Thus far, however, only mutations in rapsyn have been shown to cause a CMS associated with endplate AChR deficiency.

Rapsyn, under the influence of neural agrin, plays a critical role in concentrating the AChR in the postsynaptic membrane of the motor endplate.[49]

Rapsyn binds to the long cytoplasmic loop of each AChR subunit[80,81] and links the receptor to the subsynaptic cytoskeleton via dystroglycan[22] and an actin-binding synaptic nebulin-related anchoring

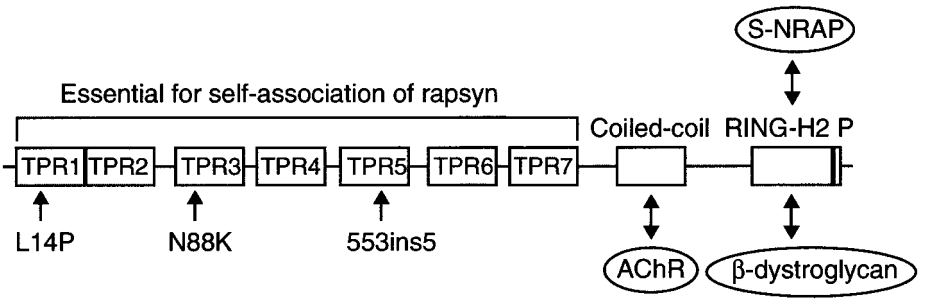


FIGURE 9. Schematic diagram showing domains of rapsyn and identified mutations. Seven TPRs are required for rapsyn self-association; the coiled-coil domain binds to the long cytoplasmic loop of AChR subunits; the RING-H2 domain links rapsyn to β-dystroglycan and to the actin-binding S-NRAP.

protein (S-NRAP).[141] The primary structure of rapsyn predicts distinct structural domains: a myristoylation signal at the N-terminus required for membrane association[124]; seven tetratrico peptide repeats (TPRs; codons 6–279) that subserve rapsyn self-association[123,124]; a coiled-coil domain (codons 298–331) whose hydrophobic surface can bind to determinants within the long cytoplasmic loop of each AChR subunit[12,123]; a cysteine-rich RING-H2 domain (codons 363–402) that binds to the cytoplasmic domain of β-dystroglycan[13] and to S-NRAP[141]; and a serine phosphorylation site at codon 406 (Fig. 9).

Self-association of rapsyn is critical for linking AChR to the cytoskeleton. Rapsyn expressed in HEK cells or other nonmuscle cells self-associates into clusters on the cell surface. When AChR, β-dystroglycan, and S-NRAP are expressed individually in HEK cells, none of these proteins aggregates into clusters, but when either AChR,[49,119,123] dystroglycan,[7,13] or S-NRAP[141] is coexpressed with rapsyn, each protein is recruited to existing rapsyn clusters.

Clinical Features. Patients carrying rapsyn mutations can have mild or severe symptoms. Moreover, among two patients homozygous for the same N88K mutations in rapsyn, one is severely affected at age 2 years, whereas the other has only mild symptoms at age 27 years. Furthermore, one patient born with multiple joint contractures shows only mild weakness at age 11 years.[107] A decremental EMG response was obtained in two severely affected patients, but in two mildly affected patients a defect of neuromuscular transmission was revealed only after exercise or by SFEMG.

Endplate Studies. The morphological and in vitro electrophysiological findings at the endplates are similar to those observed in patients whose endplate AChR deficiency stems from low-expressor mutations in AChR subunit genes. Rapsyn as well as AChR expression are both reduced, as in patients with primary AChR deficiency, so their localization at the endplate does not distinguish between a primary defect in AChR and rapsyn.

Molecular Studies. In the course of investigating CMS, we identified 37 patients with deficiency but no kinetic abnormality of AChR. A rigorous search for mutations in the AChR α, β, δ, and ε subunit genes revealed pathogenic mutations in 27 of these patients. To identify the cause of AChR deficiency in the other 10 patients, we sequenced the rapsyn gene and identified a subset of four patients, each carrying two mutant alleles arising from three mutations[107] (see Fig. 9). Expression studies of wild-type or mutant rapsyns along with wild-type AChR subunits in HEK cells revealed that none of the mutations hindered rapsyn self-association, but each mutation hindered recruitment of AChR to rapsyn clusters.

Therapy. The patients observed to date responded moderately well to anticholinesterase drugs, and three who were also given 3,4-DAP derived additional benefit from it.

Congenital Myasthenic Syndrome Associated with Plectin Deficiency. Plectin is a highly conserved and ubiquitously expressed intermediate filament-linking protein concentrated at sites of mechanical stress, such as the postsynaptic membrane of the endplate, the sarcolemma, Z-disks in skeletal muscle, hemidesmosomes in skin, and intercalated disks in cardiac muscle. Pathogenic mutations in plectin are associated with a simplex variety of epidermolysis bullosa, a progressive myopathy, and a myasthenic syndrome (reviewed by Banwell et al.[11]).

Detailed investigation of a patient with epidermolysis bullosa simplex revealed that plectin expression was absent in muscle and severely decreased in skin and was associated with a progressive myopathy; abnormal fatigability involving ocular, facial, and limb muscles; a decremental EMG response; and no anti-AChR antibodies. Morphological studies of mus-

cle demonstrated necrotic and regenerating fibers and a wide spectrum of ultrastructural abnormalities. Many endplates had an abnormal configuration with chains of small regions over the fiber surface, and a few endplates displayed focal degeneration of the junctional folds. The endplate AChR content was normal. In vitro electrophysiological studies showed normal quantal release by nerve impulse, small MEPPs, and expression of fetal as well as adult AChR at the endplates. Pyridostigmine failed to improve the patient's symptoms, but 3,4-DAP improved her strength and endurance.[11]

Work in the authors' laboratories was supported by grants from the National Institutes of Health to A. G. Engel (grant no. NS6277) and to S. M. Sine (grant no. NS 31744) and a Muscular Dystrophy Association grant to A. G. Engel.

REFERENCES

1. Abicht A, Stucka R, Karcagi V, Hercegfalvi A, Horváth R, Mortier W, Schara U, Ramaekers V, Jost W, Brunner J, Janssen G, Seidel U, Schlotter B, Pongratz D, Rüdel R, Lochmüller H. A common mutation (ϵ1267delG) in congenital myasthenic patients of Gipsy ethnic origin. Neurology 1999;53:1564–1569.
2. Abicht A, Stucka R, Song I-H, Karcagi V, Kugler K, Baumgarten-Walczak A, Stier c, Pongratz D, Mortier W, Müller-Ferber W, Lochmüller H. Genetic analysis of the entire AChR ϵ-subunit gene in 52 congenital myasthenic families. Acta Myol 2000;19:23–27.
3. Adams ME, Kramarcy M, Krall SP, Rossi SG, Rotundo RL, Sealock R, Froehner SC. Absence of α-syntrophin leads to structurally aberrant neuromuscular synapses deficient in utrophin. J Cell Biol 2000;150:1385–1398.
4. Akabas MH, Kaufmann C, Archdeacon P, Karlin A. Identification of acetylcholine receptor channel-lining residues in the entire M2 segment of the alpha subunit. Neuron 1994; 13:919–927.
5. Altiok N, Altiok K, Changeux J-P. Heregulin-stimulated acetylcholine receptor gene expression in muscle—requirement for MAP kinase and evidence for parallel inhibitory pathway independent electrical activity. EMBO J 1997;16: 717–725.
6. Apel ED, Glass DJ, Moscosco LM, Yancopoulos GD, Sanes JR. Rapsyn is required for MuSK signaling and recruits synaptic components to a MuSK-containing scaffold. Neuron 1997; 18:623–625.
7. Apel ED, Roberds SL, Campbell KP, Merlie JP. Rapsyn may function as a link between the acetylcholine receptor and the agrin-binding dystrophin-associated glycoprotein complex. Neuron 1995;15:115–126.
8. Apparsundaram S, Ferguson SM, George ALJr, Blakely RD. Molecular cloning of a human, hemicholinium-3–sensitive choline transporter. Biochem Biophys Res Commun 2000; 276:862–867.
9. Auerbach A, Lingle CJ. Heterogeneous kinetic properties of acetylcholine receptor channels in *Xenopus* oocytes. J Physiol (Lond) 1986;378:119–140.
10. Bady B, Chauplannaz G, Carrier H. Congenital Lambert–Eaton myasthenic syndrome. J Neurol Neurosurg Psychiatry 1987;50:476–478.
11. Banwell BL, Russel J, Fukudome T, Shen X-M, Stilling G, Engel AG. Myopathy, myasthenic syndrome, and epidermolysis bullosa simplex due to plectin deficiency. J Neuropathol Exp Neurol 1999;58:832–846.
12. Bartoli M, Cohen JB. Identification of the modular domains of rapsyn binding to nicotinic acetylcholine receptor (AChR) and to dystroglycan. Soc Neurosci Abstract 2001;27: Program No. 904.16.
13. Bartoli M, Ramarao MK, Cohen JB. Interactions of the rapsyn RING-H2 domain with dystroglycan. J Biol Chem 2001;276:24911–24917.
14. Bon S, Coussen F, Massoulié J. Quaternary associations of acetylcholinesterase. II. The polyproline attachment domain of the collagen tail. J Biol Chem 1997;272:3016–3021.
15. Bööj S, Larsson P-A, Dahllöf A-G, Dahlström A. Axonal transport of synapsin I and cholinergic synaptic vesicle-like material. Further immunohistochemical evidence for transport of axonal cholinergic transmitter vesicles in motor neurons. Acta Physiol Scand 1986;128:155–165.
16. Bouzat C, Bren N, Sine SM. Structural basis of different gating kinetics of fetal and adult acetylcholine receptors. Neuron 1994;13:1395–1402.
17. Brejc K, van Dijk WV, Schuurmans M, van der Oost J, Smit AB, Sixma TK. Crystal structure of ACh-binding protein reveals the ligand-binding domain of nicotinic receptors. Nature 2001;411:269–276.
18. Brengman JM, Ohno K, Milone M, Friedman RL, Feldman RG, Engel AG. Identification and functional characterization of eight novel acetylcholine receptor mutations in six congenital myasthenic syndrome kinships. Neurology 2000; 54(Suppl 3):A182–A183.
19. Brengman JM, Ohno K, Shen X-M, Engel AG. Congenital myasthenic syndrome due to two novel mutations in the acetylcholine receptor ϵ subunit gene. Muscle Nerve 1998; 21(Suppl 7):S120.
20. Brownlow S, Webster R, Croxen R, Brydson M, Neville B, Lin J-P, Vincent A, Newsom-Davis J, Beeson D. Acetylcholine receptor δ subunit mutations underlie a fast-channel myasthenic syndrome and arthrogryposis multiplex congenita. J Clin Invest 2001;108:125–130.
21. Byring RF, Pihko H, Shen X-M, Gustafsson B, Hackman P, Ohno K, Engel AG, Udd B. Congenital myasthenic syndrome associated with episodic apnea and sudden infant death. Neuromuscul Disord 2002;12:548–553.
22. Cartaud A, Coutant S, Petrucci TC, Cartaud J. Evidence for in situ and in vitro association between β-dystroglycan and the subsynaptic 43K rapsyn protein. Consequence for acetylcholine receptor clustering at the synapse. J Biol Chem 1998;273:11321–11326.
23. Chiara DC, Middleton RE, Cohen JB. Identification of tryptophan 55 as the primary site of [^{3}H]nicotine photoincorporation in the gamma subunit of Torpedo nicotinic acetylcholine receptor. FEBS Lett 1998;423:223–226.
24. Colquhoun D, Sakmann B. Fluctuations in the microsecond time range of the current through single acetylcholine receptor ion channels. Nature 1981;294:464.
25. Conomy JP, Levisohn M, Fanaroff A. Familial infantile myasthenia gravis: a cause of sudden death in young children. J Pediatr 1975;87:428–429.
26. Corringer JP, Bertrand S, Galzi J-L, Devillers-Thiéry A, Changeux J-P, Bertrand D. Mutational analysis of the charge selectivity filter of the α7 nicotinic acetylcholine receptor. Neuron 1999;22:831–843.
27. Corringer JP, Le Novère N, Changeux J-P. Nicotine receptors at the amino acid level. Annu Rev Pharmacol Toxicol 2000;40:431–458.
28. Croxen R, Newland C, Beeson D, Oosterhuis H, Chauplanaz G, Vincent A, Newsom-Davis J. Mutations in different functional domains of the human muscle acetylcholine receptor α subunit in patients with the slow-channel congenital myasthenic syndrome. Hum Mol Genet 1997;6:767–774.
29. Croxen R, Newland C, Betty M, Vincent A, Newsom-Davis J, Beeson D. Novel functional ϵ-subunit polypeptide generated by a single nucleotide deletion in acetylcholine receptor deficiency congenital myasthenic syndrome. Ann Neurol 1999;46:639–647.

30. Croxen R, Newsom-Davis J, Beeson D. Endplate acetylcholine receptor deficiency syndrome: two new mutations. Acta Myol 2000;19:45–48.
31. Croxen R, Young C, Slater C, Haslam S, Brydson M, Vincent A, Beeson D. Endplate γ- and ϵ-subunit mRNA levels in AChR deficiency syndrome due to ϵ subunit null mutations. Brain 2001;124:1362–1372.
32. Czajkowski C, Karlin A. Structure of the nicotinic receptor acetylcholine binding site. J Biol Chem 1995;270:3160–3164.
33. Dennis M, Giraudat J, Kotzyba-Hilbert F, Goeldner M, Hirth C, Chang J, Lazure C, Cretien M, Changeux J-P. Amino acids of Torpedo marmorata acetylcholine receptor alpha subunit labeled by a photoaffinity ligand for the acetylcholine binding site. Biochemistry 1988;27:2346–2357.
34. Deprez PN, Inestrosa NC. Two heparin-binding domains are present on the collagenic tail of asymmetric acetylcholinesterase. J Biol Chem 1995;270:11043–11046.
35. Dettbarn WD. Pesticide induced muscle necrosis: mechanism and prevention. Fund Appl Toxicol 1984;4:S18–S26.
36. Deymeer F, Serdaroglu P, Gülsen-Parman Y, Öztürk A, Özdemir C. Clinical characteristics of a group of Turkish patients having a benign CMS phenotype with ptosis and marked ophthalmoparesis and mutations in the acetylcholine receptor epsilon subunit gene. Acta Myol 2000;19:29–32.
37. Deymeer F, Serdaroglu P, Özdemir C. Familial infantile myasthenia: confusion in terminology. Neuromuscul Disord 1999;9:129–130.
38. Donger C, Krejci E, Serradell P, Eymard B, Bon S, Nicole S, Chateau D, Gary F, Fardeau M, Massoulié J, Guicheney P. Mutation in the human acetylcholinesterase-associated gene, *COLQ*, is responsible for congenital myasthenic syndrome with end-plate acetylcholinesterase deficiency. Am J Hum Genet 1998;63:967–975.
39. Eiden LE. The cholinergic gene locus. J Neurochem 1998; 70:2227–2240.
40. Engel AG, Lambert EH. Congenital myasthenic syndromes. Electroencephalogr Clin Neurophysiol Suppl 1987;39:91–102.
41. Engel AG, Lambert EH, Gomez MR. A new myasthenic syndrome with end-plate acetylcholinesterase deficiency, small nerve terminals, and reduced acetylcholine release. Ann Neurol 1977;1:315–330.
42. Engel AG, Lambert EH, Mulder DM, Torres CF, Sahashi K, Bertorini TE, Whitaker JN. A newly recognized congenital myasthenic syndrome attributed to a prolonged open time of the acetylcholine-induced ion channel. Ann Neurol 1982; 11:553–569.
43. Engel AG, Ohno K, Bouzat C, Sine SM, Griggs RG. End-plate acetylcholine receptor deficiency due to nonsense mutations in the ϵ subunit. Ann Neurol 1996;40:810–817.
44. Engel AG, Ohno K, Milone M, Wang H-L, Nakano S, Bouzat C, Pruitt JN, Hutchinson DO, Brengman JM, Bren N, Sieb JP, Sine SM. New mutations in acetylcholine receptor subunit genes reveal heterogeneity in the slow-channel congenital myasthenic syndrome. Hum Mol Genet 1996;5:1217–1227.
45. Engel AG, Ohno K, Sine SM. Congenital myasthenic syndromes. In: Engel AG, editor. Myasthenia gravis and myasthenic disorders. New York: Oxford University Press; 1999. p 251–297.
46. Erickson JD, Varoqui H, Eiden LE, Schafer MK, Modi W, Diebler M, Weihe E, Rand J, Bonner TI, Usdin TB. Functional identification of a vesicular acetylcholine transporter and its expression from a 'cholinergic' gene locus. J Biol Chem 1994;269:21929–21932.
47. Finer-Moore J, Stroud RM. Amphipathic analysis and possible formation of the ion channel in an acetylcholine receptor. Proc Natl Acad Sci USA 1984;81:155–159.
48. Frauenfelder H, Lesson DT. The energy landscape of nonbiological and biological molecules. Nat Struct Biol 1998;5: 757–759.
49. Froehner SC, Luetje CW, Scotland PB, Patrick J. The postsynaptic 43K protein clusters muscle nicotinic acetylcholine receptors in *Xenopus* oocytes. Neuron 1990;5:403–410.
50. Fukudome T, Ohno K, Brengman JM, Engel AG. Quinidine normalizes the open duration of slow-channel mutants of the acetylcholine receptor. Neuroreport 1998;9:1907–1911.
51. Galzi J-L, Revah F, Black D, Goeldner M, Hirth C, Changeux J-P. Identification of a novel amino acid α-tyrosine 93 within the cholinergic ligand-binding sites of the acetylcholine receptor by photoaffinity labeling. J Biol Chem 1990;265:10430–10437.
52. Gautam M, Noakes PG, Moscoso L, Rupp F, Scheller RH, Merlie MP, Sanes JR. Defective neuromuscular synaptogenesis in agrin-deficient mutant mice. Cell 1996;85:525–535.
53. Gautam M, Noakes PG, Mudd J, Nichol M, Chu GC, Sanes JR, Merlie JP. Failure of postsynaptic specialization to develop at neuromuscular junctions of rapsyn-deficient mice. Nature 1995;377:232–236.
54. Glass DJ, Bowen DC, Stitt TN, Radziejewski C, Bruno J, Ryan TE, Gies DR, Shah H, Mattson K, Burden SJ, Distefano PS, Valenzuela DM, Dechiara TM, Yancopoulos GD. Agrin acts via MuSK receptor complex. Cell 1996;85:513–523.
55. Gomez CM, Maselli R, Gammack J, Lasalde J, Tamamizu S, Cornblath DR, Lehar M, McNamee M, Kuncl R. A beta-subunit mutation in the acetylcholine receptor gate causes severe slow-channel syndrome. Ann Neurol 1996;39:712–723.
56. Gomez CM, Maselli R, Staub J, Day JW, Cens T, Wollman RL, Charnet PC. Novel δ and β subunit acetylcholine receptor mutations in the slow-channel syndrome demonstrate phenotypic variability. Soc Neurosci Abstr 1998;24:484.
57. Gomez CM, Maselli R, Vohra BPS, Navedo M, Stiles JR, Charnet P, Schott K, Rojas L, Keesey J, Verity A, Wollman RW, Lasalde-Dominici J. Novel delta subunit mutation in slow-channel syndrome causes severe weakness by novel mechanism. Ann Neurol 2002;51:102–112.
58. Grady RM, Merlie JP, Sanes JR. Subtle neuromuscular defects in utrophin-deficient mice. J Cell Biol 1997;136:871–882.
59. Greer M, Schotland M. Myasthenia gravis in the newborn. Pediatrics 1960;26:101–108.
60. Grosman C, Salamone FN, Sine SM, Auerbach A. The extracellular linker of muscle acetylcholine receptor channels is a gating control element. J Gen Physiol 2000;116:327–339.
61. Harper CM. Electrodiagnosis of endplate disease. In: Engel AG, editor. Myasthenia gravis and myasthenic disorders. New York: Oxford University Press; 2002. p 65–84.
62. Harper CM, Engel AG. Quinidine sulfate therapy for the slow-channel congenital myasthenic syndrome. Ann Neurol 1998;43:480–484.
63. Harper CM, Engel AG. Treatment of 31 congenital myasthenic syndrome patients with 3,4-diaminopyridine. Neurology 2000;54(Suppl 3):A395.
64. Harper CM, Engel AG, Fukudome T, Zochodne DW, Friedli WG. Treatment of slow channel congenital myasthenic syndrome (SCCMS) with fluoxetine. Neurology 2002;58(Suppl 3):A329.
65. Hart Z, Sahashi K, Lambert EH, Engel AG, Lindstrom J. A congenital, familial, myasthenic syndrome caused by a presynaptic defect of transmitter resynthesis of mobilization. Neurology 1979;29:559.
66. Hercegfalvi A, Abicht A, Karcagi V, Lochmüller H. Case report: congenital myasthenic syndrome in a Gipsy family showing pseudodominant pattern of inheritance. Acta Myol 2000;19:49–51.
67. Hutchinson DO, Walls TJ, Nakano S, Camp S, Taylor P, Harper CM, Groover RV, Peterson HA, Jamieson DG, Engel AG. Congenital endplate acetylcholinesterase deficiency. Brain 1993;116:633–653.
68. Ishigaki K, Nicolle D, Krejci E, Osawa M, Guicheney P, Koenig J, Fardeau M, Eymard B, Hantai D. Two novel mutations in the ColQ gene causing endplate acetylcholinesterase deficiency. Neuromuscul Disord 2001;11:666–667.

69. Jackson MB. Perfection of a synaptic receptor: kinetics and energetics of the acetylcholine receptor. Proc Natl Acad Sci USA 1989;86:2199–2203.
70. Karlin A, Akabas MH. Toward a structural basis for the function of nicotinic acetylcholine receptors and their cousins. Neuron 1994;15:1231–1244.
71. Katz B, Miledi R. The binding of acetylcholine to receptors and its removal from the synaptic cleft. J Physiol (Lond) 1973;231:549–574.
72. Katz B, Thesleff S. A study of the 'desensitization' produced by acetylcholine at the motor end-plate. J Physiol (Lond) 1957;138:63–80.
73. Kiene L-M, Stadler H. Synaptic vesicles in electromotoneurons. I. Axonal transport, site of transmitter uptake and processing of a core proteoglycan during maturation. EMBO J 1987;6:2209–2215.
74. Kimbell LM, Ohno K, Rotundo RL, Engel AG. Transplanting mutant human collagenic tailed acetylcholinesterase onto the frog neuromuscular junction: evidence for an attachment defect in a congenital myasthenic syndrome. Mol Biol Cell 2001;12:(Suppl) 161a.
75. Labarca C, Nowak MW, Zhang H, Tang L, Deshpande P, Lester HA. Channel gating governed symmetrically by conserved leucine residues in the M2 domain of nicotinic receptors. Nature 1995;376:514–516.
76. Lambert EH, Elmqvist D. Quantal components of end-plate potentials in the myasthenic syndrome. Ann NY Acad Sci 1971;183:183–199.
77. Le Novère N, Corringer PJ, Changeux J-P. Improved secondary structure predictions for a nicotinic receptor subunit: incorporation of solvent accessibility and experimental data into a two-dimensional representation. Biophys J 1999;76: 2329–2345.
78. Llinás R, Sugimori M, Lin J-W, Leopold P-L, Brady ST. ATP-dependent directional movement of rat synaptic vesicles injected into the presynaptic terminal of squid giant synapse. Proc Natl Acad Sci USA 1989;86:5656–5660.
79. Lundh H, Nilsson O, Rosen I. Treatment of Lambert–Eaton syndrome: 3,4-diaminopyridine and pyridostigmine. Neurology 1984;34:1324–1330.
80. Maimone MM, Merlie JP. Interaction of the 43 kd postsynaptic protein with all subunits of the muscle nicotinic acetylcholine receptor. Neuron 1993;11:53–66.
81. Maimone MM, Merlie JP. The intracellular domain of the nicotinic acetylcholine receptor α subunit mediates its coclustering with rapsyn. Mol Cell Neurosci 1999;14:340–354.
82. Maselli R, Chen M, Chen T-Y, Kong D, Constance B, McDonald C, Fairclough RH, Gomez CM, Wollman RL. A heteroallelic nonsense/missense mutation in the acetylcholine receptor α-subunit causing severe endplate AChR deficiency. Neurology 2002;58(Suppl 3):A230.
83. Maselli R, Chen M, Chen T-Y, Kong DZ, Agius MA, Bowe C, Wollman RL, Gomez CM. Severe low-affinity fast-channel syndrome due to a heteroallelic nonsense/missense mutation in the acetylcholine receptor ϵ-subunit. Neurology 2002; 58(Suppl 3):A228.
84. Maselli RA, Kong DZ, Bowe CM, McDonald CM, Ellis WG, Agius MA, Gomez CM, Richman DP, Wollman RL. Presynaptic congenital myasthenic syndrome due to quantal release deficiency. Neurology 2001;57:279–289.
85. Maselli RA, Soliven BC. Analysis of the organophosphate-induced electromyographic response to repetitive nerve stimulation: paradoxical response to edrophonium and d-tubocurarine. Muscle Nerve 1991;14:1182–1188.
86. McQuillen MP. Familial limb–girdle myasthenia. Brain 1966;89:121–132.
87. Middleton L, Ohno K, Christodoulou K, Brengman JM, Milone M, Neocleous V, Serdaroglu P, Deymeer F, Özdemir C, Mubaidin A, Horany K, Al-Shebab A, Mavromatis I, Mylonas I, Tsingis M, Zamba E, Pantzaris M, Kyriallis K, Engel AG. Congenital myasthenic syndromes linked to chromosome 17p are caused by defects in acetylcholine receptor ϵ subunit gene. Neurology 1999;53:1076–1082.
88. Milone M, Ohno K, Pruitt JN, Brengman JM, Sine SM, Engel AG. Congenital myasthenic syndrome due to frameshifting acetylcholine receptor epsilon subunit mutation. Soc Neurosci Abstr 1996;22:1942–1942.
89. Milone M, Shen X-M, Ohno K, Harper CM, Fukudome K, Stilling G, Brengman JM, Engel AG. Unusual congenital myasthenic syndrome with endplate AChR deficiency caused by alpha subunit mutations and a remitting–relapsing course. Neurology 1999;52(Suppl 2):185–186.
90. Milone M, Wang H-L, Ohno K, Fukudome T, Pruitt JN, Bren N, Sine SM, Engel AG. Slow-channel syndrome caused by enhanced activation, desensitization, and agonist binding affinity due to mutation in the M2 domain of the acetylcholine receptor alpha subunit. J Neurosci 1997;17:5651–5665.
91. Milone M, Wang H-L, Ohno K, Prince RJ, Shen X-M, Brengman JM, Griggs RC, Engel AG. Mode switching kinetics produced by a naturally occurring mutation in the cytoplasmic loop of the human acetylcholine receptor ϵ subunit. Neuron 1998;20:575–588.
92. Mishina M, Tobimatsu T, Imoto K, Tanaka K, Fujita Y, Fukuda K, Kurasaki M, Takahashi H, Morimoto Y, Hirose T. Location of functional regions of acetylcholine receptor alpha-subunit by site directed mutagenesis. Nature 1985;313: 364–369.
93. Mittaud P, Marangi A, Erb-Vögtli S, Fuhrer C. Agrin induced activation of acetylcholine receptor-bound Src family kinases requires rapsyn an correlates with acetylcholine receptor clustering. J Biol Chem 2001;276:14505–14513.
94. Miyazawa A, Fujiyoshy Y, Unwin N. Nicotinic acetylcholine receptor at 4.6Å resolution: transverse tunnels in the channel wall. J Mol Biol 1999;288:765–786.
95. Monod J, Wyman J, Changeux J-P. On the nature of allosteric transitions: a plausible model. J Mol Biol 1965;12:88–118.
96. Mora M, Lambert EH, Engel AG. Synaptic vesicle abnormality in familial infantile myasthenia. Neurology 1987;37:206–214.
97. Naranjo D, Brehm P. Modal shifts in acetylcholine receptor channel gating confer subunit-dependent desensitization. Science 1993;260:1811–1814.
98. Newey SA, Gramolini AO, Wu J, lzfeind G, Smin BJ, Vies KE, Ake DJ. A novel mechanism for modulating synaptic gene expression: differential localization of α-dystrobrevin transcripts in skeletal muscle. Mol Cell Neurosci 2001;17:127–140.
99. Nichols P, Croxen R, Vincent A, Rutter R, Hutchinson M, Newsom-Davis J, Beeson D. Mutation of the acetylcholine receptor ϵ-subunit promoter in congenital myasthenic syndrome. Ann Neurol 1999;45:439–443.
100. Oda Y, Nakanishi I, Deguchi T. A complementary DNA for human choline acetyltransferase induces two forms of enzyme with different molecular weights in cultured cells. Brain Res Mol Brain Res 1992;16:287–294.
101. Ohno K, Anlar B, Engel AG. Congenital myasthenic syndrome caused by a mutation in the Ets-binding site of the promoter region of the acetylcholine receptor ϵ subunit gene. Neuromuscul Disord 1999;9:131–135.
102. Ohno K, Anlar B, Özdirim E, Brengman JM, De Bleecker J, Engel AG. Myasthenic syndromes in Turkish kinships due to mutations in the acetylcholine receptor. Ann Neurol 1998; 44:234–241.
103. Ohno K, Brengman JM, Felice KJ, Cornblath DR, Engel AG. Congenital endplate acetylcholinesterase deficiency caused by a nonsense mutation and an A-to-G splice site mutation at position 3 of the collagen-like tail subunit gene (*COLQ*): How does G at position 3 result in aberrant splicing? Am J Hum Genet 1999;65:635–644.
104. Ohno K, Brengman JM, Tsujino A, Engel AG. Human endplate acetylcholinesterase deficiency caused by mutations in the collagen-like tail subunit (ColQ) of the asymmetric enzyme. Proc Natl Acad Sci USA 1998;95:9654–9659.

105. Ohno K, Engel AG, Brengman JM, Harper CM, Shen X-M, Heidenreich FR, Vincent A, Milone M, Tan E, Demirci M, Walsh P, Nakano S, Akiguchi I. The spectrum of mutations causing endplate acetylcholinesterase deficiency. Ann Neurol 2000;47:162–170.
106. Ohno K, Engel AG, Milone M, Brengman JM, Sieb JP, Iannaccone S. A congenital myasthenic syndrome with severe acetylcholine receptor deficiency caused by heteroallelic frameshifting mutations in the epsilon subunit. Neurology 1995;45 (Suppl 4):A283.
107. Ohno K, Engel AG, Shen X-M, Selcen D, Brengman JM, Harper CM, Tsujino A, Milone M. Rapsyn mutations in humans cause endplate acetylcholine receptor deficiency and myasthenic syndrome. Am J Hum Genet 2002;70:875–885.
108. Ohno K, Fukudome T, Nakano S, Milone M, Feasby TE, Tyce GM, Engel AG. Mutational analysis in a congenital myasthenic syndrome reveals a novel acetylcholine receptor epsilon subunit mutation. Soc Neurosci Abstr 1996;22:234–234.
109. Ohno K, Hutchinson DO, Milone M, Brengman JM, Bouzat C, Sine SM, Engel AG. Congenital myasthenic syndrome caused by prolonged acetylcholine receptor channel openings due to a mutation in the M2 domain of the ε subunit. Proc Natl Acad Sci USA 1995;92:758–762.
110. Ohno K, Milone M, Brengman JM, Lo Monaco M, Evoli A, Tonali P, Engel AG. Slow-channel congenital myasthenic syndrome caused by a novel mutation in the acetylcholine receptor ε subunit. Neurology 1998;50:A432.
111. Ohno K, Quiram P, Milone M, Wang H-L, Harper CM, Pruitt JN, Brengman JM, Pao L, Fischbeck KH, Crawford TO, Sine SM, Engel AG. Congenital myasthenic syndromes due to heteroallelic nonsense/missense mutations in the acetylcholine receptor ε subunit gene: identification and functional characterization of six new mutations. Hum Mol Genet 1997; 6:753–766.
112. Ohno K, Shen X-M, Fukudome T, Tsujino A, Brengman JM, De Vivo DC, Engel AG. First report of acetylcholine receptor δ subunit mutation causing endplate AChR deficiency. Neurology 2001;56(Suppl 3):232–233.
113. Ohno K, Tsujino A, Anlar B, Engel AG. A 16-bp duplication of splice-acceptor site results in silencing of the downstream copy of the splice acceptor site. How are duplicated splice-acceptor sites selected in pre-mRNA splicing? Am J Hum Genet 2001;69(Suppl.):368.
114. Ohno K, Tsujino A, Brengman JM, Harper CM, Bajzer Z, Udd B, Beyring R, Robb S, Kirkham FJ, Engel AG. Choline acetyltransferase mutations cause myasthenic syndrome associated with episodic apnea in humans. Proc Natl Acad Sci USA 2001;98:2017–2022.
115. Ohno K, Wang H-L, Milone M, Bren N, Brengman JM, Nakano S, Quiram P, Pruitt JN, Sine SM, Engel AG. Congenital myasthenic syndrome caused by decreased agonist binding affinity due to a mutation in the acetylcholine receptor ε subunit. Neuron 1996;17:157–170.
116. Ohno K, Wang H-L, Shen X-M, Milone M, Bernasconi L, Sine SM, Engel AG. Slow-channel mutations in the center of the M1 transmembrane domain of the acetylcholine receptor α subunit. Neurology 2000;54(Suppl 3):A183.
117. Okada Y, Yamazaki H, Sekine-Aizawa Y, Hirokawa N. The neuron-specific kinesin superfamily protein KIF1A is a unique monomeric motor for anterograde axonal transport of synaptic vesicle precursors. Cell 1995;81:769–780.
118. Okuda T, Haga T, Kanai Y, Endou H, Ishihara T, Katsura I. Identification and characterization of the high-affinity choline transporter. Nat Neurosci 2000;3:120–125.
119. Phillips WD, Kopta C, Blount P, Gardner PD, Steinbach JH, Merlie JP. ACh-rich receptor membrane domains organized in fibroblasts by recombinant 43-kilodalton protein. Science 1991;251:568–570.
120. Prince RJ, Sine SM. The ligand binding domains of the nicotinic acetylcholine receptor. In: Barrantes FJ, editor. The nicotinic acetylcholine receptor: current views and future trends. Austin: Landes Bioscience; 1998. p 31–59.
121. Prockop DJ, Kivirikko KI. Collagens: molecular biology, diseases, and potentials for therapy. Annu Rev Biochem 1995; 64:403–434.
122. Quiram P, Ohno K, Milone M, Patterson MC, Pruitt NJ, Brengman JM, Sine SM, Engel AG. Mutation causing congenital myasthenia reveals acetylcholine receptor β/δ subunit interaction essential for assembly. J Clin Invest 1999; 104:1403–1410.
123. Ramarao MK, Bianchetta MJ, Lanken J, Cohen JB. Role of rapsyn tetratrichopeptide repeat and coiled-coil domains in self-association and nicotinic acetylcholine receptor clustering. J Biol Chem 2001;276:7475–7483.
124. Ramarao MK, Cohen JB. Mechanism of nicotinic acetylcholine receptor cluster formation by rapsyn. Proc Natl Acad Sci USA 1998;95:4007–4012.
125. Reimer RJ, Fon AE, Edwards RH. Vesicular neurotransmitter transport and the presynaptic regulation of quantal size. Curr Opin Neurobiol 1998;8:405–412.
126. Robertson WC, Chun RWM, Kornguth SE. Familial infantile myasthenia. Arch Neurol 1980;37:117–119.
127. Sadeh M. Benign congenital myasthenic syndrome with facial malformations. Acta Myol 2000;19:33–36.
128. Salpeter MM, Kasprzak H, Feng H, Fertuck H. End-plates after esterase inactivation in vivo: correlation between esterase concentration, functional response and fine structure. J Neurocytol 1979;8:95–115.
129. Sandrock AW, Dryer SE, Rosen KM, Gozani SM, Kramer R, Theill LE, Fischbach GD. Maintenance of acetylcholine receptor number by neuregulins at the neuromuscular junction in vivo. Science 1997;276:599–603.
130. Shapira YA, Sadeh ME, Bergtraum MP, Tsujino A, Ohno K, Shen X-M, Brengman JM, Edwardson S, Matoh I, Engel AG. The novel *COLQ* mutations and variation of phenotypic expressivity due to G240X. Neurology 2002;58:603–609.
131. Shen X-M, Ohno K, Fukudome T, Brengman JM, Engel AG. Deletion of a single codon from the long cytoplasmic loop of the nAChR subunit gene causes brief single channel currents. Soc Neurosci Abstr 1999;25:1721.
132. Shen X-M, Ohno K, Milone M, Brengman JM, Spilsbury PR, Engel AG. Fast-channel syndrome. Neurology 2001; 56(Suppl. 3):A60.
133. Shen X-M, Tsujino A, Ohno K, Brengman JM, Gingold M, Engel AG. A novel fast-channel congenital myasthenic syndrome caused by a mutation in the Cys-loop domain of the acetylcholine receptor ε subunit. Neurology 2000;54(Suppl 3):A138.
134. Si J, Luo Z, Mei L. Induction of acetylcholine receptor gene expression by ARIA requires activation of mitogen-activated protein kinase. J Biol Chem 1996;271:19752–19759.
135. Sieb JP, Kraner S, Rauch M, Steinlein OK. Immature endplates and utrophin deficiency in congenital myasthenic syndrome caused by epsilon-AChR subunit truncating mutations. Hum Genet 2000;107:160–464.
136. Sieb JP, Kraner S, Schrank B, Reitter B, Goebel TH, Tzartos SJ, Steinlein OK. Severe congenital myasthenic syndrome due to homozygosity of the 1293insG epsilon-acetylcholine receptor subunit mutation. Ann Neurol 2000;48:379–383.
137. Sieb JP, Milone M, Engel AG. Effects of the quinoline derivatives quinine, quinidine, and chloroquine on neuromuscular transmission. Brain Res 1996;712:179–189.
138. Sine SM, Claudio T, Sigworth FJ. Activation of *Torpedo* acetylcholine receptors expressed in mouse fibroblasts: single-channel current kinetics reveal distinct agonist binding affinities. J Gen Physiol 1990;96:395–437.
139. Sine SM, Ohno K, Bouzat C, Auerbach A, Milone M, Pruitt JN, Engel AG. Mutation of the acetylcholine receptor α subunit causes a slow-channel myasthenic syndrome by enhancing agonist binding affinity. Neuron 1995;15:229–239.

140. Südhof TC, Jahn R. Proteins of synaptic vesicles involved in exocytosis and membrane recycling. Neuron 1991;6:665–677.
141. Tseng CN, Yao Y, Wang JM, Viroonchatapan N, Rothe E, Wang ZZ. A synaptic isoform of NRAP interacts with the postsynaptic 43K protein rapsyn and links it to the cytoskeleton at the neuromuscular junction. Soc Neurosci Abstr 2001;27:Program No. 694.6.
142. Unwin N. Acetylcholine receptor channel imaged in the open state. Nature 1995;373:37–43.
143. Walls TJ, Engel AG, Nagel AS, Harper CM, Trastek VF. Congenital myasthenic syndrome associated with paucity of synaptic vesicles and reduced quantal release. Ann NY Acad Sci 1993;681:461–468.
144. Wang D, Chirar DC, Xie Y, Cohen JB. Probing the structure of the nicotinic acetylcholine receptor with 4-benzoylbenzoyl choline, a novel photoaffinity competitive antagonist. J Biol Chem 2000;275:28666–28674.
145. Wang H-L, Auerbach A, Bren N, Ohno K, Engel AG, Sine SM. Mutation in the M1 domain of the acetylcholine receptor alpha subunit decreases the rate of agonist dissociation. J Gen Physiol 1997;109:757–766.
146. Wang H-L, Milone M, Ohno K, Shen X-M, Tsujino A, Batocchi AP, Tonali P, Brengman JM, Engel AG, Sine SM. Acetylcholine receptor M3 domain: stereochemical and volume contributions to channel gating. Nat Neurosci 1999;2:226–233.
147. Wang H-L, Ohno K, Milone M, Brengman JM, Evoli A, Batocchi AP, Middleton L, Christodoulou K, Engel AG, Sine SM. Fundamental gating mechanism of nicotinic receptor channel revealed by mutation causing a congenital myasthenic syndrome. J Gen Physiol 2000;116:449–460.
148. Zhang Y, Chen J, Auerbach A. Activation of recombinant mouse acetylcholine receptors by acetylcholine, carbamylcholine and tetraethylammonium. J Physiol (Lond) 1995; 486:189–206.