The congenital myasthenic syndromes (CMS) are a diverse group of genetic disorders caused by abnormal signal transmission at the motor endplate, a special synaptic contact between motor axons and each skeletal muscle fibre. Most CMS stem from molecular defects in the muscle nicotinic acetylcholine receptor, but they can also be caused by mutations in presynaptic proteins, mutations in proteins associated with the synaptic basal lamina, defects in endplate development and maintenance, or defects in protein glycosylation. The specific diagnosis of some CMS can sometimes be reached by phenotypic clues pointing to the mutated gene. In the absence of such clues, exome sequencing is a useful technique for finding the disease gene. Greater understanding of the mechanisms of CMS have been obtained from structural and electrophysiological studies of the endplate, and from biochemical studies. Present therapies for the CMS include cholinergic agonists, long-lived open-channel blockers of the acetylcholine receptor ion channel, and adrenergic agonists. Although most CMS are treatable, caution should be exercised as some drugs that are beneficial in one syndrome can be detrimental in another.

Introduction

The congenital myasthenic syndromes (CMS) are a group of inherited disorders in which neuromuscular transmission is impaired at the motor endplate (a special synaptic contact between motor axons and each skeletal muscle fibre) by one or more specific mechanisms (panel 1, appendix). The CMS have been recognised as clinical entities since the 1970s, after the autoimmune basis of myasthenia gravis and of the Lambert-Eaton myasthenic syndromes were first described. The different CMS were first identified by combined clinical, electrophysiological, and structural studies. Initially, the CMS were classified according to the location of the mutant protein as presynaptic, synaptic basal lamina-associated, or postsynaptic. The study of CMS gained further impetus in the early 1990s when Sanger sequencing was used to identify the gene sequence of the endplate associated proteins.

Since 2011, whole-exome sequencing has accelerated the discovery of novel CMS and, to date, 20 CMS disease genes have been identified (table 1, figure 1). The most common cause of CMS is defects in the acetylcholine receptor; the next most common cause is mutations that affect endplate development and maintenance. However, some CMS have been reported in only single cases.

In this Review we consider the factors that affect neuromuscular transmission at the endplate, the CMS identified to date and their distinguishing features and pathogenesis, and discuss available therapies.

Diagnosis

A generic diagnosis of CMS can be made on the basis of onset at birth to early childhood, fatigable weakness especially affecting the ocular and other cranial muscles, a positive family history, and a decremental electromyographic response, defined as a greater than 10% decrease of the amplitude or area of the fourth, compared to the first, evoked compound motor action potential, or an abnormal single-fibre electromyographic response (panel 2). However, some CMS present later in life, the weakness can affect proximal and torso, rather than cranial, muscles, and the decremental electromyographic response might be detected only after prolonged subtetanic stimulation. Tests for anti-acetylcholine receptor and anti-muscle-specific tyrosine kinase (MuSK) antibodies should be done in sporadic patients after the age of 1 year and in arthrogrypotic
infants, even if the mother has no myasthenic symptoms to exclude autoimmune myasthenia.

The genetic diagnosis of a specific CMS is greatly helped when clinical and electromyographic studies point to a candidate gene (panel 3). If a sufficient number of affected and unaffected relatives are available, linkage analysis can point to a candidate chromosomal locus. This approach works best in consanguineous populations and multiplex families.14

Tests for CMS-causing mutations in previously identified CMS genes are now commercially available, but these are best used in a targeted manner based on specific phenotypes. Whole-exome sequencing has been used to identify novel CMS-causing mutations. This approach captures about 97% of the entire exome but reads only 75% of the exome with more than 20-times coverage. Analysis is improved if DNA from both parents and more than one affected family member is sequenced. Exome sequencing with bioinformatic analysis tools is still expensive, and the putative mutations must be confirmed by Sanger sequencing. Exome sequencing analysis can miss pathogenic non-coding variants and large deletions or duplications that can be identified by array-based comparative genomic hybridisation.1 Also, synonymous variants that can cause exon skipping are often not registered. If a novel CMS disease gene is discovered, in-vitro expression studies can be performed with bioinformatic analysis tools to help understand the pathogenic mechanisms in novel CMS are listed in the appendix.

**Presynaptic syndromes**

**Choline acetyltransferase deficiency**

Choline acetyltransferase (ChAT) catalyses the synthesis of acetylcholine from acetyl coenzyme A and choline in cholinergic neurons. A diagnosis of ChAT deficiency is suggested by sudden episodes of apnoea that can be provoked by stress, but sometimes have no apparent cause and can also occur in patients with few or no myasthenic symptoms. Some patients are apnoic and hypotonic at birth, whereas others are normal at birth and develop apnoic attacks during infancy or childhood.7–11 However, apnoic episodes are not limited to patients with ChAT deficiency, and can also occur in patients with sodium-channel myasthenia1 and with mutations in rapsyn.15,10 In some children with ChAT deficiency, an apnoic attack is followed by ventilatory failure for weeks.16 A few patients never breathe spontaneously, and some develop cerebral atrophy from hypoxaemia.16

A clue to the identity of the disease gene came from the observation that subtenatic stimulation decreased the amplitude of the compound muscle action potential (CMAP)—the sum of several, almost simultaneous, muscle action potentials from several muscle fibres in the same area—and of EPP to 50% below baseline (normal decrease is <30%), followed by slow recovery over 5–10 mins. A marked decrease of the CMAP amplitude after subtetanic stimulation also occurs in other CMS but is followed by recovery in less than 5 mins. The slow recovery implicated a defect in acetylcholine resynthesis and provided the clue to discovery of mutations in ChAT10 (figure 2).

The severity of this CMS has been associated with the position of the mutant residues in the atomic structural model of ChAT, which in experimental models can affect the level of expression, catalytic activity, and structural stability of the enzyme.10 The most severe kinetic abnormalities are caused by mutations in the active-site tunnel of the enzyme, mutations close to the substrate-binding site, or mutations that exert their effect allosterically (figure 2).

<table>
<thead>
<tr>
<th>Classification of the recognised congenital myasthenic syndromes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Index cases</strong></td>
</tr>
<tr>
<td>Presynaptic</td>
</tr>
<tr>
<td>Choline acetyltransferase deficiency</td>
</tr>
<tr>
<td>Paucity of synaptic vesicles and reduced quantal release‡</td>
</tr>
<tr>
<td>SNAP258 deficiency</td>
</tr>
<tr>
<td>Synaptotagmin 2 deficiency‡</td>
</tr>
<tr>
<td>Synaptic basal lamina-associated</td>
</tr>
<tr>
<td>Endplate acetylcholinesterase deficiency</td>
</tr>
<tr>
<td>Laminin-β2 deficiency</td>
</tr>
<tr>
<td>Defects in acetylcholine receptor</td>
</tr>
<tr>
<td>Primary acetylcholine receptor deficiency</td>
</tr>
<tr>
<td>Kinetic defects in the acetylcholine receptor</td>
</tr>
<tr>
<td>Slow-channel syndrome</td>
</tr>
<tr>
<td>Fast-channel syndrome</td>
</tr>
<tr>
<td>Defects in endplate development and maintenance</td>
</tr>
<tr>
<td>Agrin deficiency</td>
</tr>
<tr>
<td>LRP4 myasthenia</td>
</tr>
<tr>
<td>MuSK deficiency</td>
</tr>
<tr>
<td>Dok-7 myasthenia</td>
</tr>
<tr>
<td>Rapsyn deficiency</td>
</tr>
<tr>
<td>Congenital defect of glycosylation</td>
</tr>
<tr>
<td>GFT1 myasthenia</td>
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<tr>
<td>DPAGT1 myasthenia</td>
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</tbody>
</table>
| ALG2† and ALG14† | 0 | 0%
| Other myasthenic syndromes | 1.4% |
| PREPL deletion syndrome | 1 | 0.3% |
| Na-channel myasthenia | 1 | 0.3% |
| Plectin deficiency | 2 | 0.6% |
| Myasthenic associated with centronuclear myopathies | 1 | 0.3% |
| Myasthenic symptoms associated with defects in the mitochondrial citrate carrier | 0 | 0%

Classification based on a cohort of patients with CMS investigated at the Mayo Clinic between 1988 and 2014. In another series of 680 patients with CMS, the frequencies of mutations in choline acetyltransferase, GFT, acetylcholine receptor subunits, rapsyn, and Dok7 were similar to those investigated at the Mayo Clinic. CMS=congenital myasthenic syndromes. ‡No gene defect identified. †Defects in ALG2 and ALG14 and in the mitochondrial citrate synthesis carrier were identified at other medical centres.

Table 1: Classification of the recognised congenital myasthenic syndromes
These mutations compromise the safety margin of neuromuscular transmission through incomplete filling of the synaptic vesicles with acetylcholine.

Parents of children with acute apnoeic attacks should be instructed in the use of a portable respirator and an apnoea monitor at home.7

SNAP25B deficiency
SNAP25B is one of the three SNARE proteins essential for initiation of synaptic vesicle exocytosis at the endplate and the central synapse. SNAP25B deficiency was reported in an African-American patient who was hypomotile in utero and stiff and cyanotic at birth, requiring oxygen therapy, and had multiple joint contractures. She learned to walk with a walker after age 7 years. She has periods of staring and unresponsiveness, her EEG showing generalized spike and wave discharges that did not change during the staring spells. At age 11 years, she has fatigable weakness, flaccid and ataxic gait, spastic and ataxic dysarthria, and her development is at the level of a child aged 3–4 years.

She harbours a de-novo dominant–negative Ile67Asn mutation in SNAP25B. In-vitro endplate studies show a reduced number of acetylcholine-containing quanta released from the nerve terminal by each nerve impulse, and a reduced miniature endplate potential (MEPP) frequency. Therapy with amifampridine improved the patient’s strength but not her ataxia.16

Synaptotagmin 2 deficiency
Synaptotagmin 2 is a synaptic vesicle-associated calcium sensor. Dominant mutations in the calcium-binding domain of synaptotagmin 2 reported in two kinships were associated with childhood-onset foot deformities, fatigable muscle weakness, reduced-to-absent tendon reflexes, and low amplitude compound motor action potentials with post-exercise facilitation.17

Synaptic basal lamina-associated syndromes
Endplate acetylcholinesterase deficiency due to mutations in COLQ
The isoform of acetylcholinesterase at the endplate is an asymmetric enzyme composed of one, two, or three homotetramers of globular catalytic subunits anchored to the synaptic basal lamina by ColQ, a triple-stranded collagenic tail (figure 3).18,19 Mutations in COLQ result in prolonged synaptic currents and action potentials owing to the extended residence of acetylcholine in the synaptic space.20 The nerve terminals are abnormally small, which curtails the number of quanta available for release, and some junctional folds are degenerating because the prolonged synaptic currents allow excessive Ca2+ ingress into the junctional folds.20,21 Mutations have been identified in each of the three ColQ domains (figure 3).21–23 Mutations in the N-terminal domain prevent the collagen domain from associating with the catalytic subunits; mutations in the collagen domain...
 DEVELOPMENT AMPLITUDE WAS ATTRIBUTED TO THE PAUCITY OF DECREASED QUANTAL RELEASE. THE REDUCED MINIATURE EPP IS ACCOUNTED FOR BY THE NEUROTRANSMISSION BEING COMPROMISED BY THE DEFICIENCY OF ACETYLCHOLINESTERASE, WHICH RESULTS IN THE SMALL SIZE OF NERVE TERMINALS, SIMILAR TO ENDPLATE MINIATURES EVOKED BY NERVE STIMULATION. THE NEUROTRANSMISSION IS ALSO IMPAIRED BY THE DEFICIENCY OF THE INTRACELLULAR PROTEIN WITH THE SUBUNIT COMPOSITION αβδε, WHICH RESULTS IN THE ABSENCE OF THE acetylcholine binding pockets located at the α/ε (or α/γ) and α/δ subunit interfaces (appendix p 6). RESIDUES FROM SEVEN DISTINCT REGIONS OF THE PRIMARY SEQUENCES OF EACH SUBUNIT CONVERGE TO FORM THE acetylcholine binding pocket: three regions originate from the α subunit and four from the δ, ε, or γ subunits. These regions are highly conserved across species; for an adult human acetylcholine receptor the binding affinities differ by roughly five-fold, with the α/δ site showing high affinity and the α/ε site showing low affinity. The mechanisms of acetylcholine receptor activation are described and discussed in the appendix.

**Laminin-β2 deficiency**

Laminin β2, encoded by **LAMB2**, is highly expressed in the basal lamina of the eye and kidney, and at the endplate. At the endplate, laminin β2 is involved in the alignment of the nerve terminal with the postsynaptic region and differentiation of the presynaptic region. Only one report describes this CMS. A 20-year-old woman had renal and ocular malformations (Pierson syndrome) and a CMS due to two frameshift mutations in **LAMB2**. Neuromuscular transmission was compromised by reduced quantal release evoked by nerve stimulation. The nerves were small and similar to endplate miniature action potentials, which accounts for the decreased quantal release. The reduced miniature EPP amplitude was attributed to the paucity and poor development of the junctional folds.

**Defects in the acetylcholine receptor**

**Muscle nicotinic acetylcholine receptor**

Most CMS stem from molecular defects in the muscle nicotinic acetylcholine receptor, an integral membrane protein with the subunit composition αβε at the adult endplate or αβδε at the foetal endplate and extrajunctional regions. The genes encoding the α (**CHRNA1**), δ (**CHRND**), and γ (**CHRNG**) subunits are at different loci on chromosome 2q, and those encoding the β (**CHRNA2** and **CHRNA2** and **CHRNA4** and **CHRNA5**) subunit pairs converge to form the acetylcholine binding pocket: three regions originate from the α subunit and four from the δ, ε, or γ subunits. Because each acetylcholine-binding site contains different α subunits, the rates of acetylcholine association and dissociation differ at each site, resulting in distinct agonist binding affinities. The extent to which the binding affinities differ depends on the species; for an adult human acetylcholine receptor the affinities differ by roughly five-fold, with the α/ε site showing high affinity and the α/δ site showing low affinity. The mechanisms of acetylcholine receptor activation are described and discussed in the appendix.

**Primary acetylcholine receptor deficiency**

These CMS can result from recessive missense, nonsense, or splice site and promoter region mutations in any of the acetylcholine receptor subunits, but most occur in the ε subunit (figure 4). The high frequency of mutations in the ε subunit compared with other subunits has been attributed to phenotypic rescue by substitution of the foetal γ subunit for the defective ε subunit. Despite this phenotypic rescue, the endplate acetylcholine receptor complement is decreased to about 10% of normal, and the synaptic response to acetylcholine,}

www.thelancet.com/neurology Vol 14 April 2015 423
reflected by the low amplitude of the miniature EPP and miniature endplate current, is markedly decreased. Evoked quantal release is increased and partly compensates for the acetylcholine receptor deficiency. Single-channel patch-clamp recordings from patient endplates reveal low-amplitude, long-duration channel openings, which are typical of the fetal acetylcholine receptor. The synaptic contacts consist of many small regions over an extended length of the fibre surface, but the structural integrity of the presynaptic and postsynaptic regions is preserved.

Nearly all patients have eyelid ptosis, oculoparesis that often becomes fixed, and variable but frequently moderate-to-severe limb muscle weakness. Most patients with low-expressor mutations in the ε subunit respond to cholinergic agonists and some derive additional benefit from salbutamol. Individuals harbouring null mutations in both alleles of CHRNA1, CHRNB, or CHRND cannot be rescued because no substituting subunits exist and hence these individuals probably die in utero. Patients with heterozygous or homozygous low-expressor mutations in the non-ε subunits are severely affected and have high mortality in infancy or early childhood.

Kinetic defects in acetylcholine receptor

Slow-channel syndrome

This syndrome is caused by dominant mutations in the ligand-binding or pore domains of the acetylcholine receptor (appendix) that result in prolonged synaptic currents and action potentials. The reported mutations in the ligand-binding domain enhance affinity for acetylcholine and slow its dissociation rate from the doubly-liganded receptor, whereas most mutations in the pore domain enhance receptor activation after agonist binding. Both mechanisms prolong the single-channel currents (appendix p 7), endplate currents, and EPPs, while mutations in the pore domain also cause spontaneous channel openings. Similar to endplate acetylcholinesterase deficiency, the prolonged EPP triggers a repetitive compound motor action potential (appendix p 7), that is enhanced by edrophonium. Also,
during repetitive stimulation, successive prolonged EPPs progressively depolarise the postsynaptic membrane, causing a depolarisation block. The prolonged and spontaneous openings of the acetylcholine receptor channel result in Ca²⁺ accumulation in the postsynaptic region, leading to endplate myopathy (appendix p 7). The safety margin of neuromuscular transmission is compromised by the depolarisation block of the action potential, desensitisation of the acetylcholine receptors at physiological rates of neuromuscular activity, and by endplate myopathy that causes loss of acetylcholine receptor from the junctional folds and widens the synaptic space allowing for acetylcholine loss by diffusion.

The slow-channel syndrome usually presents in the first decade of life, but severely affected patients present in the neonatal period. There is severe involvement of the cervical, scapular, and dorsal forearm muscles. The ocular muscles are usually spared. Some patients have mild, asymmetric ptosis.

Fast-channel syndrome
This syndrome is caused by a recessive mutation in one allele of an acetylcholine receptor subunit, accompanied by a null or low-expressor mutation, or rarely by another fast-channel mutation, on the other allele (appendix). This is the physiological opposite of the slow-channel syndrome in that the endplate currents decay abnormally fast, the channel openings are abnormally brief and the amplitude of the synaptic currents and potentials is reduced, owing to a decreased probability that the acetylcholine receptor channel is opened by physiological concentrations of acetylcholine (appendix p 8).

The causative mutations are in different domains and subunits of acetylcholine receptor (appendix p 8) and exert their effects by different mechanisms. In the extracellular domains, εPro121Leu and εTrp55Arg decrease affinity for acetylcholine, whereas αVal132Leu, αVal188Met, and δLeu42Pro decouple agonist binding from channel opening. In the third transmembrane domain, αVal285Ile decreases gating efficiency. In the long cytoplasmic loop of the ε subunit, Asn436del shortens channel openings by reducing the stability of the diliganded receptor, and c.1254ins18 and Ala411Pro both in the long cytoplasmic loop, produce a wide range of channel-gating kinetics resulting in an increased proportion of brief channel open times. Some mutations are pathogenic through a combination of these factors. 41,42,48

Figure 3: Endplate acetylcholinesterase deficiency due to mutations in ColQ, the triple-stranded collagenic tail that anchors acetylcholinesterase to the synaptic basal lamina

(A) The three domains of a ColQ strand with 18 identified mutations. Conserved domains of ColQ include an N-terminal proline-rich attachment domain (PRAD) that associates each ColQ strand with an acetylcholinesterase tetramer, a central collagen domain, and a C-terminal region needed for assembly of the ColQ strands in a triple helix. ColQ is anchored in the synaptic space by binding to the extracellular domain of muscle-specific kinase (MuSK) and by its cationic residues binding to perlecan. (B) Asymmetrical isoform of acetylcholinesterase. Each ColQ strand associates with four catalytic subunits of acetylcholinesterase (red circles). PRAD=proline-rich attachment domain. HSPBD=heparan sulfate proteoglycan binding domain. Cationic residues of this domain help anchoring ColQ by electrostatic interaction with anionic residues in the synaptic basal lamina. Electron cytochemical localisation of acetylcholinesterase in a healthy control participant (C) and in an acetylcholinesterase-deficient patient (D), in whom there is no reaction for acetylcholinesterase at the endplate.

www.thelancet.com/neurology  Vol 14   April 2015 425

1 μm
Defects in endplate development and maintenance

To date, mutations in genes that encode proteins essential for endplate development and maintenance, such as MUSK, AGRN, LRP4, DOK7, and RAPSN, have been detected with CMS. The products of these genes are components of a signalling network essential for endplate development and maintenance. Agrin, the product of AGRN, is secreted into the synaptic space by the nerve terminal and binds to the lipoprotein-related protein LRP4 in the postsynaptic membrane. The agrin–LRP4 complex binds to and activates the receptor tyrosine kinase MuSK. This binding enhances MuSK phosphorylation and leads to clustering of LRP4 with MuSK. Activated MuSK phosphorylates Dok-7; this leads to recruitment of two adaptor proteins, Crk and CrkL, that serve as downstream activators of Dok-7. Full activation of MuSK results in activation of rapsyn, which induces rapsyn to concentrate acetylcholine receptor on the postsynaptic membrane, enhances synapse-specific gene expression in postsynaptic nuclei, and promotes postsynaptic differentiation. Clustered LRP4, in turn, promotes differentiation of motor axons. The agrin–LRP4–MuSK–Dok-7 signalling system is also essential for maintaining the structure of the adult neuromuscular junction.84

Agrin deficiency

Three reports describe a CMS caused by defects in agrin. In one report, a 42-year-old woman and her 36-year-old brother had had eyelid ptosis and mild weakness of the facial and limb-girdle muscles since early childhood.90 They carry a homozygous missense mutation (Gly709Arg) in the laminin G-like 2 domain needed for MuSK activation. The endplates showed no decrease of acetylcholine or agrin expression, but were misshapen or regenerating. Some postsynaptic regions were denuded of their nerve terminal but the ultrastructure of the junctional folds was preserved. In vitro expression studies revealed no effect on MuSK activation by mutant agrin; therefore, the Gly709Arg mutation perturbs endplate maintenance without preventing postsynaptic differentiation. The second report describes a severe CMS in a 39-year-old man caused by two heteroallelic mutations: Gln353X in the N-terminal region and Val1727Phe located in the second laminin G-like domain of agrin.91 In vitro, Gln353X abolished agrin expression and Val1727Phe substantially reduced agrin-induced acetylcholine receptor clustering in C2 muscle cells. The synaptic contacts were dispersed and fragmented, the postsynaptic junctional folds were sparse and poorly developed, the nerve terminals were small, and the junctional plasma had degenerative changes.

A third report is of five patients in three kinships with a distinct phenotype associated with wasting that first affected the lower and later the upper limbs, with fat
replacing the posterior leg compartments, sparing of the axial and cranial muscles, and slow progression of weakness.\textsuperscript{59–61} The age at onset ranged from birth to early adulthood. In two siblings in the first kinship there was evidence of a Glu1871Arg mutation in the C-terminal region of \textit{AGRN}. The one patient in the third kinship had homozygous for a Glu1871Arg mutation in the C-terminal region of \textit{AGRN}. Thus, in each case the phenotype was determined by a single missense mutation. Acetylcholine receptor expression at the end-plates was not noticeably reduced, but neuromuscular transmission was not examined in any patient.

**LRP4 deficiency**

One study\textsuperscript{53} identified heteroallelic Glu1233Lys and Arg1277His mutations at the edge of the third beta-propeller domain of LRP4 in a 14-year-old girl. Both mutations decrease binding affinity of LRP4 for both MuSK and agrin. The patient had a respiratory arrest at birth, delayed motor development, and fatigable weakness of the limb-girdle muscles.

**MuSK deficiency**

In the past decade, five reports have described CMS caused by mutations in \textit{MUSK} (figure 5). MuSK deficiency presents at birth or early life with eyelid ptosis or respiratory distress.\textsuperscript{44–46} Subsequently, it affects the ocular, facial, and proximal limb muscles, and in some kinships the bulbar muscles as well. Structural studies in human beings and transgenic mice\textsuperscript{59} show extensive remodelling of the endplates due to recurrent cycles of focal denervation and reinnervation. No clear genotype–phenotype correlations have been observed.

**Dok-7 deficiency**

The in-vitro observation that Dok-7 is a muscle-intrinsic activator of MuSK\textsuperscript{60} prompted a search for CMS caused by mutations in \textit{DOK7}. Many mutations were detected in \textit{DOK7},\textsuperscript{61–65} but the frameshifting c.1124_1127dupTGCC in exon 7 emerged as a common variant. Other mutations prevent Dok-7 from associating with and becoming activated by Crk1 and Crk1L. The clinical course of Dok-7 deficiency can be from mild to severe. Although no consistent phenotype–genotype correlations have been detected, all patients have limb-girdle weakness, with lesser facial and cervical muscle involvement;\textsuperscript{66} a few have severe bulbar weakness and significant oculoparesis.\textsuperscript{67} Some were initially misdiagnosed as having limb-girdle muscular dystrophy. Type 1 fibre preponderance, type 2 fibre atrophy, mild myopathic changes, and presence of target formations are associated features. The endplates in all patients consist of one to many small synaptic contacts. Impaired maintenance of the endplates is evidenced by ongoing destruction and remodelling (figure 6). Neuromuscular transmission is compromised by the decreased quantal content of the EPP and the reduced miniature EPP amplitude.\textsuperscript{68}

**Rapsyn deficiency**

Rapsyn concentrates and anchors the acetylcholine receptor in the postsynaptic membrane\textsuperscript{69} and is needed for the development of the junctional folds.\textsuperscript{70} Distinct regions of rapsyn have different functions: the myristoylated N-terminal region links rapsyn to the postsynaptic membrane; seven tetratricopeptide (TPR1–7) repeats are important for rapsyn self-aggregation and to bind to the cytoplasmic domain of MuSK; a coiled-coil domain interacts with long-cytoplasmic loops of the acetylcholine receptor subunits; and a C-terminal domain binds to β dystroglycan, which links rapsyn to the actin cytoskeleton\textsuperscript{71,72} (figure 7). Cryoelectron tomography studies show that rapsyn and the acetylcholine receptor form a complex, with up to three rapsyn dimers contacting the cytoplasmic domain of each acetylcholine receptor.\textsuperscript{73}

Most patients with rapsyn deficiency present in the first year of life, with a few presenting in childhood or adult life.\textsuperscript{11} Arthrogryposis at birth and other congenital malformations occur in nearly a third of patients.\textsuperscript{12,13,70} Intercurrent infections or fever can trigger respiratory crises, resulting in anoxic encephalopathy.\textsuperscript{12,13,70} The
clinical features can suggest autoimmune myasthenia gravis but the ocular ductions are intact in most patients.13,73 Many synaptic contacts are present on individual muscle fibres (figure 7). The endplate acetylcholine receptor deficiency is milder than in primary acetylcholine receptor deficiency,70 but the junctional folds are not well differentiated (figure 7).

Patients of European or Indian subcontinent origin harbour a common Asn88Lys mutation in RAPSN.73 Other mutations have been detected in the promoter region or throughout the entire open reading frame of RAPSN. Different mutations, hinder rapsyn self-association or binding to acetylcholine receptor, impede agrin–MuSK–LRP4-mediated clustering of acetylcholine receptor, or decrease rapsyn expression.12,74 There are no genotype–phenotype correlations except that oriental Jewish patients with a homozygous E-box mutation (c.38A>G) have a mild phenotype with ptosis, prognathism, severe masticatory and facial muscle weakness, and hypernasal speech.75

**Congenital defects of glycosylation**

Glycosylation increases solubility, folding, stability, assembly, and intracellular transport of nascent peptides. O-glycosylation occurs in the Golgi apparatus, with addition of sugar residues to the hydroxyl groups of serine and threonine residues; N-glycosylation occurs in the endoplasmic reticulum in sequential reactions that link the amino group of asparagine residues with a core glycan composed of two glucose, nine mannose, and two N-acetylglucosamine (GlcNAc) moieties.76,77 Defects in four enzymes that catalyse glycosylation have been found to cause CMS: GFPT1 (glutamine fructose-6-phosphate transaminase),4,78 DPAGT1 (dolichyl-phosphate [UDP-N-acetylglucosamine] N-acetylglucosaminylphosphotransferase 1),79,80 ALG2 (alpha-1,3-mannosyl transferase), and ALG14 (UDP-N-acetylglucosaminyltransferase subunit).81 Tubular aggregates of the sarcoplasmic reticulum seen with microscopy of muscle fibres are a phenotypic clue to the diagnosis, but these are not present in all patients. Because glycosylated proteins are present at all endplate sites, neuromuscular transmission is compromised by the combination of presynaptic and postsynaptic defects.

**Defects in GFPT1**

GFPT1 regulates the entry of glucose into the hexosamine pathway, and hence the formation of precursors for N-linked and O-linked protein glycosylation. CMS caused by defects in GFPT1, causing limb-girdle muscle weakness that was responsive to pyridostigmine, was reported in 16 patients in 2011.4 A subsequent study of 11 patients revealed slow progressive weakness in ten patients; one patient with mutations disrupting the muscle-specific exon of GFPT1 never moved in utero, was arthrogrypotic at birth, and was bedridden and tube-fed at age 8 years. This patient has a severe myopathy with numerous diluted and degenerating membrane bound vesicles, autophagic vacuoles, and apoptotic nuclei identified by electron microscopy.78 Muscle specimens from six of nine patients with less severe forms of the disease showed tubular aggregates of the sarcoplasmic reticulum. Electron

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**Figure 7:** Congenital myasthenic syndrome due to rapsyn deficiency

Rapsyn concentrates and anchors the acetylcholine receptor in the postsynaptic membrane and is needed for the development of the junctional folds. (A) Rapsyn domains. The myristoylated N-terminal region links rapsyn to the postsynaptic membrane. Seven tetrapeptide repeat (TPR1–7) repeats are important for rapsyn self-aggregation and to bind to the cytoplasmic domain of muscle-specific kinase (MuSK). The coiled-coil domain interacts with long-cytoplasmic loops of the acetylcholine receptor subunits. The C-terminal domain binds to β dystroglycan, which links rapsyn to the actin cytoskeleton. (B) Small cholinesterase reactive endplate regions (labelled by enzyme histochemistry) are distributed over an extended length of the muscle fibre in patients with rapsyn deficiency compared with the normal pretzel-shaped endplate. (C,D) Many small nerve terminals are seen over poorly developed junctional folds. In (C), the distribution of acetylcholine receptor on the postsynaptic membrane, visualised with peroxidase-labeled α-bungarotoxin, is patchy (red asterisk). Reproduced from reference 70 by permission of Lippincott, Williams and Wilkins. AcHR=acetylcholine receptor. β-DG=β dystroglycan.
microscopy showed abnormally small endplate regions and poorly developed junctional folds. In-vitro electrophysiology studies showed a decreased synaptic response to acetylcholine and decreased acetylcholine release in the most severely affected patient. The observation that many endplates in this syndrome are underdeveloped implicates hypoglycosylation and altered function of endplate-associated glycoproteins, such as MuSK, agrin, and dystroglycans.

**Defects in DPAGT1**

DPAGT1 catalyses the first step of N-linked protein glycosylation. DPAGT1 deficiency results in impaired N-linked glycosylation of many proteins distributed throughout the body, but in the first five patients to be reported with CMS harbouring DPAGT1 mutations, only neuromuscular transmission was adversely affected. This was speculated to be due to decreased acetylcholine receptor expression at the endplate, although the patient endplates were not analysed.

Another study of two siblings and a third patient with DPAGT1 deficiency extended the phenotypic spectrum of the disease. These patients have moderately severe weakness and intellectual disability. The siblings responded poorly to pyridostigmine and amifampridine; the third patient responded partly to pyridostigmine and edrophonium injection increased her strength transiently, in many skipped and few non-skipped alleles and reduced expression of the wild-type protein. Intercostal muscle weakness and intellectual disability. The siblings harboured a Met1Leu mutation that abolished enzyme activity and a synonymous Leu120Leu mutation, which decreased DPAGT1 activity. The third patient carried a Val264Met mutation that abolishes the synthetic activity and a synonymous Leu120Leu mutation that profoundly augments exon skipping, resulting in many skipped and few non-skipped alleles and reduced expression of the wild-type protein. Intercostal muscle studies in these patients showed fibre type disproportion with the mean diameter of the type 1 fibres less than 50% of the diameter of type 2 fibres, small tubular aggregates, and autophagic vacuolar myopathy. Electron microscopy showed that 6 of the 62 imaged endplate regions were degenerating and most endplates had small presynaptic and postsynaptic regions. Evoked acetylcholine release, postsynaptic response to acetylcholine, and endplate acetylcholine receptor concentration were all decreased to about 50% of normal. Immunoblots of muscle extracts with two antibodies detecting glycosylated, non-specific proteins showed decreased or absent glycosylation of several proteins, including STIM1, a sarcoplasmic reticulum-associated calcium sensor that operates in concert with the calcium release-activated, calcium channel protein ORAI1 on the plasma membrane to homoeostatically regulate the sarcoplasmic reticulum calcium concentration. Because mutations in STIM1 cause a tubular aggregate myopathy, STIM1 hypoglycosylation might be a cause of the tubular aggregates in muscle in N-glycosylation disorders.

**Defects in ALG2 and ALG14**

ALG2 catalyses the second and third committed steps of N-glycosylation. ALG14 forms a multiglycosyltransferase complex with ALG13 and DPAGT1 and thus contributes to the first committed step of N-glycosylation. In one family, four affected siblings presenting with myasthenic symptoms in early childhood were homozygous for an insertion-deletion mutation. In another family, two sisters with myasthenic symptoms since around age 7 years carried heteroallelic Pro65Leu and Val68Gly mutations in ALG14. Another unrelated 4-year-old boy was homozygous for a low-expressor Val68Gly mutation. Endplate ultrastructure and parameters of neuromuscular transmission were not investigated in this study.

**Other myasthenic syndromes**

**PREPL deletion syndrome**

The hypotonia-cystinuria syndrome is caused by recessive deletions in both SLC3A1 and PREPL on chromosome 2p21. The major clinical features are type A cystinuria, growth hormone deficiency, muscle weakness, ptosis, and poor feeding. Isolated PREPL deficiency has not been investigated in detail. A female patient presented at birth with marked hypotonia, eyelid ptosis and facial and bulbar muscle weakness. She and her mother harboured a heterozygous deletion at the 2p21 chromosomal locus involving the SLC3A1 and PREPL genes suggesting the cystinuria—hypotonia syndrome, but she had no cystinuria. Her father carried a heterozygous nonsense mutation in PREPL; hence her phenotype was determined by the paternal mutation. An edrophonium injection increased her strength transiently and she responded to pyridostigmine during infancy. PREPL expression was absent from the patient’s muscle and endplates. Further endplate studies revealed decreased evoked acetylcholine release and small miniature EPPs, despite normal endplate acetylcholine receptor expression. Because PREPL is an essential activator of the clathrin-associated adaptor protein 1 (AP1), which is needed for the vesicular acetylcholine transporter to fill the synaptic vesicles with acetylcholine, the reduced miniature EPP is attributed to a decreased vesicular content of acetylcholine. The cause of the reduced evoked quantal release remains undefined.

**Plectin deficiency**

Organelle and tissue-specific isoforms of plectin, encoded by PLEC, crosslink intermediate filaments to their targets in different tissues and thereby provide cytoskeletal support. Defects in plectin can cause epidermolysis bullosa simplex, muscular dystrophy, and a myasthenic syndrome. The muscular dystrophy is caused by loss of cytoskeletal support of the muscle fibre organelles, which become dislocated within the fibre, and of the sarcolemma, resulting in many small defects that allow calcium ingress into the fibres. The two patients investigated by us harbour Glyn2057X and Arg2319X mutations, respectively.
and a common c.12043dupG mutation. Both patients had epidermolysis bullosa simplex since infancy and later developed a progressive myopathy, a CMS that was refractory to pyridostigmine, a decremental electromyographic response on repetitive nerve stimulation, and half-normal amplitude of miniature EPPs, attributed to degeneration of the junctional folds with loss of acetylcholine receptor and altered endplate geometry.

**Defects in sodium channels**
This CMS was detected in a 20-year-old woman who had brief and abrupt attacks of muscle weakness and respiratory arrest since birth that caused an anoxic encephalopathy. Evoked quantal release from the nerve terminal and the synaptic response to the released quanta were normal, but normal amplitude EPP failed to generate muscle action potentials, pointing to a defect in action potential generation. Mutation analysis of SCN4A, the gene encoding the Na,1.4 sodium channel, revealed two mutations (Ser246Leu in the S4/S5 linker in domain I and Val1442Glu in S4/S5 linker in domain IV). Na,1.4 expression at the endplates was normal. Expression studies of the Val1442Glu sodium channels in HEK cells revealed abnormally rapid decay of the Na+ current in response to short depolarizations near the resting membrane potential (fast inactivation) and enhanced decay of the Na+ current on high frequency stimulation for seconds or minutes (use-dependent inactivation). The Ser246Leu mutation had only minor kinetic effects and is probably a benign mutation. The safety margin of neuromuscular transmission is compromised because most Na,1.4 channels are inexcitable in the resting state.

**Myasthenias associated with congenital myopathies**
Eyelid ptosis, ophthalmoparesis, weakness of facial muscles, exercise intolerance, decremental electromyographic response, and response to pyridostigmine have been documented in some patients with centronuclear myopathies caused by mutations in amphiphysin (BIN1), myotubularin (MTM1), dynamin 2 (DNM2), and in other patients with centronuclear myopathies with no identified mutations. Knockdown of MTM1 or DNM2 in zebrafish causes decreased spontaneous and touch-evoked movements that are dramatically increased in response to edrophonium. Detailed investigation of neuromuscular transmission in a 39-year-old man with centronuclear myopathy with myasthenic symptoms, but no identified mutations, showed endplate remodelling, mild endplate acetylcholine receptor deficiency, simplified postsynaptic regions, a 60% reduction of the miniature EPP amplitude, and a 40% decrease of evoked acetylcholine release attributed to a decreased number of synaptic vesicles. Two patients, a 19-year-old woman and a 7-year-old boy with a congenital myopathy caused by tropomyosin 3 deficiency, had mild abnormalities on single fibre EMG suggesting a defect of neuromuscular transmission. Finally, two siblings, aged 9 and 7 years, harboring mutations in RYR1 (encoding ryanodine receptor 1) had fatigable ptosis as well as fatigable facial and general weakness suggesting myasthenia since infancy. Pyridostigmine therapy initially improved their symptoms but the response was unsustainable. Muscle specimens showed atrophy of type 1 and, to a lesser extent, of type 2 fibers, with occasional loss of mitochondria and myofilibrillar degeneration in central fiber regions. EMG studies revealed no defect of neuromuscular transmission.

**Myasthenic symptoms associated with defects in the mitochondrial citrate carrier**
The mitochondrial protein SLC25A1 mediates the exchange of mitochondrial citrate/isocitrate with cytosolic maleate, which is cleaved to form acetyl-CoA and oxaloacetate by ATP-citrate lyase. Mutations of SLC25A1 were shown to interfere with brain, eye and psychomotor development. Exome sequencing of two siblings with CMS and intellectual disability born to consanguineous parents revealed homozygous Arg247Gln mutation in SLC25A1. Molecular studies indicated the mutation impairs the transport activity of the enzyme, and knockdown of SLC25A1 orthologues in zebrafish hindered motor axons from innervating muscle fibres. A third patient, an 18-month-old girl, harboring heteroallelic Arg282His and Gly130Asp mutations in SLC25A1, also had myasthenic symptoms as well as hypoplastic optical nerves, agenesia of the corpus callosum, and 2-hydroxyglutaric aciduria.

**Available therapies**
Current therapies for CMS include cholinergic agonists, namely pyridostigmine and amifampridine, long-lived open-channel blockers of the acetylcholine receptor ion channel such as fluoxetine and quinidine, and adrenergic agonists, such as salbutamol and ephedrine. Pyridostigmine acts by inhibiting acetylcholinesterase in the synaptic basal lamina, which increases the number of acetylcholine receptors activated by a single quantum. Amifampridine increases the number of acetylcholine quanta released by each nerve impulse. Alone, and especially in combination, these agonists increase the amplitude of the endplate potential and thereby meet the safety margin of neuromuscular transmission. Therefore, they are beneficial in patients with endplate acetylcholine-receptor deficiency and those with fast-channel syndromes, in which the safety margin of transmission is compromised by a decreased synaptic response to acetylcholine and an abnormally fast decay of the synaptic current. Fluoxetine and quinidine are long-lived open-channel blockers of the acetylcholine receptor used in the treatment of the slow-channel syndrome. By shortening the duration of the prolonged synaptic currents, they prevent a depolarisation block and desensitisation of acetylcholine receptor at physiological rates of stimulation and mitigate the cationic overloading.
of the postsynaptic region that causes degeneration of the junctional folds and alters the endplate geometry. Salbutamol and ephedrine were empirically effective in CMS caused by mutations in the ColQ component of acetylcholinesterase, Dok-7, and laminin β2, and in some patients harbouring low-expressor mutations of the acetylcholine receptor. The mechanisms by which these open-channel-blockers improve neuromuscular transmission are not understood.

Drugs that benefit one type of CMS can be ineffective or harmful in another type. For example, patients harbouring low-expressor or fast-channel mutations in acetylcholine receptors show improvement with cholinergic agonists, whereas the condition of patients with slow-channel mutations in acetylcholine receptors deteriorates on these drugs. Patients harbouring mutations in Dok-7 get rapidly worse with treatment with cholinergic agonists but improve with treatment with adrenergic agonists. Therefore a molecular diagnosis is essential to inform the choice of therapy. Finally, the cholinergic agonists pyridostigmine and amifampridine exert their effect as soon as the drug is absorbed, whereas the adrenergic agonists and the acetylcholine-receptor-channel blockers act more slowly over days, weeks, or months. Table 2 summarises the pharmacotherapy of the recognised CMS.

Conclusions and future directions
In the absence of a clear cause for many CMS, identification of their molecular bases with in-depth clinical evaluation combined with electrophysiological and morphometric analyses of the patient endplates, is essential. With the introduction of gene cloning and the sequencing of complementary DNAs, the candidate gene approach has provided a powerful complement to these analyses. When a variant in a candidate gene is identified, the goal is to understand its pathogenicity. This could be achieved by incorporating the wild type and mutant genes into a heterologous expression system and then assessing the level of expression of the mutant protein and its functional properties by methods such as patch-clamp studies, to dissect the kinetic effects of acetylcholine receptor mutants, and enzyme assays of the wild type and mutant species of ChAT, DPAGT1, and acetylcholinesterase. These approaches have demonstrated that the CMS are caused by a diversity of disease targets and molecular mechanisms, which together guide individualised therapy.

Despite the power of the candidate gene approach, in some CMS the disease gene has remained elusive. For these CMS, whole-exome sequencing, which has become available in the past 3 years, proved to be a lodestone to discovery of unsuspected defects in genes encoding proteins involved in protein glycosylation. The power of this approach is enhanced if many family members, and especially trios, are analysed, and interpretation of the results is greatly helped by phenotypic clues. Although whole-exome sequencing fails to identify large scale duplications or deletions, these can now be detected by microarray-based comparative gene hybridisation. A future approach will be the use of custom-made microarrays designed to detect mutations in previously identified CMS disease genes. A drawback of this method

### Table 2: Pharmacotherapy of the congenital myasthenic syndromes

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Therapy</th>
<th>Caveats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choline acetyltransferase deficiency</td>
<td>Pyridostigmine; parenteral neostigmine methyl sulphate for acute apnoic episodes</td>
<td>Treatment must be continued even in asymptomatic patients. The most severely affected patients do not respond to any cholinergic drugs and depend on permanent respiratory support.</td>
</tr>
<tr>
<td>SNAP25β deficiency</td>
<td>Amifampridine</td>
<td>—</td>
</tr>
<tr>
<td>Synaptotagmin 2 deficiency</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Acetylcholinesterase deficiency</td>
<td>Salbutamol or ephedrine</td>
<td>Avoid pyridostigmine and amifampridine</td>
</tr>
<tr>
<td>Laminin-β2 deficiency</td>
<td>Ephedrine</td>
<td>Avoid pyridostigmine</td>
</tr>
<tr>
<td>Primary acetylcholine receptor deficiency</td>
<td>Pyridostigmine; amifampridine also helps; salbutamol can help if refractory to above</td>
<td>—</td>
</tr>
<tr>
<td>Slow-channel syndrome</td>
<td>Quinine, quinidine, or fluoxetine</td>
<td>Avoid pyridostigmine and amifampridine</td>
</tr>
<tr>
<td>Fast-channel syndrome</td>
<td>Pyridostigmine and amifampridine</td>
<td>Avoid quinine, quinidine, or fluoxetine</td>
</tr>
<tr>
<td>Agrin deficiency</td>
<td>No response to pyridostigmine or amifampridine in one patient; partial response to ephedrine in a second patient</td>
<td>Use pyridostigmine with caution</td>
</tr>
<tr>
<td>LRP4 deficiency</td>
<td>—</td>
<td>Avoid pyridostigmine and amifampridine</td>
</tr>
<tr>
<td>MuSK deficiency</td>
<td>Variable response to amifampridine; good response to salbutamol in one patient</td>
<td>Conventional doses of pyridostigmine can worsen symptoms</td>
</tr>
<tr>
<td>Dok-7 deficiency</td>
<td>Salbutamol or ephedrine</td>
<td>Avoid pyridostigmine</td>
</tr>
<tr>
<td>Rapsyn deficiency</td>
<td>Pyridostigmine, amifampridine salbutamol</td>
<td>—</td>
</tr>
<tr>
<td>GFPT1, DAPG1, ALG2 and ALG14 associated congenital myasthenic syndrome</td>
<td>Pyridostigmine; amifampridine might confer additional benefit</td>
<td>—</td>
</tr>
<tr>
<td>PREPL deletion syndrome</td>
<td>Pyridostigmine beneficial in infancy</td>
<td>Old patients are refractory to pyridostigmine</td>
</tr>
<tr>
<td>Plectin deficiency</td>
<td>Refractory to pyridostigmine and amifampridine</td>
<td>—</td>
</tr>
<tr>
<td>Defects in sodium channels</td>
<td>Pyridostigmine and acetazolamide</td>
<td>—</td>
</tr>
<tr>
<td>MuSK=muscle-specific kinase</td>
<td>—</td>
<td>no data available</td>
</tr>
</tbody>
</table>

Search strategy and selection criteria
PubMed was searched for articles published on congenital myasthenic syndromes between Jan 01 2004 and August 31 2014 with no language restrictions. The primary search term was: “congenital myasthenic syndrome”. The secondary search terms were: “acetylcholine receptor”; “choline acetyltransferase”; “ColQ”; “laminin beta-2”; “agrin”; “LRP4”; “MuSK”; “Dok-7”; “rapsyn”; “GFPT1”; “DPAGT1”; “ALG2”; “ALG14”; “Prepl”; “plectin”; “centronuclear myopathies”; “Na1.4 channel”; “Synaptotagmin”; and “SNAP25”. The authors also searched their own reprint files, clinical histories of their patients, and data files of their own research studies.
will be that it cannot identify newly emerging CMS disease genes. However, in many patients it will obviate the need for more expensive whole-exome sequencing.

More CMS disease genes are very likely to be discovered, but demonstration of pathogenicity associated with individual mutations will still be a necessity. This demonstration will take advantage of tried and true methods for gene expression and functional comparison of the wild type and mutant gene products. Clinicians armed with knowledge of the molecular mechanism behind the aberrant gene product will be in an advantageous position to formulate rational and individualised therapies for each form of CMS.

Contributors
All authors contributed equally to the literature search and preparation of the manuscript.

Declaration of interests
The authors declare no competing interests.

Acknowledgments
AGE and X-MS were supported by US National Institute of Health (NIH) grant NS06277. SMS was supported by NIH grant NS031744.

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