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Genetic heterogeneity and pathophysiological mechanisms in congenital myasthenic syndromes

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Review article

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Abstract

Congenital myasthenic syndromes (CMS) are a rare heterogeneous group of inherited neuromuscular disorders associated with distinctive clinical, electrophysiological, ultrastructural and genetic abnormalities. These genetic defects either impair neuromuscular transmission directly or result in secondary impairments, which eventually compromise the safety margin of neuromuscular transmission. In this report we will explore the significant progress made in understanding the molecular pathogenesis of CMS, which is important for both patients and clinicians in terms of reaching a definite diagnosis and selecting the most appropriate treatment.

Introduction

The neuromuscular junction is a specialized synapse with a complex molecular architecture, which serves to achieve reliable transmission between the nerve terminal and muscle fibre. It is composed of three main compartments: the presynaptic part formed by the nerve terminal is responsible for acetylcholine synthesis, storage and release. The synaptic cleft contains the basal lamina, which constitutes a sound extracellular matrix, both structurally and functionally. This maintains good adhesion between the synaptic membranes and facilitates neuromuscular communication. The postsynaptic muscle membrane, also called endplate has a multitude of folds, which contains several acetylcholine receptor clusters on its surface.¹

Depolarisation at the nerve terminal opens the presynaptic voltage-gated calcium (Ca^{++}) channels allowing influx of Ca^{++} into the presynaptic nerve bud, which triggers release of multiple vesicles (quanta) of acetylcholine by exocytosis. Acetylcholine diffuses across the synaptic space and binds to the acetylcholine receptors. This in turn results in influx of cations, mainly sodium (Na^+) but also Ca^{++} that induces depolarisation of the muscle endplate generating endplate potentials (EPP) and ultimately muscle contraction. An important concept in neuromuscular junction functional integrity is the safety margin, which reflects the extent of interference of the synaptic mechanisms that can exist without failure of transmission.²⁻³

Congenital myasthenic syndromes (CMS) are a heterogeneous group of inherited neuromuscular disorders in which the safety margin of neuromuscular transmission is compromised.⁴⁻⁵ To date 13 genes have been found to cause CMS when mutated. These are the pre-synaptic acetyltransferase *CHAT*⁶, the gene *COLQ*⁷⁻⁸⁻⁹ encoding the triple stranded collagenic tail of the synaptic acetylcholinesterase (AChE), the genes encoding the different subunits of the postsynaptic acetylcholine receptors (AChR) *CHRNA1*, *CHRNA1*, *CHRNB1*, *CHRND*, *CHRNE* and *CHRNG*, the *RAPSN* gene encoding the postsynaptic protein rapsyn¹⁰, the postsynaptic muscle specific kinase (*MUSK*) gene¹¹, the postsynaptic sodium channel (*SCN4*)¹², the *DOK7* gene encoding the postsynaptic Dok-7 protein¹³⁻¹⁴, the *LAMB2* encoding the synaptic Laminin B2 protein¹⁵, the *AGRN* gene encoding the postsynaptic agrin protein¹⁶ and the *PLEC1* gene encoding the postsynaptic protein plectin.¹⁷⁻¹⁸⁻¹⁹

Genetic classification of the different CMS cohorts reveals that the majority of CMS patients have mutations in genes expressing postsynaptic endplate proteins⁴⁻²⁰ (**table 1**). Despite tremendous progress in identifying the molecular basis of the different CMS subtypes, almost half of the CMS population remains genetically undiagnosed. For instance, limb girdle CMS with tubular aggregates remains a fascinating but as yet genetically uncharacterized group of patients waiting to be unravelled.²¹ Furthermore, there is significant variability in clinical phenotype, onset and course of disease and response to therapy in patients sharing the same genetic defect. In spite of this, treatment efficacy and safety seem closely linked to the underlying molecular aetiology.

In this report we will classify CMS based on the site of molecular defect in the neuromuscular junction. We will focus on recent progress in understanding the pathomechanisms underlying the different CMS categories and highlight any relevant clinical features that can guide clinicians in making the correct diagnosis and selecting the most efficacious and least harmful treatment.

Presynaptic CMS

Choline acetyltransferase (ChAT) Deficiency

A decrease in ChAT activity has been associated with a number of developmental and neurodegenerative disorders, including Alzheimer's disease, Huntington disease, amyotrophic lateral sclerosis, Schizophrenia, Rett syndrome and Sudden Infant Death Syndrome (SIDS).²² However, the most striking association of ChAT deficiency causing disease is seen in CMS with episodic apnoea (CMS-EA)⁶.

The *CHAT* gene encodes the choline acetyltransferase protein, which is located at the pre-synaptic nerve terminal and is responsible for acetylcholine synthesis from acetyl coenzyme A and choline⁶. Mutations in this gene have been shown to be associated with a reduction in ChAT synthesis or impairment in ChAT catalytic function.³ Analysis of the crystal structure of ChAT rat protein reveals that *CHAT* mutations are located either at the substrate binding site where the enzyme catalytic site is found or alternatively these mutations alter the function of the enzyme allosterically or by changing the folding or stability of the enzyme.²³⁻²⁴ The mutations identified to date have been scattered throughout the gene with no suggestion of "hotspots".

The CMS clinical phenotype in ChAT deficiency is associated with sudden and recurrent episodes of apnoea and bulbar weakness precipitated by infection, fever, excitement or no identifiable trigger against a background of variable fatigable weakness.⁶ The course of disease seems to follow two distinct trends with either neonatal onset respiratory distress with progressive improvement over time but then further respiratory relapses in adulthood or late onset (during infancy or childhood) respiratory crises with a more unpredictable course of disease.²⁵⁻²⁶

Treatment is with AChE inhibitor therapy in variable doses with or without addition of 3, 4-diaminopyridine (3, 4 DAP)²⁷. There is some recent anecdotal evidence that midazolam may mitigate the severity of apnoeic episodes but further study is needed.²⁸

Synaptic CMS

Acetylcholinesterase deficiency (AChE)

The synaptic basal lamina associated CMS is caused by the absence of the asymmetric form of acetylcholinesterase (AChE) from the synaptic space.⁷⁻⁸ One, two or three globular subunits form the main structure of this synaptic enzyme, which needs to be anchored to the basal lamina for its proper function. The ColQ protein is responsible for attaching acetylcholinesterase to the basal lamina. It is worth noting that endplate AChE deficiency is not caused by mutations in the *ACHE* gene but by recessive mutations in the *COLQ* gene encoding the collagenic tail subunit.

ColQ is composed of an N-terminal proline-rich region attachment domain (PRAD), a collagenic central domain and a C-terminal region enriched in charged residues and cysteines. Two heparan sulphate proteoglycan

binding domains in the collagen domain and the C-terminal domain anchor AChE in the synaptic space.²⁹⁻³⁰⁻³¹ No fewer than 30 mutations have been reported so far, most of which are frameshift and nonsense mutations that truncate the ColQ protein distal to the PRAD domain.⁴⁻³²

Absence of AChE results in excess ACh in the synaptic cleft causing abnormal activation of the AChR and prolonged duration of the synaptic current. This in turn evokes repetitive compound action potentials, also seen in slow channel syndromes, and instigates an endplate myopathy through cation overload.

The course of disease in ColQ deficient patients can either be associated with a progressive trend leading to significant disability or alternatively can present with a later onset of disease with a much milder progression over the years. Despite the clear clinical heterogeneity, a number of distinguishing features have been observed in this subgroup including the onset of symptoms in the neonatal period, evidence of delayed motor milestones, frequent early respiratory complications, sometimes needing ventilator support, and ocular involvement with evidence of ptosis, ophthalmoparesis and sometimes abnormally slow pupillary reactions.⁹ The neurophysiological findings are invariably abnormal and can be diagnostically helpful when repetitive CMAPs are evoked. Treatment with choline esterase inhibitors has been shown to be inefficacious or even dangerous in this CMS subgroup. In contrast, ephedrine seems to lead to clinical improvement with fewer tendencies to respiratory complications.²⁷

Laminin B2 deficiency

Laminins are glycoproteins found in the basal lamina composed of an alpha, beta and gamma subunits. The beta2 chain encoded by the *LAMB2* gene is found in all the laminin heterotrimers located at the neuromuscular junction and seems to play an important role in synaptogenesis.³³ Mutations in the laminin β 2 subunit have previously been described in relation to Pierson syndrome typically associated with severe renal and ocular defects leading to death within weeks or months.

There is one case report in the literature describing a female adult patient presenting with renal impairment in early childhood needing kidney transplantation. She also had delayed motor milestones, ptosis, ophthalmoparesis, abnormal pupils and macular abnormalities. Neurophysiological studies confirmed a neuromuscular transmission defect. Treatment with cholinesterase inhibitors led to worsening neuromuscular weakness. Ephedrine was more beneficial. Two novel recessive truncating mutations in the *LAMB2* gene were confirmed.¹⁵

Postsynaptic CMS

This is by far the commonest group of CMS. It is caused by the interruption of an important pathway involving several postsynaptic proteins including agrin, muscle specific tyrosine kinase (MuSK), downstream of kinase (Dok-7), acetylcholine receptors (AChRs) and the AChR-clustering protein rapsyn. This pathway is believed to be responsible for the stability of the synaptic architecture and for the aggregation and positioning of AChR on the post-synaptic membrane.³⁴ Thus postsynaptic CMS related to AChR deficiency are not only caused by mutations in the different subunits of AChR but also includes other postsynaptic proteins.

Defects in acetylcholine receptor subunits

Molecular defects involving the postsynaptic AChR lead to either deficiency of the receptor with or without kinetic defects or cause kinetic abnormalities with or without minor AChR deficiency. The kinetic mutations fall into two categories according to whether they induce slow or fast channel syndromes⁴⁻²⁰.

Primary AChR deficiency

This is associated with a recessively inherited form of CMS, most commonly associated with mutations in the epsilon subunit. This predilection is probably related to the presence of the gamma foetal isoform, which can partially compensate for the absence of epsilon subunit. Moreover, the gene encoding the epsilon subunit contains multiple GC repeats that are likely to predispose to DNA rearrangement.⁴⁻²⁰ A multitude of private mutations have been identified in this subgroup although a few founder mutations are recognised in certain geographical or ethnical distributions such as the North African, Spanish/Portuguese and Gypsy populations **(table2)**.

Clinically, ptosis with ophthalmoparesis and global weakness are invariably found in this CMS group. The course of disease is fairly static, although transient exacerbation can be triggered by infection. The spectrum of clinical severity varies greatly and both intrafamilial and interfamilial differences are seen.⁴⁻⁵⁻²⁰

Kinetic defects in AChR

Fast channel syndromes

Fast channel mutations cause "loss of function" of the AChR leading to reduced affinity for ACh, and abnormally brief channel opening events. This is a relatively rare form of CMS associated with mostly recessive mutations in the AChR α , δ and ϵ subunits.²⁴ Great clinical variability is observed but patients often present at birth with respiratory and/or bulbar problems which can be associated with increased mortality in the neonatal period. Ocular symptoms are invariably present. A partial response to anticholinesterases and 3,4-DAP has been noted.

Slow channel CMS

This category of CMS arises from “gain of function” mutations, which are typically dominant in inheritance and can involve any of the AChR subunits.⁴ These mutations cause sustained activation of the AChR either by enhancing its affinity for ACh or delaying channel closure.

The prolonged AChR channel activation outlasts the refractory period of the muscle fibre action potential so that a single nerve stimulus evokes repetitive compound action potentials. Moreover, this abnormal channel opening causes postsynaptic cation overload that leads to an endplate myopathy.

Clinically, this form of CMS usually presents in childhood with evidence of delayed motor milestones and ophthalmoparesis with ptosis. The neck muscles and the long finger and wrist extensors seem to be preferentially weaker. Conventional treatment with anticholinesterases or 3,4-DAP can worsen symptoms. Long acting open channel blockers such as fluoxetine, quinidine, and ephedrine have been shown to be beneficial²⁷.

Escobar syndrome

The AChR has 5 subunits, 2 α , 1 β , 1 δ and 1 ϵ or γ subunits. By substituting the γ to ϵ subunits in late foetal life, foetal receptors are gradually replaced by adult receptors. The foetal AChR seems to play a pivotal role in neuromuscular organogenesis by acting as a guide for the primary encounter of axon and muscle *in utero*.³⁵⁻³⁶

Escobar syndrome is an antenatal congenital myasthenic disorder in which neuromuscular organogenesis is flawed due to mutation in the γ subunit, encoded by *CHRNA3* gene. Although clinically variable, this syndrome typically presents with arthrogryposis multiplex congenita with joint contractures, pterygia, cryptochism in males and respiratory distress. Because γ subunit expression does not extend beyond 33 weeks of gestation, patients with this molecular defect have no typical myasthenic symptoms after birth.³⁵ There is some suggestion that certain homozygous mutations in the γ subunit result in recurrent miscarriages. In the right context, this may well be worth exploring when counselling affected families.

It worth mentioning that mutations in the other subunits of the AChR (*CHRNA1*, *CHRNA1*, *CHRNA1*) as well as *RAPSN* and *DOK7* genes have also been linked to in utero defects such as foetal akinesia syndrome.³⁷⁻³⁸

Rapsyn deficiency

This is another important cause of CMS. Rapsyn is a postsynaptic protein that comprises several functional domains: a myristoylated N-terminal is required for membrane interaction.³⁹ 7 tetratricopeptide repeats (TPR) are important for rapsyn self-aggregation⁴⁰ and binding to the cytoplasmic portion of the specific muscle kinase Musk⁴¹; the coiled coil domain interacts with the cytoplasmic loops of AChR subunits⁴²; and the C terminal domain binds to β dystroglycan and thereby links the rapsyn-AChR complex to the cytoskeleton⁴³. Recessive mutations in the *RAPSN* gene are a relatively common cause of CMS and result in postsynaptic AChR

deficiency. Until recently, most patients harbouring mutations in *RAPSN* were either homozygous or heterozygous for the N88K mutation in exon 2⁴⁴⁻⁴⁵. In a recent French CMS series of 20 patients with confirmed causative *RAPSN* mutations, three patients had the N88K substitution but the sequencing failed to depict a second pathogenic mutation. Using qPCR analysis, three chromosomal microdeletions were found. All these multi-exon deletions corresponded to the missing pathogenic allelic mutation⁴⁶. Of note, a similar finding was reported by Müller et al in one patient with a chromosomal microdeletion event in the *RAPSN* gene.⁴⁷

The clinical phenotype of rapsyn-CMS varies from severe hypotonia, arthrogryposis, dysmorphism and the presence of a high arched palate at birth to mild limb muscle weakness. Early and late onset phenotypes have been described. Recurrent apnoeic episodes and absence of ocular features have been suggested as possible discerning features between rapsyn-CMS and low expressor mutations in the AChR ϵ subunit.⁴⁸ This form of CMS improves during childhood with respiratory crises disappearing and weakness improving over time. Furthermore, patients often respond dramatically to anticholinesterase medication. Thus recognition of this CMS genotype early can prevent death in a condition with a good long-term outcome.

MuSK deficiency

During NMJ development, Musk plays a key role in the organisation of the postsynaptic actin cytoskeleton and AChR clustering through recruitment of several downstream kinases and phosphorylation of the AChR β subunit.⁴⁹⁻⁵⁰ Three different kinships have been described so far in association with Musk deficiency.¹¹⁻⁵¹⁻⁵² Despite significant variability in clinical phenotype and disease severity, most patients had ocular symptoms and fatigable limb weakness. Respiratory complications were also noted either in the neonatal period or later in adulthood. Cholinesterase inhibitors and 3, 4 DAP were of some limited benefit.

Dok-7 deficiency

In 2006, Okada et al functionally characterised Dok-7 and showed that it could induce aneural activation of MuSK and subsequent clustering of AChRs. During the same year, Beeson et al identified *DOK7* mutations in 21 patients with a clinical diagnosis of CMS exhibiting a limb girdle phenotype.¹³⁻¹⁴ Since then *DOK7* mutations have been confirmed in a significant number of other series.⁵³⁻⁵⁴⁻⁵⁵⁻⁵⁶⁻⁵⁷ The pathophysiological repercussions of *DOK7* mutations appear to arise from abnormal activation of MuSK signalling leading to an abnormally unstable and simplified neuromuscular junction affecting both pre and postsynaptic structures.¹⁴⁻⁵⁵⁻⁵⁸ More recent animal studies using a zebrafish model suggest that Dok-7 deficiency may also impair slow muscle organisation, independent of Musk.⁵⁹

The clinical phenotype is typically associated with normal initial motor milestones with weakness manifesting either in childhood or adulthood with predominant limb-girdle involvement, often sparing the extra-ocular muscles. Occasionally, congenital stridor with idiopathic bilateral cord palsy can be the presenting symptoms in this form of CMS.⁶⁰ Patient can report prolonged periods of worsening weakness and fatigue. There is also anecdotal evidence of exacerbation of weakness in the context of pregnancy.

Dok-7 CMS is associated with poor response and potentially worsening weakness with cholinesterase inhibitors and a rather erratic response to 3, 4 DAP. However, there is reasonable evidence that ephedrine provides benefit.⁵⁶⁻⁶²

Agrin deficiency

Although agrin's site of action is at the postsynaptic level, this protein is released by the presynaptic nerve terminal. Agrin is a heparan sulphate proteoglycan that binds to low-density lipoprotein receptor-related protein 4 (LRP4) expressed in muscle, leading to activation of MuSK and subsequent clustering of essential postsynaptic proteins.¹⁶

Agrin associated CMS has been described in only 2 adult patients with a fairly mild phenotype and no suggestion of progression. Delayed motor milestones, ptosis with no ophthalmoparesis, mild to minimal proximal limb weakness were reported. The patients had no bulbar or respiratory difficulties. Repetitive CMAPS were evoked on EMG. Treatment with cholinesterase inhibitors and 3, 4 DAP was ineffective but ephedrine was beneficial.

Defects in the postsynaptic sodium channel (Na_v1.4)

There is one report of an adult female presenting with a congenital myasthenic syndrome associated with recurrent apnoeic crises, needing ventilatory support and learning difficulties. The latter were attributed to anoxic brain damage. Neurophysiology was consistent with a myasthenic syndrome and pointed to the Na channel as a potential candidate gene. Two mutations were identified one of which was felt to be pathogenic. The inheritance pattern remains unclear.¹²

Plectin deficiency

This is another very rare and emerging cause of CMS with only 3 case reports found in the literature.¹⁷⁻¹⁸⁻¹⁹ The *PLEC1* gene encodes for plectin, a cytoskeleton linker protein widely distributed in muscle and epithelial tissues.⁶² Mutations in this gene have classically been associated with a skin disorder (epidermolysis bullosa simplex) with and without associated features such as cardiomyopathy, respiratory failure, oesophageal

atresia and late onset muscular dystrophy. All reported patients had myasthenic symptoms from early infancy and subsequently developed mild to severe skin blistering in keeping with epidermolysis bullosa. Treatment with 3, 4 DAP may be of some benefit in this subgroup.

Summary

Although a large number of CMS genes have been discovered, most patients have harboured mutations in AChR subunits especially the ϵ subunit, *RAPSN*, *COLQ*, *CHAT* and *DOK7* genes. Being aware of common founder mutations in certain populations can expedite the process of making the correct genetic diagnosis and providing safe and efficacious treatment. As in many neuromuscular disorders, a particular focus on respiratory and bulbar functions is essential in preventing serious and potentially fatal complications in a highly treatable condition like CMS.

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Table 1: Estimated frequency of CMS subtypes based on individuals with CMS investigated at the Munich laboratory

Molecular defect	Percentage of CMS attributed to specific gene mutations
Presynaptic	
<i>CHAT</i>	5%
Synaptic	
<i>COLQ</i>	10-15%
<i>LAMB2</i>	<1%
Postsynaptic	
Primary AChR deficiency ± kinetic defects	Up to 60%
<i>RAPSN</i>	20%
<i>MUSK</i>	<1%
<i>DOK7</i>	10%
<i>AGRN</i>	<1%
<i>SCN4A</i>	<1%
<i>PLEC1</i>	<1%

Table 2: Genetic heterogeneity and treatment options in CMS depending on the underlying molecular defect

Gene		Gene size (number of exons)	Common/founder mutations	Geographical distribution	Therapy
<i>CHAT</i>		18 exons	-	-	Cholinesterase inhibitors, 3,4 DAP, close monitoring of respiratory function
<i>COLQ</i>		18 exons	-	-	Ephedrine , avoid cholinesterase inhibitors
<i>LAMB2</i>		32 exons	-	-	Ephedrine
<i>AChR subunits deficiency</i>	<i>CHRNE</i>	12 exons	ε1267delG (exon 12)	European Roma and/or south eastern European	Cholinesterase inhibitors, 3,4 DAP
			ε1293insG (exon 12)	Maghreb (Algeria and Tunisia)	Cholinesterase inhibitors, 3,4 DAP
			c.70insG (exon 2)	Spanish/Portuguese	Cholinesterase inhibitors, 3,4 DAP
	<i>CHRNA1</i>	9 exons	-	-	Cholinesterase inhibitors, 3,4 DAP
	<i>CHRNA1</i>	11 exons	-	-	Cholinesterase inhibitors, 3,4 DAP
	<i>CHRND</i>	12 exons	-	-	Cholinesterase inhibitors, 3,4 DAP
<i>AChR subunits kinetic defects</i>	<i>Fast channels</i>	Any of the AChR subunits			Cholinesterase inhibitors, 3,4 DAP
	<i>Slow channels</i>	Any of the AChR subunits	<i>CHRNA1</i> p.G153S		Fluoxetine , quinidine
<i>RAPSN</i>		8 exons	p.N88K (exon 2)	-	Cholinesterase inhibitors, 3,4 DAP
<i>MUSK</i>		14 exons	-	-	Cholinesterase inhibitors, 3,4 DAP
<i>DOK7</i>		7 exons	1124_1127dupTGCC (exon 7)	-	Ephedrine
<i>AGRN</i>		36 exons	-	-	Ephedrine
<i>SCN4A</i>		24 exons	-	-	Unclear
<i>PLEC1</i>		Many isoforms present	-	-	3, 4 DAP