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Current Status of the Congenital Myasthenic Syndromes

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Abstract

Congenital myasthenic syndromes (CMS) are heterogeneous disorders in which the safety margin of neuromuscular transmission is compromised by one or more specific mechanisms. Clinical, electrophysiologic, and morphologic studies have paved the way for detecting CMS-related mutations in proteins residing in the nerve terminal, the synaptic basal lamina, and in the postsynaptic region of the motor endplate. The disease proteins identified to date include choline acetyltransferase (ChAT), the endplate species of acetylcholinesterase (AChE), β 2-laminin, the acetylcholine receptor (AChR), rapsyn, plectin, $\text{Na}_v1.4$, the muscle specific protein kinase (MuSK), agrin, downstream of tyrosine kinase 7 (Dok-7), and glutamine-fructose-6-phosphate transaminase 1 (GFPT1). Myasthenic syndromes associated with centronuclear myopathies were recently recognized. Analysis of properties of expressed mutant proteins contributed to finding improved therapy for most CMS. Despite these advances, the molecular basis of some phenotypically characterized CMS remains elusive. Moreover, other types of CMS and disease genes likely exist and await discovery.

Keywords

Congenital myasthenic syndrome; neuromuscular junction; EMG; choline acetyltransferase; ColQ; β 2-laminin; acetylcholine receptor; rapsyn; agrin; MuSK; Dok-7; GFPT1; plectin; fetal akinesia syndrome

1. Introduction

In each congenital myasthenic syndrome (CMS) the safety margin of neuromuscular transmission is compromised by one or more mechanisms. These mechanisms involve the synthesis or packaging of acetylcholine (ACh) quanta into synaptic vesicles, the Ca^{2+} -dependent evoked release of ACh from the nerve terminal, and the efficiency of released quanta in generating a postsynaptic depolarization. Quantal efficiency depends on the endplate (EP) geometry, the density and functional state of acetylcholinesterase (AChE) in the synaptic space, and the density, affinity for ACh, and kinetic properties of the acetylcholine receptor (AChR). Table 1 presents a classification for CMS based on 321 unrelated index patients investigated at the Mayo Clinic. The genetic basis of the CMS was

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determined in 318 patients. In 220 other index patient the molecular basis of the CMS awaits identification.

Table 1 indicates that the purely presynaptic CMS are least frequent, accounting for only 6% of all cases. Of note, however, a defect in presynaptic quantal release is also present in EP AChE deficiency [1], Dok-7 myasthenia [2,3], β 2-laminin deficiency [4], and in the CMS associated with centronuclear myopathy [5]. The purely postsynaptic CMS account for most patients in this group and mutations in AChR subunits account for more than one-half of all cases. Figure 1 shows the distribution of the CMS disease proteins at the neuromuscular junction.

2. The investigation and diagnosis of the CMS

A full understanding of how the safety margin of neuromuscular transmission is compromised in a given CMS is based on clinical, morphologic, in vitro electrophysiologic, and molecular genetic studies. The clinical evaluation must include detailed electromyographic (EMG) studies to demonstrate a defect in neuromuscular transmission, tests for anti-AChR and anti-MuSK antibodies in sporadic patients presenting after the age of 1 year and in infants born with contractures, even if the mother has no symptoms to suggest an autoimmune myasthenia. The morphologic evaluation of the EP includes localization of AChR and AChE and ultrastructural analysis. In vitro electrophysiologic studies must be sufficiently complete so they provide information on parameters of quantal release and the factors affecting the efficiency of the released quanta. A surprising number of CMS stem from a kinetic abnormalities of the AChR. These can be recognized by examination of the decay phase of the miniature EP current (MEPC), and analyzed by patch-clamp recordings of currents flowing through single AChR channels.

Because only few medical centers are able to perform all or some of the above studies, and mutations analysis of DNA isolated from blood or other tissues has been increasingly used to identify CMS disease genes and mutations. Indeed, automated sequencing methods of currently identified CMS genes are widely available and morphologic and functional studies are only indicated when mutation analysis of known CMS genes yields negative results.

3. Genetic analysis

This is greatly facilitated when clinical, physiologic, or morphologic studies point to a candidate protein or gene. For example, a kinetic abnormality of AChR detected at the single channel level [6], or severe EP AChR deficiency revealed by α -bungarotoxin binding studies [7], predicts mutations in an AChR subunit gene. Table 2 lists generic and specific clinical clues that facilitate targeted mutation analysis.

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 expensive and time intensive than the candidate gene approach.

In patients with strong phenotypic clues for a given recessive CMS but only one or no identified mutations in the open reading frame, cDNA isolated from muscle can reveal an intronic mutation. This was the case for some patients with Dok-7 myasthenia.[3] cDNA analysis was also useful in deciphering the consequences of a frameshifting mutation in ColQ. [8]

Genetic testing for CMS is now commercially available and facilitates diagnosis and management by neuromuscular specialists. It is best used in a targeted manner based on specific clinical features, as listed in Table 2, or beginning with the most frequently mutated

genes, as shown in Table 1. However, this approach has a number of drawbacks: (1) it is expensive, especially if used in a shotgun manner; (2) it does not establish that recessive mutations are heteroallelic unless they are homozygous; therefore DNA from both parents also must be analyzed; (3) it will miss intronic mutations not close to exons; (4) evaluation of pathogenicity is based on software programs whose reliability is still debated; (5) it does not inform on kinetic consequences of mutations in AChR or ChAT, or on pathogenicity of mutations that render the disease protein structurally unstable; and (6) negative results do not exclude the diagnosis of a CMS because only previously identified disease genes are sequenced.

Another approach is linkage analysis if a sufficient number of informative relatives are available. If successful, it will point to a candidate chromosomal locus. If the physical map of the locus shows an attractive candidate gene, then mutation analysis by direct sequencing becomes feasible. This approach seldom works for CMS because large informative CMS kinships are seldom available; however, it has been successful in inbred populations with multiple consanguineous families [9].

A direct and efficient approach is the use microarrays specifically designed for screening multiple candidate disease loci in known CMS genes. One publication finds this approach has a 73.3% overall sensitivity and a 95.5% sensitivity for missense mutation, but it is not recommended for detecting insertion or deletion mutations [10]. Also, this approach will miss mutations in novel CMS disease genes.

A novel approach to mutation discovery is whole exome sequencing that searches for mutations in exons. Kits available for this method presently capture only ~97% of the entire exome but read only 75% of the exome with more than 20x coverage. The enormous amount of generated data need to be filtered against previously identified variants deemed nonpathogenic and scrutinized for mutations in genes encoding EP related genes. The putative mutations must be confirmed by capillary sequencing and the non-truncating mutations examined by expression studies. Also, the cost of exome sequencing with the required bioinformatics analysis is still high [11]. Other pitfalls in this approach are that (1) disease causing variants in noncoding regions and some large deletions or duplications can be missed, (2) pathogenic variants causing rare diseases may have previously been entered in dbSNP, (3) synonymous variants in good candidate genes that might affect a splice enhancer/repressor are often filtered out. Exome sequencing is most efficient when large and/or multiple kinships are available for analysis. Whole genome sequencing is also feasible but is even more expensive and more complicated to interpret than exome sequencing.

Large deletion or duplication mutations can be missed both by Sanger sequencing or whole exome sequencing. Although rare, they can be identified by array based comparative genomic hybridization.[12]

4. Presynaptic CMS

4.1. Endplate choline acetyltransferase (ChAT) deficiency

ChAT catalyzes the synthesis of ACh by transfer of an acetyl group from acetyl-CoA to choline in cholinergic neurons. Pathogenic mutations, alone or in combination, alter expression, catalytic efficiency, or structural stability of the enzyme [13,14]. The decreased rate of ACh resynthesis progressively depletes the ACh content of the synaptic vesicles, and hence the amplitude of the of the miniature EP potential (MEPP), when neuronal impulse flow is increased.

Some patients present with hypotonia, bulbar paralysis and apnea at birth. Others patients are normal at birth and develop apneic attacks during infancy or childhood precipitated by infections, excitement, or no apparent cause [14–18]. In some children an acute attack is followed by respiratory insufficiency that lasts for weeks [19]. Few patients are apneic, respirator dependent, and paralyzed since birth [14], and some develop cerebral atrophy after episodes of hypoxemia [14,14]. Others improve with age, but still have variable ptosis, ophthalmoparesis, fatigable weakness, and recurrent cyanotic episodes, or complain only of mild to moderately severe fatigable weakness. The symptoms are worsened by exposure to cold likely because this further reduces the catalytic efficiency of the mutant enzyme [16].

Mutations with severe kinetic effects are located in the active-site tunnel of the enzyme, or adjacent to its substrate binding site, or exert their effect allosterically. Some mutations have no kinetic effects and express well but impair the thermal stability of ChAT by introducing a proline residue into an α -helix [14]. Genotype-phenotype correlations are hindered in patients with biallelic mutations. Interestingly, however, in three very severely affected patients with life-long apnea, permanent paralysis, and failure to respond to pyridostigmine, one of the two mutations was near the active-site of ChAT.

Although some patients fail to respond to AChE inhibitors, these should be tried in the initial management of all patients. Therapy should be continued prophylactically even when patients are asymptomatic. The parents should be provided with a portable ventilatory device, instructed in the intramuscular injection of neostigmine methylsulfate, and advised to install an apnea monitor in the home [20].

4.2. Paucity of synaptic vesicles and reduced quantal release

In this congenital myasthenic syndrome, the number of quanta released by nerve impulse is decreased due to a decreased store of quanta available for release, and this decrease is proportionate to a reduced density of synaptic vesicles in the nerve terminal. The probability of quantal release is normal [21]. The molecular basis of the disease awaits identification.

4.3. Congenital Lambert-Eaton-like syndrome

In this CMS, repetitive nerve stimulation shows marked facilitation of the compound muscle action potential (CMAP) at physiologic rates of stimulation [22]. In a single patient investigated by the author, the number of quanta released by nerve impulse was decreased due to a decreased probability of quantal release. EP ultrastructure was normal [20]. The patient failed to respond to 3,4-DAP, which enhances quantal release in the acquired forms of the Lambert-Eaton syndrome.

5. Synaptic basal-lamina-associated CMS

5.1. Endplate acetylcholinesterase deficiency

The EP species of AChE is an asymmetric enzyme composed of catalytic subunits encoded by *ACHE_T* and a collagenic structural subunit encoded by *COLQ*. No spontaneous mutations have been observed in *AChE_T*. The ColQ protein anchoring the complex in the synaptic basal lamina is composed of three identical strands. The N-terminal residues of each strand bind a catalytic homotetramer. Mutations in the N-terminal region of ColQ prevent its association with the catalytic subunits; frameshift or nonsense mutations in collagenic midsection of ColQ produce an insertion incompetent single stranded enzyme. Mutations of critical residues in the globular C-terminal region of ColQ prevent ColQ insertion into the synaptic basal lamina or hinder the triple helical assembly of the ColQ strands [23,24] (Fig 2).

Absence of AChE from the EP prolongs the lifetime of ACh in the synaptic cleft because each ACh binds multiple AChRs before leaving the synaptic space by diffusion. This prolongs the duration of the MEPP and EPP, and when the EPP outlasts the absolute refractory period of the muscle fiber, it generates a second (or repetitive) muscle action potential, reflected by a repetitive compound muscle action potential (CMAP) that is not affected by edrophonium. In study, however, detected a repetitive CMAP in only 12 of 18 patients.[25] Cholinergic overactivity at the EP results in cationic overloading of the postsynaptic region and causes degeneration of the junctional folds with loss of AChR. The nerve terminals are abnormally small and often encased by Schwann cells, which reduces the number of releasable quanta. The safety margin of neuromuscular transmission is compromised by decreased quantal release, loss of AChR from degenerating junctional folds, altered endplate geometry, and desensitization of AChR from overexposure to ACh [1,26]

The clinical course is variable. [23,24,27] Most patients present in early infancy and are severely disabled. These patients typically harbor mutations in the N-terminal or rod domain of ColQ which abolish the expression of AChE in the synaptic space. Patients with the C-terminal missense mutations that do not abolish the triple helical assembly of the ColQ **harboring the Y430S mutation in the C-terminal domain of ColQ strands or the insertion of ColQ into synaptic basal lamina present later in childhood and have a milder course. [28,29]. Another 15-year-old patient harboring C-terminal Y440D and I447M mutations was a cheer-leader at age 11 and developed progressive weakness only after age 12. She expressed the triple-helical ColQ protein on density gradient centrifugation and showed reduced rather than absent immunostaining for AChE the EPs (AG Engel, unpublished observation).

Therapy is still unsatisfactory, but ephedrine has had a significant beneficial effect in some patients [25,29,30]. More recently, albuterol was found to be as or more effective than ephedrine [31].

5.2. CMS associated with β 2-laminin deficiency

β 2-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 β 2-laminin governs the appropriate alignment of the axon terminal with the postsynaptic region and, hence, pre- and postsynaptic trophic interactions. Defects in β 2-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 [4]. 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 reduced MEPP amplitude. Electron microscopy showed the nerve terminals were abnormally small and often encased by Schwann cells, accounting for the decreased quantal release. The synaptic space was widened and the junctional folds were simplified, accounting for the decreased MEPP amplitude.

6. Postsynaptic CMS

6.1 Primary AChR deficiency

CMS with severe EP AChR deficiency result from different types of homozygous or more frequently heterozygous recessive mutations in AChR subunit genes. The mutations are concentrated in the ϵ subunit. The main reason for this is that expression of the fetal type γ subunit, although at a low level, partially compensates for absence of the ϵ subunit [32–34] whereas patients harboring null mutations in subunits other than ϵ might not survive for lack of a substituting subunit. Some mutations cause premature termination of the translational

chain. These mutations are frameshifting [7,33,35–37], occur at a splice site [7,36], produce a stop codon directly [33], or arise from a chromosomal microdeletion [38]. An important mutation in this group is ϵ 1369delG because it results in loss of a C-terminal Cys470 residue crucial to maturation and surface expression of the adult receptor [39]. Thus any mutation that truncates the ϵ subunit upstream of Cys470 abrogates expression of the ϵ subunit.

Other mutations in the ϵ subunit reside in the promoter region [38,40,41], the signal peptide region [36,42], or involve residues essential for subunit assembly. Mutations hindering subunit assembly appear in the ϵ subunit at an N-glycosylation site (ϵ S143L) [42], in Cys128 (ϵ C128S) in the canonical Cys-loop [34], in Arg147 (ϵ R147L) which is part of a short extracellular span of residues that contributes to subunit assembly [33], in Thr51 (ϵ T51P) [36], and in the long cytoplasmic loop of the β subunit causing the deletion of three codons [43]. A missense mutation in the long cytoplasmic loop of the δ subunit (δ E381K) presents with symptoms typical of rapsyn deficiency likely because the mutated Glu381 is a binding partner for rapsyn [44].

Epsilon subunit mutations occurring at homozygosity are endemic in Mediterranean or other Near Eastern countries [36,45]. For example, the frameshifting ϵ 1267delG mutation occurring at homozygosity is endemic in Gypsy families where it derives from a common founder [35].

Morphologic studies show an increased number of small endplate regions distributed over an increased span of the muscle fiber, and the distribution of AChRs on the junctional folds is patchy and attenuated [32,33]. The structural integrity of the junctional folds is preserved, but at some EP regions the junctional folds are less complex so that the postsynaptic membrane is less folded. The safety margin of neuromuscular transmission is compromised by the AChR deficiency and by simplification of the junctional folds which reduces the synaptic response to ACh.

Most patients respond favorably but incompletely to AChE inhibitors. The additional use of 3,4-DAP results in further improvement but the limited ocular ductions, pronounced in most patients with AChR deficiency, are typically refractory to any therapy. Recently, albuterol was found to be effective in patients responding poorly to pyridostigmine and 3,4-DAP [46].

6.2. Slow-channel syndromes

These syndromes arise from dominant gain of function mutations that either enhance the receptor's affinity for ACh or increase its gating efficiency (β/α) due to an accelerated channel opening rate (β) or abnormally slow channel closings rate (α) [47]. Either mechanism prolongs the EPPs. As in EP AChE deficiency, when the length of the EPP exceeds the absolute refractory period of the muscle fiber, it triggers a repetitive CMAP, but unlike in AChE deficiency the repetitive response is accentuated 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 (Figure 3). In addition, the mutant channels open even in the absence of ACh [48], causing a continuous cation leak into the postsynaptic region. The safety margin of synaptic transmission is compromised by the progressive depolarization block during physiologic activity, the EP myopathy, and by an 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 [49] or quinidine, or fluoxetine [50–52].

6.3. Fast-channel syndromes

The fast-channel syndromes are physiologic opposites of the slow-channel syndromes. They are recessively inherited disorders caused either by decreased affinity for ACh, or by reduced gating efficiency, or by destabilization of the channel kinetics, or by a combination of these mechanisms, and leave no anatomic foot prints. Each of these derangements results in abnormally brief channel openings reflected by an abnormally fast decay of the EP potentials and currents. A fast-channel mutation dominates the clinical phenotype when the second allele harbors a null mutation or if it occurs at homozygosity. 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. Hence these mutations point to vital spots of the receptor that participate in signal transmission from the agonist binding site to residues that effect channel opening [53,54]. 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 introducing a positive charge into one of the two anionic ACh binding sites of the receptor (ϵ W55R) greatly reduced affinity for cationic ACh and rendered the patient refractory to clinically tolerated doses of cholinergic agonists [55].

6.4. Prenatal syndromes caused by mutations in AChR subunits and other EP specific proteins

The first identified prenatal myasthenic syndrome was traced to mutations in the fetal AChR γ -subunit. In humans, AChR harboring the fetal γ subunit appears on myotubes around the ninth developmental week and becomes concentrated at nascent nerve-muscle junctions around the sixteenth developmental week. Subsequently, the γ subunit is replaced by the adult ϵ subunit and is no longer present at fetal EPs after the thirty-first developmental week [56]. Thus pathogenic mutations of the γ -subunit result in hypomotility in utero mostly during the sixteenth and thirty-first developmental week. The clinical consequences at birth are multiple joint contractures, small muscle bulk, multiple pterygia (webbing of the neck, axilla, elbows, fingers, or popliteal fossa), fixed flexion contractures of the fingers (campodactyly), rocker-bottom feet with prominent heels, and characteristic facies with mild ptosis and a small mouth with downturned corners. Myasthenic symptoms are absent after birth because by then the normal adult ϵ subunit is expressed at the EPs [56,57]. More recently, lethal fetal akinesia syndromes arising from biallelic null mutations in the AChR α , β , and δ subunits as well as in rapsyn [58,59] and Dok-7 [60] were also identified.

6.5. CMS caused by defects in rapsyn

Rapsyn, under the influence of agrin, LRP4, MuSK and Dok-7 concentrates AChR in the postsynaptic membrane and links it to the subsynaptic cytoskeleton through dystroglycan [61–65]. Mutations have now been detected in the entire open reading frame and promoter region of *RAPSN* [66–73]. Nearly all Indo-Europeans harbor a common N88K mutation in *RAPSN* [71], but one patient carried 2 heteroallelic non-N88K mutations [74]. Expression studies in different cell lines reveal that different rapsyn mutations hinder rapsyn colocalization with AChR, prevent formation of agrin-induced AChR clusters, impede rapsyn self-association, or reduce rapsyn expression [66,72]. Despite these differences, there are no consistent genotype-phenotype correlations except Near-Eastern Jewish patients harboring a homozygous E-box mutation ($-38A>G$) have masticatory and facial muscle weakness, ptosis, prognathism, and hypernasal speech. Cervical, axial and limb muscles are usually spared [67].

In most patient, myasthenic symptoms present at birth or infancy; in a few they present in the second or third decade [69]. Arthrogryposis at birth and other congenital malformations occur in nearly one-third [66,69,75] but are not associated with specific mutations. Respiratory infections or other febrile illnesses precipitate increased weakness and respiratory crises and can cause an anoxic encephalopathy [66,69,76,77]. Mutations in the open reading frame of *RAPSN* result in clinical features that resemble those of autoimmune myasthenia except ophthalmoparesis is uncommon [69]; in our series, only 9 of 39 patients had constant or episodic involvement of the extraocular muscles [75]. Most patients have ptosis of varying severity. Facial and bulbar weakness is common, often associated with neck muscle weakness. Proximal muscle weakness is more severe than distal weakness. Out-of-proportion weakness of the foot dorsiflexors is a feature of the late-onset phenotype [69]; it was absent in 39 early-onset patients [75].

The morphologic features of the EP and the factors that impair the safety margin of neuromuscular transmission are the same as in primary AChR deficiency but the EP AChR deficiency is relatively mild in most patients (Figure 4). In some patients single-fiber EMG is required to demonstrate a defect in neuromuscular transmission.

Most patients respond well to AChE inhibitors; some derive additional benefit from the use of 3,4-DAP [76] and some observed by the author benefited from the added use of ephedrine or albuterol.

6.6. CMS caused by defects in plectin

Plectin, encoded by *PLEC*, is a highly conserved and ubiquitously expressed intermediate filament-linking protein. Owing to tissue and organelle-specific transcript isoforms, plectin is a versatile linker of cytoskeletal components to target organelles in cells of different tissues [78–80]. It is 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. In skeletal muscle, multiple alternatively spliced transcripts of the exon preceding a common exon 2 link cytoskeletal intermediate filaments to specific targets: the outer nuclear membrane (isoform 1), the outer mitochondrial membrane (isoform 1b), Z disks (isoform 1d), and in sarcolemmal costameres (isoform 1f) [80]. Pathogenic mutations in plectin result in epidermolysis bullosa simplex (EBS), a progressive muscular dystrophy in many patients, and a myasthenic syndrome in some patients (reviewed in [Refs.81,82]). Heteroallelic nonsense, frameshift, or splice-site mutations in *PLEC* were recently reported in 4 unrelated patients with documented defects in neuromuscular transmission [82–84]. In two patients investigated by the author microelectrode studies of intercostal muscle EPs showed low-amplitude MEPPs. Morphologic studies revealed dislocated and degenerating muscle fiber organelles, plasma membrane defects resulting in Ca^{2+} overloading of the muscle fibers as in Duchenne dystrophy, and extensive degeneration of the junctional folds, all attributable to lack of cytoskeletal support [82]. One patient harbored homozygous frameshift mutations in both *PLEC* and in *CHRNE* [84]. Interestingly, a recent study identified a homozygous deletion mutation in isoform 1f that caused limb-girdle muscular dystrophy but neither EBS nor myasthenia [85].

6.7. Na-channel myasthenia

A normokalemic patient had abrupt attacks of respiratory and bulbar paralysis since birth lasting 3 to 30 minutes similar to those caused by ChAT deficiency. Detailed electrophysiology analysis of patient EPs revealed that suprathreshold EPPs failed to generate muscle action potentials pointing to Nav1.4, encoded by *SCN4A*, as the culprit. EP structure and Nav1.4 expression at the EPs were normal, but *SCN4A* harbored 2 mutations

(S246L in the S4/S5 linker in domain I and V1442E in S4/S5 linker in domain IV). Recombinant V1442E-sodium channels expressed in HEK cells showed marked enhancement of fast inactivation close to the resting potential and enhanced use-dependent inactivation on high frequency stimulation; S246L had only minor kinetic effects and is likely a benign mutation. The safety margin in this congenital myasthenic syndrome is impaired because a large fraction of the Nav1.4 channels are inexcitable in the resting state [86].

7. Defects in mechanisms governing endplate development and maintenance

7.1. CMS caused by defect in agrin

Agrin, encoded by *AGRN*, is a multidomain proteoglycan secreted into the synaptic basal lamina by the nerve terminal. The muscle isoform of agrin harbors A and B regions near its C-terminal. Agrin phosphorylates and thereby activates MuSK by way of its receptor LRP4 (Kim et al 2008; Zhang et al 2008). Two siblings with eyelid ptosis but normal ocular ductions and only mild weakness of the facial and hip-girdle muscles carried a homozygous missense mutation in *AGRN* at codon 1709 (G1709R). The mutation is in the laminin G-like 2 domain, upstream of the γ and ζ inserts of neural agrin required for MuSK activation and EP formation. AChR and agrin expression at the EP were normal. Structural studies showed EPs with misshaped synaptic gutters partially filled by nerve endings and formation of new EP regions. The postsynaptic regions were preserved. Expression studies revealed the mutation did not affect activation of MuSK by agrin or agrin binding to α -dystroglycan. Forced expression of a 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 [87]. The index patient failed to respond to a cholinesterase inhibitor and 3,4-DAP but responded partially to ephedrine [87].

7.2. CMS Caused by Defects in MuSK

MuSK (muscle specific receptor tyrosine kinase), under the influence of agrin mediated by LRP4 and in concert with Dok-7, plays a role in maturation and maintenance of the EP and in directing rapsyn to concentrate AChR in the postsynaptic membrane [88–90]). In one kinship, a brother and sister carried 2 heteroallelic mutations: c.220insC, which is a frame-shifting null mutation, and a missense mutation (V790M) with no effect on the catalytic kinase activity of MuSK but decreased its expression and stability and resulted in decreased agrin-dependent AChR aggregation. The EPs consisted of multiple small regions linked by nerve sprouts. AChR expression per endplate was reduced to approximately 45% of normal [91]. In vitro electrophysiologic recordings and EM studies of patient EPs were unavailable. When the missense mutation was overexpressed in mouse muscle by electroporation, it reduced synaptic AChR expression and resulted in aberrant axonal outgrowth similar to that observed in the patient [92]. The safety margin in this CMS is likely compromised by the AChR deficiency.

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 sparse secondary synaptic clefts. The patient failed to respond to pyridostigmine, ephedrine or 3,4-DAP but responded partially to albuterol.[93]

A third kinship harbored a homozygous P344R missense mutation in the cysteine-rich extracellular domain of MuSK [94]. The clinical course was progressive. Low doses of pyridostigmine and 3,4-DAP led to gradual improvement; ephedrine or higher doses of pyridostigmine were not tolerated.

7.3. CMS caused by defects in Dok-7

After the discovery in 2006 of Dok-7 as a muscle-intrinsic activator of MuSK [64], numerous CMS-related mutations were identified in *DOK7* [2,3,95–97] and Dok-7 myasthenia is now recognized as a common cause of CMS.

Dok-7 is strongly expressed at the postsynaptic region of skeletal muscle and in heart. It harbors an N-terminal pleckstrin homology domain (PH) important for membrane association, a phosphotyrosine-binding (PTB) domain, and C-terminal sites for phosphorylation. The PTB and PH domains are required for association with and phosphorylation of MuSK. Phosphorylation of two of the C terminal residues is a requisite for Dok-7 activation by Crk and Crk-L [98].

The weakness in Dok-7 myasthenia typically has a limb-girdle distribution but mild ptosis and facial weakness are not infrequent [3,95–97,99–101]. Severe bulbar symptoms are uncommon except for laryngeal stridor in infants [102] but were present in a patient who carries a readthrough mutation in the last codon of *DOK7* [3].

The disease may present with hypomotility in utero, at birth, or later in infancy. In 16 patients investigated by us, the age at onset ranged from birth to 5 years (mean, 1.6 years, median, 1 year) [3]. The clinical course varied from mild static weakness limited to the limb-girdle muscles to severe generalized progressive disease with conspicuous muscle atrophy. All had short-term fatigability on exertion. Ten patients experienced intermittent worsenings lasting from days to weeks, as also observed by others [95,100]. Seven patients had significant respiratory embarrassment. The overall course was progressive in 12 of the 16 patients.

Repetitive stimulation studies reveal a decremental response of the CMAP but not in all muscles. An abnormal response is most consistently detected in the facial and trapezius muscles [3].

Type 1 fiber preponderance and type 2 fiber atrophy are common findings. Sparse necrotic and regenerating fibers, pleomorphic decreases in oxidative enzyme activity and target formations appear in some patients. The synaptic contacts are small relative to fiber size and are single or multiple on a given fiber. Most EPs lack the normal pretzel shape indicating impaired differentiation of the postsynaptic region [3,99]. The expression of Dok-7 at the EP is normal or reduced and does not consistently correlate with the clinical state; moreover, Dok-7 expression is also attenuated other CMS that reduce AChR expression [3].

In vitro microelectrode studies of intercostal muscle EPs in 14 patients observed by us showed that the mean MEPP and MEPC amplitudes were reduced to approximately two-thirds of normal and the predicted amplitude of the EPP was significantly decreased. Some patients had a marked reduction in the quantal content of the EPP. That multiple parameters of neuromuscular transmission are affected is likely related to both pre- and postsynaptic structural defects at the junction. However, there was no correlation between the altered parameters of neuromuscular transmission and the clinical state [3].

Electron microscopy analysis shows ongoing destruction of existing endplates and attempts to form new endplates (Fig. 5). Some EPs are normal but many display one or more of the

following abnormalities: degeneration of junctional folds, partial occupancy by or absence of nerve terminal; highly simplified junctional folds; and degeneration of subsynaptic organelles. Some nerve terminals are partly or completely encased by Schwann-cell, and few are degenerating. Nerve sprouts appear near degenerating or simplified EPs. Interestingly, the density and distribution of AChR on nondegenerate junctional folds is normal. Taken together, the structural findings indicate that Dok-7 is required not only for the normal development of the EP but is also crucial for maintaining its structural integrity throughout life [3].

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 [98,103]. Mutations disrupting or eliminating the PH and PTB domains of Dok-7 prevent dimerization and association of Dok-7 with MuSK [104]. Some mutations causing exons skipping were traced to intronic mutations. Detection of other mutations required analysis of cDNA or cloned cDNA [3]. A recent review lists all Dok-7 mutations reported since 2006 [97].

7.4. Myasthenic syndrome associated with centronuclear myopathy

Centronuclear myopathies (CNM) 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 (*BINI*), and the ryanodine receptor (*RYR1*) [105]. Features suggesting a myasthenic disorder, ptosis, ophthalmoparesis, abnormal fatigability, decremental EMG response [106] or abnormally increased jitter [107] have been observed in clinically and genetically different CNM patients.

A recently investigated 39-year-old man with CNM and a myasthenic syndrome [108] had a 19–35% EMG decrement and responded partially to pyridostigmine. Serologic tests for AChR and MuSK antibodies were negative. No mutations were detected in *MTM1*, *DNM2*, *BINI*, 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 [108]. Four other CNM patients with myasthenic features responding to pyridostigmine with no known mutation were also reported but EP structure and parameters of neuromuscular transmission were not evaluated [109].

7.5. CMS caused by defect in the hexosamine biosynthetic pathway

This CMS was reported in 2011 by Senderek and co-workers [9]. It is caused by mutations in *GFPT1* coding for glutamine-fructose-6-phosphate transaminase 1. GFPT1 controls the flux of glucose into the hexosamine pathway, and thus the formation of hexosamine products and the availability of precursors for N- and O-linked glycosylation of proteins. The disease gene was discovered by linkage and homozygosity analysis studies of multiplex kinships with a limb-girdle CMS 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 AChE inhibitors.

Among the 13 reported patients, most presented in the first decade, about one-fourth had elevated serum CK levels, some had distal as well as proximal weakness, but very few had ptosis or respiratory muscle involvement. Immunoblots of muscle of affected patients revealed decreased expression of O-N-acetylglucosamine residues on numerous muscle proteins. One patient was shown to have a decreased number of EP AChRs. The effects of

the different mutations on EP fine structure and the extent to which they alter parameters of neuromuscular transmission have not yet been determined [9].

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Fig. 1.

Schematic diagram of an EP with locations of presynaptic, synaptic and postsynaptic CMS disease proteins. Green line, synaptic basal lamina; red line, AChR on crests of the junctional folds; blue line, MuSK and Dok-7 closely associated with AChR. GFPT1, present in all tissues and potentially affecting multiple proteins, is not represented.

Fig. 2. Schematic diagram showing domains of a ColQ strand and components of the asymmetric species of AChE. The indicated mutations appear in each ColQ domain.

Fig. 3. Slow-channel syndrome. Repetitive compound muscle potentials (A) are generated by prolonged AChR channel opening events (B) that generate abnormally prolonged endplate currents (C). The slow-channel MEPC decays biexponentially owing to presence of both wild-type and mutant channels at the endplate. The α V249F mutation is in the second transmembrane domain of AChR. The prolonged endplate currents as well as leakiness of the receptor in the resting state result in Ca^{2+} overloading of the postsynaptic region (D and E) and in an EP myopathy (F). The slow-channel mutations occur in extracellular or transmembrane domains of each AChR subunit. Reproduced from Ref. 43, by permission.

Fig. 4.

Structural features of rapsyn deficient EPs. (A) Small cholinesterase reactive EP regions are dispersed over an extended length of the muscle fiber. These synaptic contacts differ from the compact pretzel shaped contacts at normal EPs. (B) and (C) Multiple small nerve terminals are apposed against a highly simplified postsynaptic regions with no (B) or few (C) junctional folds. In (B), note patchy distribution of AChR, visualized with peroxidase-labeled α -bungarotoxin, on the postsynaptic membrane. Bars 50 μ m in (A) and 1 μ m in (B) and (C). Reproduced from Ref. 71, by permission.

Fig. 5. Dok-7 myasthenia. EPs with pre- and postsynaptic abnormalities. (A) EP region in shows marked degeneration of its junctional folds (asterisks). Schwann cell process (SC) is present amidst relics of the folds. A nerve sprout appears near the top. (B) A highly abnormal EP region devoid of nerve terminal. Some junctional folds are degenerating (asterisk). The subsynaptic sarcoplasm harbors large myeloid structures. A nerve sprouts surrounded by Schwann cell (SC) appears above the junction. Reproduced Ref. 3, by permission.

Table 1

Classification and relative frequency of congenital myasthenic syndromes based on index patients observed at the Mayo Clinic^a

Defect site	Index cases	Relative frequency (%)
<i>Presynaptic</i> (5.9%)		
Choline acetyltransferase	17	5.3
Paucity of synaptic vesicles ^b	1	0.3
Congenital Lambert-Eaton-like syndrome ^b	1	0.3
<i>Synaptic Basal Lamina</i> (13.7%)		
Endplate AChE deficiency	43	13.4
β-2 laminin deficiency	1	0.3
<i>Postsynaptic</i> (68%)		
Primary AChR deficiency with/without kinetic abnormality	109	34
Primary kinetic abnormality with/without AChR deficiency	58	18.1
Rapsyn deficiency	48	15
Plectin deficiency	2	0.6
Na-channel myasthenia	1	0.3
<i>Defects in mechanisms governing endplate development and maintenance</i> (12.5%)		
Dok-7 myasthenia	31	9.7
Glutamine-fructose-6-phosphate transaminase deficiency (GFPT1)	8	2.5
Myasthenic syndrome associated with centronuclear myopathy ^g	1	0.3
Total	321	100

^a Mutations in MuSK [91,93,94] and agrin [87] have been identified in few kinships at other medical centers

^b No gene defect identified.

Table 2

Generic and specific clinical features of the congenital myasthenic syndromes based on patients investigated at the Mayo Clinic

Generic features

Fatigable weakness involving ocular, bulbar, and limb muscles since infancy or early childhood

Similarly affected relative

Decremental EMG response at 2- to 3-Hz stimulation, or abnormal jitter and blocking on single fiber EMG^a

Negative tests for anti-AChR antibodies, MuSK, and P/Q type calcium channels

Exceptions and caveats

In some CMS the onset is delayed

There may be no similarly affected relatives

The symptoms can be episodic

EMG abnormalities may not be present in all muscles, or are present only intermittently

Weakness may not involve cranial muscles

CHRNA7 70insG can occur in combination with autoimmune MG [110]

Anti-AChR antibodies in an autoimmune MG patient conferred slow-channel properties on the receptor [111]

Clinical clues pointing to a specific congenital myasthenic syndrome^b
Endplate acetylcholinesterase deficiency

Repetitive CMAPs

Refractoriness to cholinesterase inhibitors; negative edrophonium test

Delayed pupillary light reflex in some cases

Absence of cholinesterase reactivity from EPs in muscle specimens

Slow-channel myasthenic syndrome

Repetitive CMAPs

Selectively severe involvement of cervical and wrist and finger extensor muscles in most cases

Cranial muscles only mildly affected; slowly progressive course

Worsened by long-term pyridostigmine therapy; little or no response to edrophonium

Dominant inheritance in nearly all cases

Endplate choline acetyltransferase deficiency

Recurrent apneic episodes, spontaneous or with fever, vomiting, or excitement

No or variable myasthenic symptoms between acute episodes

Stimulation at 10 Hz for 5 min causes marked decrease of CMAP followed by slow recovery over 10 min

EMG decrement at 2 Hz can be absent at rest but appears after stimulation at 10 Hz for 5 min, then disappears slowly over 10 min

Rapsyn deficiency

Multiple congenital joint contractures or dysmorphic features in ~30%

Increased weakness and respiratory insufficiency precipitated by intercurrent infections

Ophthalmoparesis in ~25%; strabismus relatively common

Dok-7 myasthenia

Predominantly limb-girdle and axial distribution of weakness, mild facial weakness, and ptosis are common, and normal ocular ductions in most patients

Significant bulbar muscles involvement in some patients

Often worsened by pyridostigmine; responds to ephedrine and albuterol

Can present with stridor and vocal cord paralysis in neonates and infants

GFPT1 (GFAT) myasthenia^c

Tubular aggregates in muscle in most patients

Predominantly limb-girdle and axial distribution of weakness

Responds to pyridostigmine

Laminin-β2 myasthenia

Nephrotic syndrome, ocular abnormalities (Pierson syndrome)

Refractoriness to cholinesterase inhibitors

Plectin deficiency myasthenia

Epidermolysis bullosa simplex

Myasthenic syndrome associated with centronuclear myopathy

Muscle histology

^aSingle fiber EMG has high sensitivity but can also be abnormal in myopathies and some neuropathies.

^bThere are no specific clues to the diagnosis of the fast-channel congenital myasthenic syndrome, primary endplate AChR deficiency, and most cases of rapsyn deficiency.

^cGFPT1, glutamine fructose-6-phosphate transaminase 1.