Congenital myasthenic syndromes (CMS) can arise from presynaptic, synaptic, or postsynaptic defects. Mutations of the acetylcholine receptor (AChR) that increase or decrease the synaptic response to acetylcholine (ACh) are a common cause of the postsynaptic CMS. An increased response occurs in the slow-channel syndromes. Here, dominant mutations in different AChR subunits and in different domains of the subunits prolong the activation episodes of AChR by either delaying channel closure or increasing the affinity of AChR for ACh. A decreased synaptic response to ACh occurs with recessive, loss-of-function mutations. Missense mutations in the low-affinity, fast-channel syndrome and in a disorder associated with mode-switching kinetics of AChR result in brief activation episodes and reduce the probability of channel opening. Mutations causing premature termination of the translational chain or missense mutations preventing the assembly or glycosylation of AChR curtail the expression of AChR. These mutations are concentrated in the ε subunit, probably because substitution of the fetal γ for the adult ε subunit can rescue humans from fatal null mutations in ε. Recent molecular genetic studies have also elucidated the pathogenesis of the CMS caused by absence of the asymmetric form of acetylcholinesterase from the synaptic basal lamina. Endplate acetylcholinesterase deficiency is now known to be caused by mutations in the collageneic tail subunit of the asymmetric enzyme that prevents the association of the collageneic tail subunit with the catalytic subunit or its insertion into the basal lamina.

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Congenital myasthenic syndromes (CMS) are heterogeneous disorders arising from presynaptic, synaptic, or postsynaptic defects. In each CMS, the specific defect compromises the safety margin of neuromuscular transmission by one or more mechanisms. The clinical phenotypes of CMS are often similar; therefore, precise diagnosis requires correlation of clinical, in vitro electrophysiological, morphological, and, whenever possible, molecular genetic studies.1

Prior to 1990, the investigations involving patients with CMS were based on clinical, morphologic, and conventional microelectrode studies. Since then, four developments have paved the way for molecular analysis of CMS. First, by 1993, the complementary DNA sequences of the α, β, δ, and ε subunits of adult and of the γ subunit of fetal human acetylcholine receptor (AChR) were known, allowing molecular genetic analysis. Second, in the early 1990s, Milone et al2 succeeded in patch-clamping endplates in human intercostal muscles, permitting analysis of the activity of single AChR channels. Third, the use of mammalian expression systems facilitated detailed analysis of how human AChR mutants alter the kinetics of the AChR channel. Coincident with this, we1 hypothesized that a kinetic abnormal-
Fifty-six Acetylcholine Receptor Subunit Gene Mutations in 69 Kinships

<table>
<thead>
<tr>
<th>Mutation</th>
<th>α</th>
<th>β</th>
<th>δ</th>
<th>ε</th>
<th>Total</th>
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<tbody>
<tr>
<td>Point mutations</td>
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<tr>
<td>Slow-channel</td>
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<td>2</td>
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<td>13</td>
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<td>Fast-channel</td>
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<td>2</td>
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<td>5</td>
<td>11</td>
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<tr>
<td>In-frame rearrangement</td>
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<td>2</td>
<td>3</td>
<td></td>
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</tr>
<tr>
<td>Premature-chain termination (null mutations)</td>
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<td>16</td>
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<td>Frame-shifting rearrangement</td>
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<td>Splice-site</td>
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<td>3</td>
<td></td>
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<tr>
<td>Nonsense</td>
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<td>3</td>
<td></td>
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<td></td>
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<tr>
<td>Total</td>
<td>13</td>
<td>3</td>
<td>2</td>
<td>38</td>
<td>56</td>
</tr>
</tbody>
</table>

Numbers in boldface indicate recessive mutations that reduce acetylcholine receptor expression.

**MUTATIONS IN AChR SUBUNITS CAUSE POSTSYNAPTIC CMS**

Since 1994, we and other investigators have identified 56 AChR subunit gene mutations in 69 CMS kinships. The Table indicates the identified mutations according to their functional consequences and subunit locations. It includes 34 published and 17 unpublished CMS mutations observed in our laboratories, three slow-channel mutations in the α subunit and a frame-shifting rearrangement in the ε subunit described by Croxen et al., and a slow-channel mutation in the β subunit detected by Gomez et al. Interestingly, 38 of the 56 mutations and all 27 null mutations occur in the ε subunit of AChR, highlighting the susceptibility of the ε subunit gene to mutation.

**Increased Response to ACh: Slow-Channel Mutations**

The clues for the diagnosis of a slow-channel CMS consist of selectively severe weakness of the forearm extensor muscles, a repetitive compound muscle action potential response to single-nerve stimuli that is accentuated by edrophonium, and a prolonged and biexponentially decaying miniature endplate current. Eleven slow-channel CMS mutations have been reported to date. The different mutations occur in different AChR subunits and in different functional domains of the subunits (Figure, A). Each is dominant, causing a pathologic gain of function.

The phenotypic consequences of the slow-channel CMS mutations stem from prolonged opening episodes of the AChR channel. These cause (1) cationic overload-
α1254ins18 mutation, which determines the phenotype, causes mode switching in the kinetics of receptor activation in which the normal high efficiency of gating is accompanied by two new modes that gate inefficiently. In the two abnormal modes the channel opens more slowly and closes more rapidly than normal. The α1245ins18 AChR at the endplate shows abnormally brief activation episodes during steady-state agonist application, and appears electrically silent during the synaptic response to ACh. The phenotypic consequences are endplate AChR deficiency, simplification of the postsynaptic region, and compensatory expression of fetal AChR.
that restores electrical activity at the endplate and rescues the phenotype.

**AChR Deficiency Caused by Recessive Mutations in AChR Subunits**

Severe endplate AChR deficiency can result from different types of recessive mutations in AChR subunit genes. The mutations are either homozygous or, more frequently, heterozygous. Morphologic 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. Conventional microelectrode studies reveal a decreased amplitude of the miniature endplate potentials and currents, and frequently high or higher than normal quantal release by nerve impulse. Single-channel recordings at the endplate 

Different types of recessive mutations causing severe endplate AChR deficiency have now been identified (Figure, B): (1) Mutations causing premature termination of the translational chain—these mutations are frame shifting,7,11,12,19,20 occur at a splice site,9,14,28 or produce a stop codon directly.7 (2) Missense mutation in a signal peptide region (eG-8R).8 (3) Missense mutations in residues essential for assembly of the pentameric receptor—mutations of this type were observed in the e subunit at an N-glycosylation site (eSI43L),8 in cysteine 128 (eC128S), a residue that is an essential part of the C128-C142 disulfide loop in the extracellular domain,13 and in arginine 147 (eR147L) in the extracellular domain, which lies between isoleucine 145 and threonine 150, residues that contribute to subunit assembly.7 (4) Missense mutations affecting both AChR expression and kinetics. For example, eR311W and eL254ins1813 in the long cytoplasmic loop between M3 and M4 decrease, whereas eP245L in the M1 domain13 increases the open duration of channel events. In the case of eR311W and eP245L, the kinetic consequences are modest and are likely overshadowed by the reduced expression of the mutant gene.

There are two possible reasons that recessive mutations causing AChR deficiency are concentrated in the e subunit. First, expression of the fetal type γ subunit, although at a low level, may compensate for absence of the e subunit,7,12,13 whereas patients harboring null mutations in subunits other than e might not survive for lack of a substituting subunit. Second, the gene encoding the e subunit, and especially the exons coding for the long cytoplasmic loop, have a high GC content, which likely predisposes to DNA rearrangements.

**ENDPLATE AChE DEFICIENCY ARISES FROM MUTATIONS IN THE ColQ SUBUNIT OF ASYMMETRIC AChE**

In skeletal muscle, AChE exists in homomeric globular forms of type T catalytic subunits (ACHET) and heteromeric asymmetric forms composed of 1, 2, or 3 tetrameric AChE attached to a collagenic tail (ColQ). Asymmetric AChE is concentrated at the endplate where its ColQ anchors it into the basal lamina. The ACHET gene has been cloned in humans15,16; ColQ complementary DNA has been cloned in *Torpedo* and rodents,20 but not in humans. In endplate AChE deficiency (endplate AD), the normal asymmetric species of AChE are absent from muscle.31 Endplate AD could stem from a defect that prevents binding of ColQ to ACHET, or the insertion of ColQ into the basal lamina. In recent studies of six patients with endplate AD, Ohno and coworkers7 found no mutations in ACHET. They therefore cloned human ColQ complementary DNA, determined the genomic structure and chromosomal localization of ColQ, and then searched for mutations in this gene. Their search revealed six recessive truncation mutations of ColQ in the six patients. Coexpression of each ColQ mutant with wild-type ACHET in fibroblasts showed that a mutation proximal to the ColQ attachment domain for ACHET prevents association of ColQ with ACHET; mutations distal to the attachment domain generate a mutant species of AChE composed of one ACHET tetramer and a truncated ColQ strand. The mutant species lack part of the collagen domain and the entire C-terminal domain of ColQ, or only the C-terminal domain of ColQ that is required for formation of the triple collagen helix, and this likely prevents their insertion into the basal lamina. Additional observations25 indicate that endplate AD can also arise from missense mutations in ColQ. Finally, rare cases of endplate AD could stem from defects in the basal lamina that prevent the binding of ColQ.

**FUTURE PROSPECTS**

Since 1994, molecular analysis of the CMS has provided clear insights into disease mechanisms and highlighted functionally significant domains of AChR and AChE. In the coming years molecular studies will undoubtedly be applied to presynaptic CMS, like those that alter the release of ACh quanta by nerve impulse or those that prevent the filling of synaptic vesicles with ACh. It is also likely that the molecular studies will provide clues for conventional and gene therapy and lead to the identification of novel CMS.

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