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Chapter Number

Congenital Myasthenic Syndromes – Molecular Bases of Congenital Defects of Proteins at the Neuromuscular Junction

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1. Introduction

Congenital myasthenic syndromes (CMS) are heterogeneous disorders caused by mutations in molecules expressed at the neuromuscular junction (NMJ) (Fig. 1). Each mutation affects the expression level or the functional properties or both of the mutant molecule. No fewer than 11 defective molecules at the NMJ have been identified to date. The mutant molecules include (i) acetylcholine receptor (AChR) subunits that forms nicotinic AChR and generate endplate potentials (Ohno *et al.*, 1995; Sine *et al.*, 1995), (ii) rapsyn that anchors and clusters AChRs at the endplate (Ohno *et al.*, 2002; Milone *et al.*, 2009), (iii) agrin that is released from nerve terminal and induces AChR clustering by stimulating the downstream LRP4/MuSK/Dok-7/rapsyn/AChR pathway (Huze *et al.*, 2009), (iv) muscle-specific receptor tyrosine kinase (MuSK) that transmits the AChR-clustering signal from agrin/LRP4 to Dok-7/rapsyn/AChR (Chevessier *et al.*, 2004; Chevessier *et al.*, 2008), (v) Dok-7 that interacts with MuSK and exerts the AChR-clustering activity (Beeson *et al.*, 2006; Hamuro *et al.*, 2008), (vi) plectin that is an intermediate filament-associate protein concentrated at sites of mechanical stress (Banwell *et al.*, 1999; Selcen *et al.*, 2011), (vii) glutamine-fructose-6-phosphate aminotransferase 1 encoded by *GFPT1*, the function of which at the NMJ has not been elucidated (Senderek *et al.*, 2011), (viii) skeletal muscle sodium channel type 1.4 ($Na_V1.4$) that spreads depolarization potential from endplate throughout muscle fibers (Tsujino *et al.*, 2003), (ix) collagen Q that anchors acetylcholinesterase (AChE) to the synaptic basal lamina (Ohno *et al.*, 1998; Ohno *et al.*, 1999; Kimbell *et al.*, 2004), (x) β2-laminin that forms a cruciform heterotrimeric lamins-221, -421, and -521 and links extracellular matrix molecules to the β-dystroglycan at the NMJ (Maselli *et al.*, 2009), (xi) choline acetyltransferase (ChAT) that resynthesizes acetylcholine from recycled choline at the nerve terminal (Ohno *et al.*, 2001). AChR (Lang & Vincent, 2009), MuSK (Hoch *et al.*, 2001; Cole *et al.*, 2008), and LRP4 (Higuchi *et al.*, 2011) are also targets of myasthenia gravis, in which autoantibody against each molecule impairs the neuromuscular transmission.

CMS are classified into three groups of postsynaptic, synaptic, and presynaptic depending on the localization of the defective molecules. Among the eleven molecules introduced

above, AChR, rapsyn, MuSK, Dok-7, plectin, and $Na_V1.4$ are associated with the postsynaptic membrane. Agrin, ColQ, and β2-laminin reside in the synaptic basal lamina. The only presynaptic disease protein identified to date is choline acetyltransferase (ChAT). A target molecule and its synaptic localization of glutamine-fructose-6-phosphate aminotransferase 1 (GFPT1) are still unresolved but the phenotypic consequence is the postsynaptic AChR deficiency. This chapter focuses on molecular bases of these three groups of CMS.

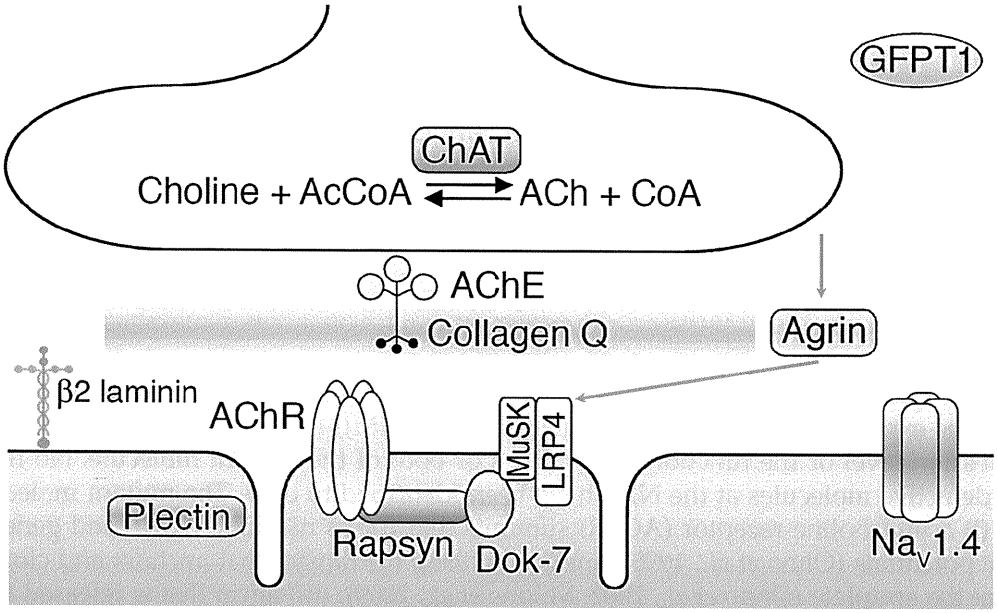

Fig. 1. Schematic of molecules expressed at the NMJ

2. Physiology of the NMJ

This section introduces molecular basis of development and maintenance of the NMJ, and physiological features of nicotinic muscle AChR.

2.1 NMJ synaptogenesis

At the NMJ, MuSK is an indirect receptor for agrin (Valenzuela *et al.*, 1995; Dechiara *et al.*, 1996). Agrin released from the nerve terminal binds to LRP4 on the postsynaptic membrane (Kim *et al.*, 2008; Zhang *et al.*, 2008). Binding of LRP4 to agrin phosphorylates MuSK. Phosphorylated MuSK recruits the noncatalytic adaptor protein Dok-7 (Okada *et al.*, 2006). Once recruited, Dok-7 further facilitates phosphorylation of MuSK, and induces clustering of rapsyn and AChR by phosphorylating the β subunit of AChR. Rapsyn self-associates and makes a homomeric cluster at the endplate, which serves as a scaffold for AChR. Rapsyn and AChR bind each other with a stoichiometry of 1:1. Rapsyn also binds to β-dystroglycan and links the rapsyn scaffold to the subsynaptic cytoskeleton (Froehner *et al.*, 1990; Cartaud *et al.*, 1998; Ramarao & Cohen, 1998; Ramarao *et al.*, 2001). Except for LRP4, each of the above molecules is a CMS target.

2.2 Physiology of the nicotinic muscle AChR

Nicotinic AChRs are pentameric ligand-gated ion channels. The family of pentameric ligand-gated ion channels includes cationic AChRs, cationic serotonergic receptors ($5HT_3$), anionic glycine receptors, and anionic $GABA_A$ and $GABA_C$ receptors (Keramidas *et al.*, 2004). Heteromeric neuronal nicotinic AChRs are comprised of various combinations of α (α2-α7) and β subunits (β2-β4), whereas homomeric AChRs are formed only by a single α subunit (e.g., α7-α9) (Mihailescu & Drucker-Colin, 2000). On the other hand, nicotinic muscle AChRs have only two forms: fetal AChR that carries the α, β, δ, and γ subunits encoded by *CHRNA1*, *CHRNB1*, *CHRND*, *CHRNG*, respectively, in the stoichiometry $\alpha_2\beta\delta\gamma$; and adult-type AChR that carries the ε subunit instead of the γ subunit in the stoichiometry $\alpha_2\beta\delta\varepsilon$ (Mishina *et al.*, 1986). The ε subunit is encoded by *CHRNE*. Nicotinic muscle AChR harbors two binding sites for ACh at the interfaces between the α–δ and α–γ/α–ε subunits (Lee *et al.*, 2009; Mukhtasimova *et al.*, 2009). Binding of a single ACh molecule opens the channel pore but for a short time. Binding of two ACh molecules stabilizes the open state of AChR, and AChR stays open for a longer time. Only cations pass through the channel pore of nicotinic AChRs. Unlike sodium, potassium, or calcium channels, AChRs, in general, have no selectivity for cations, but α7 AChRs have 10-20 times higher permeability for Ca^{2+} than for Na^+.

3. Postsynaptic CMS

Postsynaptic CMS is classified into four phenotypes: (i) endplate AChR deficiency due to defects in AChR, rapsyn, agrin, MuSK, Dok-7, plectin, glutamine-fructose-6-phosphate aminotransferase 1, (ii) slow-channel congenital myasthenic syndrome, (iii) fast-channel congenital myasthenic syndrome, and (iv) sodium channel myasthenia.

3.1 Endplate AChR deficiency

Endplate AChR deficiency is caused by defects in AChR, rapsyn, agrin, MuSK, Dok-7, plectin, and GFPT1.

3.1.1 Endplate AChR deficiency due to defects in AChR subunits

Endplate AChRs deficiency can arise from mutations in *CHRNA1*, *CHRNB1*, *CHRND*, and *CHRNE*, but not *CHRNG*.

Two different groups of mutations of the AChR subunit genes cause endplate AChR deficiency. The first group includes null mutations in *CHRNE* encoding the ε subunit. The null mutations are caused by frameshifting DNA rearrangements, *de novo* creation of a stop codon, and frameshifting splice-site mutations, or mutations involving residues essential for subunit assembly. Large-scale in-frame DNA rearrangements also abolish expression of the AChR ε subunit (Abicht *et al.*, 2002). Mutations in the promoter region (Ohno *et al.*, 1999) and most missense mutations (Ohno *et al.*, 1997) do not completely abolish expression of the ε subunit but the molecular consequences are indistinguishable from those of null mutations. Lack of the ε subunit can be compensated for by the presence of the fetal γ subunit that is normally expressed in embryos (Engel *et al.*, 1996). The patients can survive with γ-AChR even in the absence of ε-AChR. If a null mutation resides in the other AChR

subunit genes, the affected individual will have no substituting subunit and cannot survive. Indeed, two homozygous missense low expressor or null mutations in *CHRNA1* and *CHRND* caused lethal fetal akinesia (Michalk *et al.*, 2008).

The second group of mutations affecting the AChR subunit genes includes missense mutations of *CHRNA1*, *CHRNB1*, and *CHRND*. These mutations compromise expression of the mutant subunit and/or the assembly of AChRs, but do not completely abolish AChRs expression. The main difference between mutations in *CHRNE* and those in *CHRNA1*, *CHRNB1*, and *CHRND* is tolerance to low or no expression of the ε subunit whereas similar mutations in other subunits generally have devastating consequences and cause high fatality. Some missense mutations in *CHRNA1*, *CHRNB1*, *CHRND*, and *CHRNE* also affect the AChR channel kinetics and vice versa. The kinetic effects will predominate if the second mutation is a low expressor, or if the kinetic mutation has slow-channel features with dominant gain-of function effects.

In endplate AChR deficiency, the postsynaptic membrane displays a reduced binding for peroxidase- or ^{125}I-labeled α-bungarotoxin and the synaptic response to ACh, reflected by the amplitude of the miniature endplate potential, endplate potential, and endplate current, is reduced. In some but not all cases the postsynaptic region is simplified. In most cases, the muscle fibers display an increased number of small synaptic contacts over an extended length of the muscle fiber. In some patients quantal release is higher than normal. In patients with null mutations in *CHRNE*, single channel recordings of AChRs at patient endplates reveal prolonged opening bursts that open to an amplitude of 60 pS, indicating expression of the fetal γ-AChR in contrast to the adult ε-AChR that has shorter opening bursts and opens to an amplitude of 80 pS. In contrast, in most patients with low-expressor mutations in the *CHRNA1*, *CHRNB1*, or *CHRND*, single channel recordings demonstrate no or minor kinetic abnormalities.

As in autoimmune myasthenia gravis, endplate AChR deficiency is generally well controlled by regular doses of anticholinesterases. Anticholinesterase medications inhibit the catalytic activity of AChE; this prolongs the dwell time of ACh in the synaptic space and allows each ACh molecule to bind repeatedly to AChR.

3.1.2 Endplate AChR deficiency due to defects in rapsyn

Congenital defects of rapsyn also cause endplate AChR deficiency. Rapsyn makes a homomeric cluster and binds to AChR as well as to β-dystroglycan, and forms AChR clusters at the endplate (Froehner *et al.*, 1990; Cartaud *et al.*, 1998; Ramarao & Cohen, 1998; Ramarao *et al.*, 2001). The structural domains of rapsyn include an N-terminal myristoylation signal required for membrane association (Ramarao & Cohen, 1998), seven tetratrico peptide repeats at codons 6 to 279 that subserve rapsyn self-association (Ramarao & Cohen, 1998; Ramarao *et al.*, 2001), a coiled-coil domain at codons 298 to 331 that binds to the long cytoplasmic loop of each AChR subunit (Bartoli *et al.*, 2001), a Cys-rich RING-H2 domain at codons 363-402 that binds to the cytoplasmic domain of β-dystroglycan (Bartoli *et al.*, 2001) and mediates the MuSK induced phosphorylation of AChR (Lee *et al.*, 2008), and a serine phosphorylation site at codon 406. Transcription of rapsyn in muscle is under the control of helix-loop-helix myogenic determination factors that bind to the *cis*-acting E-box sequence in the *RAPSN* promoter (Ohno *et al.*, 2003).

Loss-of-function mutations in *RAPSN* have been reported in the coding region (Ohno *et al.*, 2002; Burke *et al.*, 2003; Dunne & Maselli, 2003; Maselli *et al.*, 2003; Muller *et al.*, 2003; Banwell *et al.*, 2004; Yasaki *et al.*, 2004; Cossins *et al.*, 2006; Muller *et al.*, 2006) as we as in the promoter region (Ohno *et al.*, 2003). N88K in *RAPSN* is one of the most frequently observed mutations in CMS (Muller *et al.*, 2003; Richard et al., 2003). We reported lack of a founder haplotype for N88K (Ohno & Engel, 2004), but analysis of markers closer to *RAPSN* later revealed possible presence of a shared haplotype (Muller et al., 2004) suggesting that N88K is an ancient founder mutation but subsequent multiple recombination events and divergence of microsatellite markers have narrowed the shared haplotype region. Functional analysis L14P, N88K, and 553ins5 disclosed that these mutations have no effect on self-association of rapsyn but impair colocalization of rapsyn with AChR (Ohno *et al.*, 2002). Analysis of A25V, N88K, R91L, L361R, and K373del later revealed diverse molecular defects affecting colocalization of rapsyn with AChR, formation of agrin-induced AChR clusters, self-association of rapsyn, and expression of rapsyn (Cossins *et al.*, 2006). Although there are no genotype-phenotype correlations in mutations at the coding region, arthrogryposis at birth and other congenital malformations occurs in nearly a third of the patients. In addition, the -38A>G mutation affecting an E-box in the promoter region observed in Near-Eastern Jewish patients exhibits unique facial malformations associated with prognathism and malocclusion (Ohno *et al.*, 2003).

Most patients respond well to anticholinesterase medications. Some patients further improve with addition of 3,4-diaminopyridine, ephedrine, and albuterol (Banwell *et al.*, 2004). The drug 3,4-diaminopyridine blocks the presynaptic potassium channel, which slows the repolarization of the presynaptic membrane (Wirtz *et al.*, 2010) enhancing the influx of Ca^{2+} through the presynaptic voltage-gated P/Q-type and N-type channels. This, in turn, facilitates the exocytosis of synaptic vesicles and the quantal content of the endplate potential.

3.1.3 Endplate AChR deficiency due to a defect in agrin

Neural agrin released from the nerve terminal is a key mediator of synaptogenesis at the NMJ. A reported homozygous G1709R agrin mutation, however, did not cause AChR deficiency but mutations in agrin are potential causes of AChR deficiency by interfering with the activation of MuSK and by impeding synaptic maturation.

The patient harboring the G1709R mutation was a 42-year-old woman with right lid ptosis since birth, no oculoparesis, and mild weakness of facial, hip-girdle and anterior tibial muscles, and refractoriness to pyridostigmine or 3,4-diaminopyridine (Huze *et al.*, 2009). The mutation is in the laminin G-like 2 domain, upstream of the neuron-specific y and z exons that are required for MuSK activation and AChR clustering. AChR and agrin expression at the endplate were normal. Structural studies showed endplates with misshaped synaptic gutters partially filled by nerve endings and formation of new endplate regions. The postsynaptic regions were preserved. Expression studies in myotubes using a mini-agrin construct revealed the mutation did not affect MuSK activation or agrin binding to α-dystroglycan. Forced expression of the mutant mini-agrin gene in mouse soleus muscle induced changes similar to those at patient endplates. Thus, the observed mutation perturbs the maintenance of the endplate without altering the canonical function of agrin to induce development of the postsynaptic compartment.

3.1.4 Endplate AChR deficiency due to defects in MuSK

MuSK and LRP4 form a heteromeric receptor for agrin. Five *MUSK* mutations have been reported in three papers. The first report describes heteroallelic frameshift (220insC) and missense (V790M) mutations in a patient with respiratory distress in early life, mild ptosis, decreased upward gaze, and fatigable weakness of the cervical and proximal more than distal muscles. The symptoms were worsened by pregnancy. Treatment with pyridostigmine and 3,4-diaminopyridine was ineffective (Chevessier *et al.*, 2004). The frameshift mutation prevents MuSK expression and the missense mutation decreases MuSK expression and impairs its interaction with Dok-7. Forced expression of the mutant protein in mouse muscle decreased AChR expression at the endplate and caused aberrant axonal outgrowth (Chevessier *et al.*, 2004). Interestingly, mice homozygous for MuSK V789M (which corresponds to the human MuSK V790M) are normal but mice hemizygous for V789M are severely affected suggesting that MuSK V790M in humans is a haploinsufficient only when accompanied by a null mutation (Chevessier *et al.*, 2008).

A second report describes heteroallelic M605I and A727V mutations in MuSK in a patient with severe myasthenic symptoms since early life that improved after puberty but worsened after menstrual periods. The MEPP and MEPC amplitudes in anconeus muscle were reduced to about 30% of normal and the EPP quantal content was half-normal. Synaptic contacts were small and electron microscopy showed simplified postsynaptic regions with too few secondary synaptic clefts. The patient failed to respond to pyridostigmine, ephedrine or 3,4-diaminopyuridine but responded partially to albuterol (Maselli *et al.*, 2010).

A third report describes a homozygous P31L mutation in the extracellular domain of MuSK in 5 patients in a consanguineous Sudanese kinship. The findings included ptosis from an early age, partial ophthalmoparesis, and weakness of torso and limb girdle muscles. Pyridostigmine therapy gave only slight benefit (Mihaylova *et al.*, 2009).

3.1.5 Endplate AChR deficiency due to defects in Dok-7

Phosphorylated MuSK recruits a noncatalytic adaptor protein, Dok-7. Recruited Dok-7 further facilitates phosphorylation of MuSK (Okada *et al.*, 2006). Dok-7 is highly expressed at the postsynaptic region of skeletal muscle and in heart. It harbors an N terminal pleckstrin homology domain (PH) important for membrane association, a phosphotyrosine-binding (PTB) domain, and C-terminal sites for phosphorylation. The PH and PTB domains are required for association with and phosphorylation of MuSK. Phosphorylation of two C terminal residues is a requisite for Dok-7 activation by Crk and Crk-L (Hallock *et al.*, 2010).

Numerous mutations have been identified in *DOK7* (Beeson *et al.*, 2006; Muller *et al.*, 2007; Anderson *et al.*, 2008; Selcen *et al.*, 2008; Vogt *et al.*, 2009; Ben Ammar *et al.*, 2010). Nearly all patients carry a common 1124_1127dupTGCC mutation in exon 7. This and other mutations upstream of the C-terminal phosphorylation sites abrogate the ability of Dok-7 to associate with Crk1/Crk1L and hence its activation (Hallock *et al.*, 2010; Wu *et al.*, 2010). Mutations disrupting or eliminating the PH and PTB domains of Dok-7 prevent dimerization and association of Dok-7 with MuSK (Bergamin *et al.*, 2010).

3.1.6 Endplate AChR deficiency due to defects in plectin

Plectin, encoded by *PLEC*, is a highly conserved and ubiquitously expressed intermediate filament-linking protein concentrated at sites of mechanical stress, such as the postsynaptic membrane of the endplate, the sarcolemma, Z-disks in skeletal muscle, hemidesmosomes in skin, and intercalated disks in cardiac muscle. Pathogenic mutations in *PLEC* result in epidermolysis bullosa simplex, a progressive myopathy (Smith *et al.*, 1996), and, in some patients, myasthenic syndrome (Banwell *et al.*, 1999; Selcen *et al.*, 2011). We reported two cases of CMS associated with plectin deficiency (Banwell *et al.*, 1999; Selcen *et al.*, 2011). The dystrophic changes in muscle are attributed to dislocation of the fiber organelles no longer anchored by the cytoskeletal intermediate filaments and to sarcolemmal defects allowing Ca^{2+} ingress into the muscle fibers. The myasthenic syndrome is attributed to destruction of the junctional folds lacking adequate cytoskeletal support.

3.1.7 Endplate AChR deficiency due to defects in glutamine-fructose-6-phosphate aminotransferase 1 (GFPT1)

Glutamine-fructose-6-phosphate transaminase 1, encoded by *GFPT1*, catalyzes transfer of an amino group from glutamine onto fructose-6-phosphate, yielding glucosamine-6-phosphate and glutamate. GFPT1 is a rate-limiting enzyme that controls the flux of glucose into the hexosamine biosynthesis pathway. GFPT1 thus initiates formation of UDP-N-acetylglucosamine (UDP-GlcNAc), which is a source of multiple glycosylation processes including addition of N-acetylglucosamine to serine or threonine residues (O-linked GlcNAc) (Wells *et al.*, 2001). The disease gene was discovered by linkage analysis and homozygosity mapping of 13 kinships with a limb-girdle CMS often associated with tubular aggregates in skeletal muscle (Senderek *et al.*, 2011). Immunoblots of muscle of affected patients revealed decreased expression of O-linked GlcNAc, but the responsible molecule(s) causing CMS remain elusive.

3.2 Slow-channel congenital myasthenic syndrome (SCCMS)

The second class of postsynaptic CMS due to mutations in the AChR subunit genes is SCCMS. SCCMS is an autosomal dominant disorder, in which a gain-of-function mutation on a single allele compromises the neuromuscular signal transduction (Ohno *et al.*, 1995). The mutation causes prolonged AChR channel openings and increases the synaptic response to ACh (Fig. 2). There is a single reported case of autosomal recessive SCCMS, in which an εL78P mutation minimally prolongs channel opening events but the mutant channel arising from a single allele is not sufficient to cause disease (Croxen *et al.*, 2002). In general, dominantly inherited disorders, including SCCMS, tend to present after adolescence and have a relatively mild course. Some patients with SCCMS, however, present early in life and become severely disabled even in the first decade.

In SCCMS, neuromuscular transmission is compromised by three distinct mechanisms. First, staircase summation of endplate potentials causes depolarization block of the postsynaptic membrane by rendering the voltage-gated skeletal muscle sodium channel go into an inactivated state and thereby inhibit action potential generation (Maselli & Soliven, 1991). Second, some mutant AChRs are prone to become desensitized (Milone *et al.*, 1997), which reduces the number of AChRs that respond to the released ACh quanta. Third,

prolonged opening of AChR causes excessive influx of extracellular calcium, which results in focal degeneration of the junctional folds as well as apoptosis of some of the junctional nuclei (Groshong *et al.*, 2007). In normal adult human ε-AChR, 7% of the synaptic current is carried by Ca^{2+}, which is higher than that carried by the human fetal γ-AChR or by muscle AChRs of other species (Fucile *et al.*, 2006). This predisposes endplate to Ca^{2+} overloading when the channel opening events are prolonged. In addition, at least two SCCMS mutations, εT264P (Ohno *et al.*, 1995) and αV259F (Fidzianska *et al.*, 2005), increase the Ca^{2+} permeability 1.5- and 2-fold, respectively (Di Castro *et al.*, 2007).

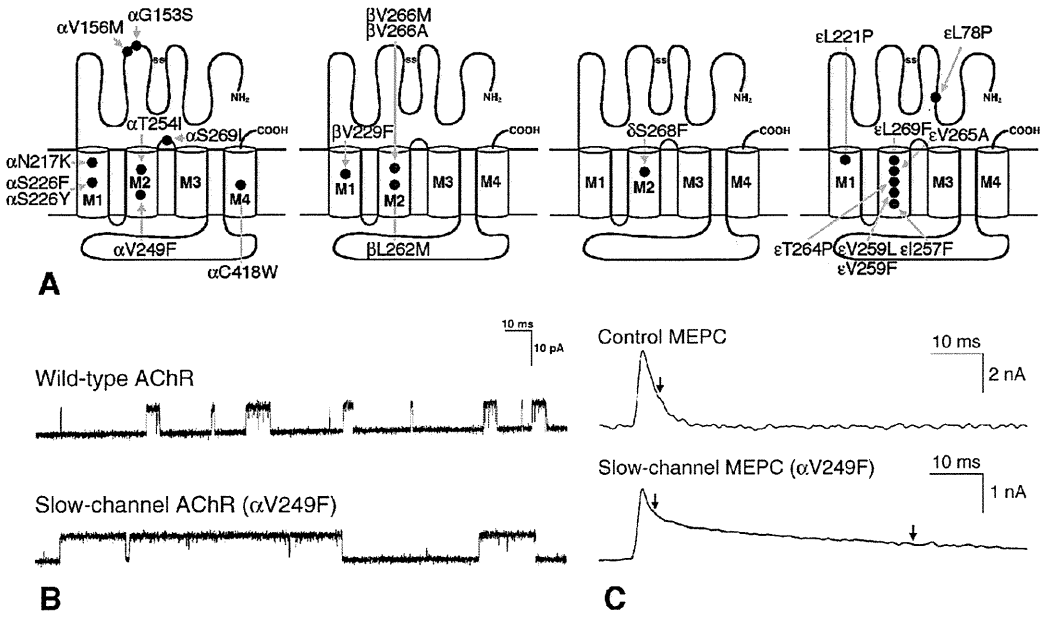

Fig. 2. Slow channel CMS. (A) Schematic diagram of AChR subunits with SCCMS mutations. (B) Single channel currents from wild-type and slow channel (αV249F) AChRs expressed on HEK293 cells. (C) Miniature endplate current (MEPC) recorded from endplates of a control and a patient harboring αV249F. The patient's MEPC decays biexponentially (arrows) due to expression of both wild-type and mutant AChRs.

Slow channel mutations can be divided into two groups. The first group includes mutations at the extracellular domain like αG153S (Sine *et al.*, 1995), as well as at the N-terminal part of the first transmembrane domain like αN217K (Wang *et al.*, 1997) and εL221F (Hatton *et al.*, 2003). These mutations increase the affinity for ACh binding, probably by retarding the dissociation of ACh from the binding site, which gives rise to repeated channel openings after a single event of ACh binding. The second group includes mutations at the second transmembrane domain (M2) that lines the ion channel pore. These mutations mostly introduce a bulky amino acid into the channel lining face, but εT264P (Ohno *et al.*, 1995) introduces a kink into the channel pore, whereas βV266A (Shen *et al.*, 2003) and εV265A (Ohno *et al.*, 1998) introduce a smaller amino acid into the pore. Mutations in M2 retard the channel closing rate α and variably enhance the channel opening rate β. Some mutations in M2 also increase affinity for ACh, which include αV249F (Milone *et al.*, 1997), εL269F (Engel *et al.*, 1996), and εT264P (Ohno *et al.*, 1995).

SCCMS can be treated with conventional doses of long-lived open channel blockers of AChR, such as the antiarrhythmic agent quinidine (Fukudome *et al.*, 1998; Harper & Engel, 1998) and the antidepressant fluoxetine (Harper *et al.*, 2003). Quinidine reduces the prolonged burst duration of SCCMS to the normal level at 5 μM (Fukudome *et al.*, 1998). As the concentration of quinidine in the treatment of cardiac arrhythmia is 6-15 μM, 5 μM is readily attainable in clinical practice and indeed demonstrates significant effects (Harper & Engel, 1998). Similarly, fluoxetine reduces the prolonged burst duration to the normal level at 10 μM, which is clinically attainable without adverse effects at 80 to 120 mg/day of fluoxetine (Harper *et al.*, 2003).

3.3 Fast-channel congenital myasthenic syndrome (FCCMS)

The third class of postsynaptic CMS due to mutations in AChR subunit genes is FCCMS. FCCMS is kinetically opposite to SCCMS (Fig. 3). In FCCMS, the closed state of AChR is stabilized compared to the open state which results in abnormally brief channel opening events which, in turn, reduces the amplitude of the endplate potential and impair the safety margin of neuromuscular transmission. The resulting pathophysiology is thus similar to endplate AChR deficiency, but abnormally small endplate potential is a qualitative instead of a quantitative defect in AChR.

FCCMS is an autosomal recessive disorder. One allele carries a missense mutation that confers a fast closure of AChRs, and the other allele usually harbors a low-expressor or null mutation, or the fast channel mutation occurs at homozygosity. As in heterozygous healthy parents of endplate AChR deficiency, we humans may completely lack 50% of each AChR subunit without any clinical symptoms. In FCCMS, a low-expressor or null mutation on one allele unmasks the deleterious effect of the fast-channel mutation on the second allele. Detailed kinetic analyses of FCCMS mutations have revealed special insights into the molecular architectures of the AChR subunits. Three such examples are presented here.

The ε1254ins18 mutation causes a duplication of STRDQE codons at positions 413 to 418 close to the C-terminal end of the long cytoplasmic loop (LCP) linking the third (M3) and fourth (M4) transmembrane domains of the receptor. ε1254ins18-AChR expressed on HEK293 cells opens in three different modes. The opening probabilities of normal AChRs are clustered into a single large peak, whereas the ε1254ins18-AChR shows three different peaks (Milone *et al.*, 1998). In all the three modes, the AChR is activated slowly and inactivated rapidly, which gives rise to an inefficient synaptic response to ACh. Another FCCMS mutation, εA411P in the LCP also destabilizes the channel opening kinetics. The channel opening probabilities of εA411P-AChRs are widely distributed and do not form any discernible peaks (Wang *et al.*, 2000). Our analysis first disclosed that the function of LCP is to stabilize the open conformation of the AChR.

εN436del is a deletion of Asn at the C-terminal end of the LCP. The deletion shortens the LCP and shifts a negatively charged Asp residue at codon 435 against M4. εN436del-AChR decreases the duration of channel opening bursts 2.7-fold compared to the wild type due to a 2.3-fold decrease in gating efficiency and a 2.5-fold decrease in agonist affinity of the diliganded closed state. A series of artificial mutations established that the effects of εN436del are not due to juxtaposition of a negative charge against M4 but to the shortening of the LCP. Deletion of the C-terminal residue of the LCP of the β and δ subunits also results in fast-