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Congenital Myasthenic Syndromes in 2012

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Abstract

Congenital myasthenic syndromes (CMS) represent a heterogeneous group of disorders in which the safety margin of neuromuscular transmission is compromised by one or more specific mechanisms. Clinical, electrophysio-logic, and morphologic studies have paved the way for detecting CMS-related mutations in proteins residing in the nerve terminal, the synaptic basal lamina, or in the postsynaptic region of the motor endplate. The disease proteins identified to date include the acetylcholine receptor, acetylcholinesterase, choline acetyltransferase, rapsyn, and Na_v1.4, muscle-specific kinase, agrin, β2-laminin, downstream of tyrosine kinase 7, and glutamine-fructose-6-phosphate transaminase 1. Analysis of electrophysiologic and biochemical properties of mutant proteins expressed in heterologous systems have contributed crucially to defining the molecular consequences of the observed mutations and have resulted in improved therapy of most CMS.

Keywords

Congenital myasthenic syndrome; Neuromuscular junction; EMG; Acetylcholine receptor (AChR); Rapsyn; ColQ; Choline acetyltransferase; Agrin; MuSK; β2-laminin; Dok-7; GFPT1; Escobar syndrome

Introduction

Congenital myasthenic syndromes (CMS) were described as early as 1937 [1] but received little attention until after the autoimmune origin of acquired myasthenia gravis was discovered. Since then no fewer than 12 clinically and genetically distinct CMS had been delineated, with five identified during the past decade. These advances had two important consequences: emerging genotype-phenotype correlations provided clues for targeted mutation analysis; and understanding the molecular basis of the different syndromes pointed to rational therapy for most types of CMS.

The first part of this review describes basic features of neuromuscular transmission, current classification of CMS, and approaches to their clinical and molecular diagnosis. This is

followed by a description of salient features of the known CMS with emphasis on the more recently identified syndromes.

Basic Features of Neuromuscular Transmission

CMS are inherited disorders of neuromuscular transmission in which the safety margin of neuromuscular transmission is compromised by one or more specific mechanisms. An understanding of the concept of the safety margin and of the mechanisms that can compromise it requires a brief overview of the anatomic and physiologic aspects of neuromuscular transmission.

At the motor endplate (EP), the nerve terminal is separated from the postsynaptic region of clefts and folds by primary and secondary synaptic clefts. The nerve terminal contains numerous synaptic vesicles that store quantal packets (5–10,000 molecules) of acetylcholine (ACh). Readily releasable vesicles abut the active zones of the presynaptic membrane. The acetylcholine receptor (AChR) is strategically deployed on the terminal expansions of the postsynaptic junctional folds at a density of about 10,000 molecules/ μm^2 . The EP species of acetylcholinesterase (AChE) is concentrated in the synaptic space where it is anchored to the basal lamina. Exocytotic release of single quanta occurs spontaneously in the resting state. Depolarization of the nerve terminal by nerve impulse opens voltage-gated Ca^{2+} channels positioned in the active zones of the presynaptic membrane. The entry of Ca^{2+} ions triggers the release of multiple quanta. The number of released quanta (m) is a function of the probability of release (p), which depends mostly on the prevailing Ca^{2+} concentration near the intraterminal release sites, and the store of quanta available for release (n), so that $m=np$.

ACh released into the synaptic space diffuses toward the postsynaptic membrane. About 20% of the released ACh is hydrolyzed by AChE before it binds to the AChR. Binding of ACh to AChR causes the cation-selective AChR channel to open for a period distributed exponentially from a fraction of a millisecond to several milliseconds, allowing Na^+ , and to a lesser extent Ca^{2+} , to enter the muscle fiber. After ACh dissociates from AChR, AChE hydrolyzes it rapidly and completely to choline and acetate. Choline is then taken up by the nerve terminal by Na^+ -dependent active transport. ACh is resynthesized in the nerve terminal from choline and acetyl-coenzyme A in a reaction catalyzed by choline acetyltransferase (ChAT). The newly synthesized ACh is concentrated in synaptic vesicles by a proton pump-dependent ACh transporter.

The postsynaptic depolarization and the concomitant current flow induced by a single quantum give rise to a miniature endplate potential (MEPP) and a miniature endplate current (MEPC). The amplitude of the MEPP depends on the number of ACh molecules per quantum, the EP geometry, the number of activatable AChRs, which is a function of postsynaptic AChR density, the depolarization per channel opening, and the input resistance of the muscle fiber. The duration of the MEPP depends on the mean open time of the AChR channel, the functional state of AChE, and the cable properties of the sarcolemma. The amplitude and duration of the MEPC are independent of the cable properties of the

sarcolemma, but are otherwise determined by the same factors that govern the amplitude and duration of the MEPP.

Multiquantal release by a nerve impulse generates an endplate potential (EPP). The EPP amplitude is a function of m and of the MEPP amplitude. When the EPP exceeds the threshold for activating perijunctional voltage-gated Na^+ channels, it triggers a muscle fiber action potential. The safety margin of neuromuscular transmission is the difference between the actual EPP amplitude and the EPP amplitude required to trigger a muscle fiber action potential. From the above discussion it follows that the safety margin will be compromised by defects that affect the 1) number of ACh molecules per quantum; 2) quantal release mechanisms; and 3) quantal efficiency. Quantal efficiency depends on the EP geometry, the functional state of AChE in the synaptic space, and the density and kinetic properties of AChR.

Classification of the CMS

The CMS may be classified according to inheritance, the EP-associated protein that is altered, or the site of the defect (presynaptic, synaptic basal lamina, or postsynaptic). A classification by inheritance is simple: the slow-channel syndromes are caused by autosomal-dominant gain-of-function mutations; all other CMS recognized to date are caused by autosomal-recessive loss-of-function mutations. Table 1 shows a classification of the CMS according to the site of the defect and the disease protein in 321 kinships investigated at the Mayo Clinic. The classification is still tentative because additional CMS might yet be discovered, and because in some presynaptic disorders the disease protein is not known.

Table 1 shows the defect was localized to the presynaptic region in 6%, resided in the synaptic basal lamina in 13.7%, was postsynaptic in 68%, and both pre- and postsynaptic in 12.5%. Moreover, all purely postsynaptic CMS, except those due to a defect in plectin [2] or caused by a kinetic abnormality of the postsynaptic Na channel [3], are caused by mutations in AChR.

Diagnosis of a CMS

In some CMS there are strong phenotypic clues pointing to a specific diagnosis. For example, in EP AChE deficiency, single nerve stimuli elicit a repetitive compound muscle action potential (CMAP), which is unaffected by edrophonium; the patients are refractory to anti-AChE therapy; and the pupillary light reflex is delayed in some patients. An even stronger clue to the diagnosis is failure of the EPs to react for AChE by cytochemical criteria.

Slow-channel syndrome patients also show a repetitive CMAP in response to single nerve stimuli, which is accentuated by edrophonium, and most patients have selectively severe weakness and atrophy of cervical and wrist and finger extensor muscles.

Patients suffering from EP ChAT deficiency have recurrent apneic episodes from early infancy occurring spontaneously or with fever, vomiting, or excitement, and variable

myasthenic symptoms between the acute episodes. The decremental electromyogram (EMG) response can be absent at rest, but appears after at 10-Hz stimulation for 5 min and then disappears slowly over the next 10–15 min.

About one fourth of the patients with rapsyn deficiency are born with multiple joint contractures and many experience increased weakness and respiratory insufficiency precipitated by intercurrent infections.

In Dok-7 (downstream of tyrosine kinase 7) myasthenia and in the CMS caused by glutamine-fructose-6-phosphate transaminase 1 (GFPT1) deficiency, the weakness has a predominantly limb-girdle distribution. Dok-7 myasthenia is worsened by pyridostigmine but improved by ephedrine or albuterol and there are minor nonspecific myopathic changes in muscle. GFPT1-deficient patients respond favorably to pyridostigmine and frequently harbor tubular aggregates in muscle.

The CMS caused by plectin deficiency is associated with epidermolysis bullosa simplex. The CMS associated with β 2-laminin deficiency is associated with a highly fatal nephrotic syndrome and ocular malformations.

There are no specific clues to the diagnosis of the fast-channel CMS, primary EP AChR deficiency, and most cases of rapsyn deficiency.

In vitro electrophysiologic analysis of parameters of neuromuscular transmission using intercostal or anconeus muscle biopsy specimens combined with cytochemical localization of AChE and estimation of the number of AChR per EP using radioiodinated α -bungarotoxin are powerful methods to identify kinetic defects or decreased expression of AChR, or EP AChE deficiency. However, these specialized tests are available only at a few medical centers.

Molecular Genetic Studies

Molecular genetic studies are greatly facilitated when the clinical and laboratory clues mentioned above point to a candidate gene or protein whose sequence is known. If a mutation is identified, its pathogenicity is confirmed by cosegregation of the mutations with disease in the investigated kinship, absence of the identified variant from at least 200 alleles of 100 control subjects, and by expression studies in human embryonic kidney (HEK) cells [4], monkey kidney fibroblasts (COS cells) [5], or mouse myotubes [6].

When no candidate genes are apparent, mutation analysis can be based on frequencies of the heretofore identified mutations in different EP proteins, as shown in Table 1. This approach is more costly and time intensive than the candidate gene approach. In our experience, about one third of the DNA samples analyzed in this manner reveal no mutations.

If a sufficient number of informative kinships are available, the disease gene can be detected by linkage analysis but most CMS patients do not have a large number of similarly affected relatives.

A novel approach to mutation discovery is whole-exome sequencing. The enormous amount of data generated by this approach need to be filtered against previously identified nonpathogenic variants and by selecting for variants in genes that code for proteins expressed at the neuromuscular junction. The identified mutations must still be confirmed by capillary sequencing and the pathogenicity of non-truncating mutations confirmed by appropriate expression studies [7].

Paucity of Synaptic Vesicles and Reduced Quantal Release

In this rare CMS, the safety margin of neuromuscular transmission is compromised by the paucity of synaptic vesicles, which correlates with electrophysiology studies that show a decreased store of quanta available for release [8]. The disease responds well to anti-AChE therapy. The molecular basis of the syndrome has not been identified.

Endplate ChAT Deficiency

As indicated above under the section on “Clinical Diagnosis of the Congenital Myasthenic Syndrome”, this syndrome is associated with sudden episodes of apnea against a background of variable myasthenic symptoms [9, 10, 11•]. Most patients respond to anti-AChE therapy but few remain apneic and paralyzed since birth, and some develop cerebral atrophy after episodes of hypoxemia [11•]. The observed mutations alter the expression or kinetic properties or structural stability of ChAT. Mutations with the most severe kinetic consequences are located close to the active site or the substrate binding site of the enzyme. Few mutations, remote from these sites, also exert severe kinetic effects by allosteric mechanisms [11•]. The safety margin of neuromuscular transmission is compromised by decreased ACh in the synaptic vesicles, which reduces the MEPP amplitude.

Endplate AChE Deficiency

The EP species of AChE is an asymmetric enzyme composed of homotetramers of catalytic subunits of AChE_T, encoded by *ACHE*_T, and a collagenic tail subunit that anchors the enzyme in the synaptic basal lamina [12]. The tail subunit consists of three strands of ColQ, encoded by *COLQ*. ColQ 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. Each ColQ strand binds an AChE_T tetramer to its PRAD [13]. Two groups of charged residues in the collagen domain of ColQ (heparan sulfate proteoglycan binding domains [HSPBD]) [14] and attachment of the C-terminal domain of ColQ to MuSK (muscle-specific kinase) on the postsynaptic membrane [15] assure insertion of the asymmetric enzyme into the synaptic basal lamina. The C-terminal region is also required for initiating the triple helical assembly of ColQ, which proceeds from a C- to an N-terminal direction in a zipper-like manner [16]. Pathologic mutations have now been identified in each *COLQ* domain [17].

In the absence of AChE, the synaptic currents are prolonged causing cationic overloading of the postsynaptic regions and degeneration of the junctional folds. The AChE-deficient EPs also have small nerve terminals, which restricts quantal release by nerve impulse. The safety margin of neuromuscular transmission is compromised by decreased quantal release by

nerve impulse, desensitization of AChR by ACh during physiologic activity, and loss of AChR from degenerating junctional folds.

For many years, EP AChE deficiency was considered untreatable. However, recent studies show that its symptoms can be mitigated by ephedrine [18, 19] and perhaps still better by albuterol [20•] at dosages used in clinical practice. The mechanism by which the adrenergic agonists improve neuromuscular transmission is not known.

CMS Caused by Defects in b2-Laminin

b2-Laminin, encoded by *LAMB2*, is a component of the basal lamina of different tissues and is highly expressed in kidney, eye, and the neuromuscular junction. Synaptic b2-laminin governs the appropriate alignment of the axon terminal with the postsynaptic region and, hence, pre- and postsynaptic trophic interactions. Defects in b2-laminin result in Pierson syndrome with renal and ocular malformations. A patient carrying heteroallelic missense and frameshift mutations in *LAMB2* had Pierson syndrome as well as severe ocular, respiratory, and proximal limb muscle weakness [21]. The renal defect was corrected by renal transplant at age 15 months. In vitro microelectrode studies revealed decreased quantal release by nerve impulse and a decreased MEPP amplitude. Electron microscopy showed the nerve terminals were abnormally small and often encased by Schwann cells, accounting for the decreased quantal release by nerve impulse; and the synaptic space was widened and the junctional folds were simplified, accounting for the decreased MEPP amplitude.

CMS Caused by Mutations in AChR

Most postsynaptic CMS are caused by one or more mutations in an AChR subunit gene that alter the surface expression or kinetic properties of the receptor. The kinetic mutations fall into two distinct groups: 1) dominant, gain-of-function mutations that prolong the openings of the AChR channel and cause slow-channel syndromes, and 2) recessive loss-of-function mutations that shorten the openings of the AChR channel and cause fast-channel syndromes. Some lowexpressor mutations also have minor kinetic effects, and some kinetic mutations also reduce AChR expression

Primary EP AChR Deficiency

Primary AChR deficiency results from mutations in the α , β , δ , or ϵ subunits of AChR that decrease expression of the pentameric receptor and have little or no kinetic effects. Primary AChR deficiency is the most common cause of CMS, accounting for one third of all CMS patients (Table 1). The pathogenic mutations cause a frameshift, involve a splice-site, introduce a stop codon in the open reading frame, or are missense mutations of residues essential for subunit assembly.

In most patients with primary AChR deficiency, the mutations reside in the ϵ subunit and patients with recessive mutations in the ϵ subunit are generally less severely affected than those with mutations in other subunits. The explanation for this is that expression of the fetal γ subunit partially compensates for low or no expression of the ϵ subunit and rescues the

phenotype. Patients harboring lowexpressor or null mutations in both alleles of non- ϵ subunits are infrequent, have very severe disease, and often die in early life.

The clinical features vary from mild to severe. The most severely affected patients have marked ocular, bulbar, and respiratory muscle weakness from birth and survive only with respiratory support and gavage feeding. Their motor milestones are severely delayed; they can seldom learn to climb steps and can walk for only a short distance. Older patients close their mouth by supporting the jaw with their hand and elevate their eyelids with their fingers. Facial deformities, prognathism, malocclusion, and scoliosis or kyphoscoliosis are apparent by the second decade. Muscle bulk is reduced. The tendon reflexes are normal or hypoactive. The least affected patients pass their motor milestones with slight or no delay and only show mild ptosis and limited ocular ductions. Patients with intermediate clinical phenotypes experience moderate physical handicaps from early childhood. Ocular palsies and ptosis appear during the first year of life.

Morphologic studies show an increased number of EP regions distributed over an increased span of the muscle fiber. The junctional folds are preserved but some EP regions are simplified and smaller than normal. Expression of AChR on the junctional folds is markedly reduced. Microelectrode studies reveal reduced amplitude MEPPs. In patients with mutations in the ϵ subunit, patch-clamp studies show low-amplitude long-duration synaptic currents typical of the AChR harboring the γ instead of the ϵ subunit.

Most patients respond favorably but incompletely to anti-AChE medications. The additional use of 3,4-diaminopyridine (3,4-DAP) results in further significant improvement in about one third of the cases [22]. Recently, albuterol was noted to have beneficial effects in two patients responding poorly to cholinergic agonists [23]. This intriguing finding needs to be explored in a controlled clinical trial in a larger number of patients.

Slow-Channel Syndromes

Slow-channel syndromes arise from dominant gain-of-function mutations of AChR that enhance the affinity for ACh, or increase the gating efficiency (β/α) by increasing the channel opening rate (β) or by decreasing channel closings rate (α) [17]. Either mechanism prolongs the EP potentials and currents. As in EP AChE deficiency, when the length of the EPP exceeds the absolute refractory period of the muscle fiber, it triggers a second muscle fiber action potential but, unlike in EP AChE deficiency, the repetitive potential is enhanced by edrophonium. Also, at physiologic rates of stimulation, each prolonged EPP arises in the wake of the preceding EPP causing a progressive depolarization block of the postsynaptic membrane. The prolonged EP currents cause cationic overloading of the postsynaptic region and an EP myopathy. In addition, the mutant channels open even in the absence of ACh [24] causing a continuous cation leak into the postsynaptic region. The safety margin of synaptic transmission is compromised by progressive depolarization block during physiologic activity, EP myopathy, and increased propensity of some mutant receptors to become desensitized.

The slow-channel syndromes are refractory to, or are worsened by, cholinergic agonists but are improved by long-lived open-channel blockers of the AChR, such as quinine, quinidine, or fluoxetine [25, 26].

Fast-Channel Syndromes

The fast-channel syndromes are physiologic opposites of the slow-channel syndromes. They are recessively inherited disorders caused by mutations that decrease affinity for ACh, reduce gating efficiency, destabilize channel kinetics, or cause a combination of these mechanisms. Each of these derangements results in abnormally brief channel openings reflected by an abnormally fast decay of the EPP and EP current. A fast-channel mutation dominates the clinical phenotype if it is homozygous or when the second allele harbors a low-expressor or null mutation. Fast-channel mutations in the extracellular domain of AChR that reduce gating efficiency exert their effect in the transitional state during which the liganded receptor isomerizes from the closed to the open state. Thus, these mutations point to vital spots of the receptor subserving signal transmission from the agonist binding site to residues that effect channel opening [27, 28].

The clinical consequences vary from mild to severe. Most patients respond to a combination of 3,4-DAP, which increases the number of ACh quanta released by nerve impulse, and anti-AChE medications, which increase the number of AChRs activated by each quantum. However, a mutation in the ϵ subunit that introduced a positive charge into one of the two anionic binding sites of AChR reduced affinity for cationic ACh to the extent that it rendered the patient refractory to clinically tolerated doses of cholinergic agonists [29].

The safety margin of neuromuscular transmission in the fast-channel syndromes is compromised by a decreased probability of channel opening, which reduces the amplitude of MEPP and EPP, and because the threshold for activating the postsynaptic voltage-gated sodium channel increases when the duration of the EPP decreases.

Rapsyn Deficiency

Rapsyn (receptor-associated protein of the synapse), under the influence of agrin, LRP4 (low-density lipoprotein receptor-related protein 4), MuSK, and Dok-7, concentrates AChR in the postsynaptic membrane and links it to the subsynaptic cytoskeleton through dystroglycan [30–33]. In most patients, myasthenic symptoms present at birth or infancy but in a few they present in the second or third decade [34]. Arthrogryposis at birth and other the congenital malformations occur in nearly a third of the patients [34–36] but are not associated with specific mutations. Respiratory infections or other intercurrent febrile illnesses precipitate increased weakness and respiratory crises. Most patients have eyelid ptosis but only 25% have ophthalmoparesis. Nearly all Indo-Europeans harbor a common N88K mutation [37]. There are no genotype-phenotype correlations except Near-Eastern Jewish patients who carry an E-box mutation ($-38A>G$) in *RAPSN* have facial deformities associated with prognathism, malocclusion, and a mild clinical course [38]. The morphologic alterations at the EP resemble those in patients with low-expressor mutation of the AChR. Most patients respond well to anticholinesterase medications; some derive

additional benefit from the use of 3,4-DAP [39, 40]. Some patients observed by the author benefited from the added use of ephedrine [39] or albuterol.

Defects in Mechanisms Governing Endplate Development and Maintenance

MuSK, activated by agrin via LRP4 (low-density lipoprotein receptor-related protein 4) [33, 41] acts in concert with Dok-7 [32] and its activators [42, 43] to regulate the development and maintenance of the neuromuscular junction. Each of the above proteins is a potential CMS target. Defects in MuSK, agrin, and Dok-7 are now known to cause CMS and are considered in the next three sections.

CMS Caused by Defect in Agrin

A homozygous G1709R mutation was identified in a 42-year-old woman with right lid ptosis since birth, no oculoparesis, and mild weakness of facial, hip-girdle, and anterior tibial muscles, and refractoriness to pyridostigmine or 3,4-DAP [44]. Structural studies showed EPs with misshaped synaptic gutters partially filled by nerve endings and formation of new EP regions. The postsynaptic regions were preserved. The mutation did not affect agrin activation by MuSK or agrin binding to α -dystroglycan. Forced expression of the mutant mini-agrin gene in mouse soleus muscle showed changes similar to those at patient EPs. Thus, the observed mutation perturbs the maintenance of the EP without altering the canonical function of agrin to induce development of the postsynaptic compartment [44].

CMS Caused by Defects in MuSK

Three reports document CMS caused by recessive mutations in the MuSK. The first report describes a heteroallelic frameshift (c.220insC) and a missense (p. V790M) mutation [45]. The frameshift mutation prevented MuSK expression; the missense mutation decreased MuSK expression and impaired its interaction with Dok-7 [32]. Forced expression of the mutant protein in mouse muscle decreased AChR expression at the EP and caused aberrant axonal outgrowth [45].

A second report describes heteroallelic p.M605I and p. A727V mutations in MuSK [46•]. 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 paucity of secondary synaptic clefts.

A third report describes a homozygous p.P31L mutation in the extracellular domain of MuSK in five patients in a consanguineous Sudanese kinship [47].

The symptoms present in early life and vary from mild [47] to severe [45]. Pregnancy [45] and menstrual periods [46•] worsen the symptoms. One patient with severe symptoms in infancy improved after puberty [45]. None of the patients responded to cholinergic agonists but one patient was improved by albuterol [46•].

CMS Caused by Defects in Dok-7

After the discovery in 2006 of Dok-7 as a muscle-intrinsic activator of MuSK [32], numerous CMS-related mutations were identified in *DOK7* [6•, 19, 48•] and Dok-7 myasthenia is now recognized as a common cause of CMS.

The weakness in Dok-7 myasthenia typically has a limb-girdle distribution but mild ptosis and facial weakness are not infrequent. Severe bulbar symptoms are uncommon. The disease may present with hypomotility in utero, at birth, or later in infancy or early childhood. The clinical course varies from mild static weakness to progressive disease with conspicuous muscle atrophy. Pyridostigmine often worsens the disease immediately or gradually. In contrast, treatment with ephedrine [49] or albuterol is effective [20].

The synaptic contacts are small relative to fiber size and are single or multiple on a given fiber. Electron microscopy reveals ongoing destruction of existing EPs and attempts to form new EPs. Therefore, Dok-7 is essential not only for EP development [32] but also for maintaining the structural integrity of the EP throughout life [48•]. Neuromuscular transmission is compromised by variable decreases in quantal release and by a reduced postsynaptic response to ACh [48•, 50].

CMS Caused by Defect in the Hexosamine Biosynthetic Pathway

This CMS was reported in 2011 [51•]. It is caused by mutations in *GFPT1* coding for glutamine-fructose-6-phosphate transaminase 1. The enzyme controls the flux of glucose into the hexosamine pathway, and thus the formation of hexosamine products and the availability of precursors for N- and O-linked glycosylation of proteins. The disease gene was discovered by linkage studies and homozygosity mapping of multiplex kinships with a limb-girdle CMS associated with tubular aggregates in skeletal muscle. The affected patients harbored no mutations in Dok-7, and unlike patients with Dok-7 myasthenia, responded favorably to anticholinesterase medications.

Among the 13 reported patients, most presented in the first decade, about one-fourth had elevated serum creatine kinase levels, some had distal as well as proximal weakness, and few had ptosis or respiratory muscle involvement. Immunoblots of muscle of affected patients revealed decreased expression of O-N-acetylglucosamine residues on numerous muscle proteins. One patient was shown to have a decreased number of EP AChRs. One patient had a reduced number of AChRs per EP; how the enzyme deficiency altered other parameters of neuromuscular transmission in other patients was not assessed [51•].

Sodium Channel Myasthenia

Only one patient with this syndrome has been observed to date [3]. A 20-year-old normokalemic woman had abrupt attacks of respiratory and bulbar paralysis since birth lasting 3 to 30 min and became cognitively challenged after repeated episodes of hypoxia. The apneic attacks were like those caused by ChAT deficiency. EMG studies revealed a decremental response of the CMAP at 2 Hz only after a conditioning train of subtetanic or tetanic stimuli. Micro-electrode recordings from intercostal muscle EPs revealed normal

EPPs that failed to trigger action potentials and pointed to a defect in $\text{Na}_v1.4$, the sodium channel in skeletal muscle. Mutation analysis of *SCN4A* that codes for $\text{Na}_v1.4$ revealed two heteroallelic missense mutations: a benign p.S246L mutation in the cytoplasmic link between the S4 and S5 segments of domain I, and a pathogenic p.V1442E in the extracellular link between the S3 and S4 segments of domain IV. Expression studies in HEK cells revealed that most V1442E channels are fast-inactivated and inexcitable at a normal resting membrane potential of -80 mV.

The phenotype in this CMS differs from that of periodic paralyses caused by other mutations of *SCN4A* because the onset is neonatal, the disorder is normokalemic, the attacks selectively involve bulbar and respiratory muscles, and the muscle fiber membrane potential is normal when action potential generation fails. After the defect in $\text{Na}_v1.4$ was established, the patient was treated with pyridostigmine, which improved her endurance, and with acetazolamide, which prevented further attacks of respiratory and bulbar weakness.

CMS Caused by Plectin Deficiency

Plectin, encoded by *PLEC*, is a highly conserved and ubiquitously expressed intermediate filament-linking protein concentrated at sites of mechanical stress, such as the postsynaptic membrane lining junctional folds, the sarcolemma, Z-disks in skeletal muscle, hemidesmosomes in skin, and intercalated disks in cardiac muscle. Pathogenic mutations in plectin result in epidermolysis bullosa simplex, a progressive myopathy and, in some patients, a myasthenic syndrome [52]. Heteroallelic nonsense, frame-shift, or splice-site mutations in *PLEC* were recently reported in four unrelated patients [2, 53, 54]. In two patients microelectrode studies of intercostal muscle EPs showed low-amplitude MEPPs. Morphologic studies revealed dislocated and degenerating muscle fiber organelles, plasma membrane defects allowing calcium ingress into the muscle fibers, and degeneration of the junctional folds, all attributable to lack of cytoskeletal support [2]. One patient harbored homozygous frameshift mutations in both *PLEC* and in *CHRNA1* [54].

Myasthenic Syndrome Associated with Centronuclear Myopathy

Centronuclear myopathies (CNMs) are clinically and genetically heterogeneous congenital myopathies in which the predominant pathologic alteration is centralization of the muscle fiber nuclei. The implicated disease proteins/ genes are myotubularin (*MTM1*), dynamin 2 (*DNM2*), amphiphysin 2 (*BIN1*), and the ryanodine receptor (*RYR1*) [55]. Features suggesting a myasthenic disorder, ptosis, ophthalmoparesis, abnormal fatigability, decremental EMG response [56], or abnormally increased jitter [57] have been observed in clinically and genetically different CNM patients but the mechanism of the putative myasthenic disorder has not been determined.

A recently investigated 39-year-old man with CNM and a myasthenic syndrome [58•] had a 19% to 35% EMG decrement and responded partially to pyridostigmine. Serologic tests for AChR and MuSK antibodies were negative. No mutations were detected in *MTM1*, *DNM2*, *BIN1*, and *RYR1*. Intercostal muscle EP studies revealed simplified postsynaptic regions, and mild AChR deficiency. The safety margin of neuromuscular transmission was compromised by reduction of the MEPP amplitude to 60% and of quantal release to 40% of

normal [58•]. Four other CNM patients with myasthenic features responding to pyridostigmine but no known mutation were also reported but EP structure and parameters of neuromuscular transmission were not evaluated [59].

Prenatal CMS with Fetal Akinesia and Deformations

Fetal hypomotility can result in intrauterine growth retardation, multiple joint contractures, subcutaneous edema, pterygia (webbing of the neck, axilla, elbows, fingers, or popliteal fossa), lung hypoplasia, and other congenital malformations. The syndrome is often lethal; the nonlethal form is referred to as the Escobar syndrome. Fetal akinesia has many causes. Those due to defects in neuromuscular transmission include transplacental transfer from mother to fetus of anti-AChR antibodies with a high-titer of anti- γ -subunit specificities [60, 61], and spontaneous mutations in EP-associated proteins.

In humans, AChR containing the γ subunit appears on myotubes around the 9th developmental week and is concentrated at nascent EPs around the 16th developmental week. The fetal γ subunit is replaced by the adult ϵ subunit and is no longer present at fetal EPs after the 31st developmental week [62]. Thus, pathogenic mutations or an autoimmune attack on the AChR γ subunit result in hypomotility in utero. The clinical consequences at birth are multiple joint contractures, small muscle bulk, multiple pterygia, camptodactyly, rocker-bottom feet with prominent heels, characteristic facies with mild ptosis, and a small mouth with downturned corners [63, 64]. If the patient survives after birth, myasthenic symptoms are absent because by then the normal ϵ subunit is expressed at the EPs. Lethal fetal akinesia syndromes have also been reported due to biallelic null mutations in the AChR α , β , and δ subunits, as well as in rapsyn and Dok-7 [65•, 66, 67].

Conclusions

Mutations in no fewer than 13 genes are now known to cause CMS. In many CMS, clinical clues point to the correct clinical diagnosis and the disease gene. However, there are no specific clues to the diagnosis of the fast-channel CMS, primary EP AChR deficiency, and most cases of rapsyn deficiency. When no candidate gene is apparent, mutation analysis can be based on frequencies of the heretofore identified mutations in different EP proteins, or on clues derived from in vitro analysis of parameters of neuromuscular transmission. In the recently discovered CMS caused by mutations in *GFPT1*, linkage analysis of phenotypically similar kinships was crucial for discovering the disease gene. It is now also recognized that myasthenic syndromes can be associated with epidermolysis bullosa simplex due to mutations in plectin, and with CNMs with or without identified disease genes. Most CMS are highly disabling but if correctly diagnosed, most can be mitigated by pharmacotherapy.

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- Of importance

- Of major importance

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Table 1

Classification of the CMS based on 321 index patients investigated at the Mayo Clinic

Defect site	Index cases
<i>Presynaptic</i> (6%)	
ChAT deficiency ^a	17
Paucity of synaptic vesicles	1
Congenital Lambert-Eaton syndrome-like	1
<i>Synaptic basal lamina</i> (13.7%)	
Endplate AChE deficiency ^a	43
β2 laminin deficiency ^a	1
<i>Postsynaptic</i> (68%)	
Primary AChR kinetic defect ^a	58
Primary AChR deficiency ^{a, b}	109
Rapsyn deficiency ^a	48
Sodium channel myasthenia ^a	1
Plectin deficiency ^a	2
<i>Combined pre- and postsynaptic</i> (12.5%)	
Dok-7 myasthenia ^a	31
CMS in centronuclear myopathy	1
GFPT1 myasthenia ^a	8
Total	321

Few patients harboring mutations in agrin [44] and MuSK [45] were identified at other medical centers.

AChE acetylcholinesterase; *AChR* acetylcholine receptor; *ChAT* choline acetyltransferase; *CMS* congenital myasthenic syndromes; *Dok-7* downstream of tyrosine kinase 7; *GFPT1* glutamine-fructose-6-phosphate transaminase 1; *MuSK* muscle-specific kinase.

^aGene defect identified.

^bMost mutations reside in the AChR ε subunit.