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Research Paper

Congenital Myasthenic Syndromes in Belgium: Genetic and Clinical Characterization of Pediatric and Adult Patients

Nathalie Smeets, MD [a,*], Alexander Gheldof, PhD [b], Bart Dequeker, MSc [b], Margaux Poleur, MD [c], Sofia Maldonado Slootjes, MD [d], Vinciane Van Parijs, MD [d], Nicolas Deconinck, MD, PhD [e], Pauline Dontaine, MD [e], Alicia Alonso-Jimenez, MD, PhD [f], Jan De Bleecker, MD, PhD [g], Willem De Ridder, MD, PhD [f], Sarah Herdewyn, MD, PhD [g], Stéphanie Paquay, MD [h], Arnaud Vanlander, MD, PhD [i], Liesbeth De Waele, MD, PhD [j,k], Geertrui Peirens, MD [j,k], Diane Beysen, MD, PhD [l], Kristl G. Claeys, MD, PhD [m,n], Nicolas Dubuisson, MD [d], Isabelle Hansen, MD [o], Gauthier Remiche, MD, PhD [p], Sara Seneca, PhD [b], Véronique Bissay, MD, PhD [q], Luc Régal, MD [a]

[a] Child Neurology Unit, Department of Pediatrics, Vrije Universiteit Brussel, Universitair Ziekenhuis Brussel, Brussels, Belgium
[b] Center of Medical Genetics, Vrije Universiteit Brussel, Universitair Ziekenhuis Brussel, Brussels, Belgium
[c] University Department of Neurology, Citadelle Hospital of Liège, Liège, Belgium
[d] Department of Neurology, Cliniques Universitaires Saint-Luc, Brussels, Belgium
[e] Department of Pediatric Neurology, Queen Fabiola Children's University Hospital, Université Libre De Bruxelles, Brussels, Belgium
[f] Department of Neurology, University of Antwerp, Antwerp University Hospital, Antwerp, Belgium
[g] Department of Neurology, Ghent University Hospital, Ghent, Belgium
[h] Department of Neuropediatrics, Cliniques Universitaires Saint-Luc, Brussels, Belgium
[i] Department of Pediatric Neurology and Metabolic Diseases, Ghent University Hospital, Ghent, Belgium
[j] Department of Development and Regeneration, KU Leuven, Leuven, Belgium
[k] Department of Pediatric Neurology, University Hospitals Leuven, Leuven, Belgium
[l] Department of Pediatric Neurology, University of Antwerp, Antwerp University Hospital, Antwerp, Belgium
[m] Department of Neurology, University Hospitals Leuven, Leuven, Belgium
[n] Laboratory for Muscle Diseases and Neuropathies, Department of Neurosciences, KU Leuven, Leuven Brain Institute (LBI), Leuven, Belgium
[o] Department of Neurology, University of Liège, Centre Hospitalier Universitaire de Liège, Liège, Belgium
[p] Department of Neurology, Hôpital Universitaire de Bruxelles - Hôpital Erasme, Université Libre de Bruxelles, Brussels, Belgium
[q] NEUR Research Group and Department of Neurology, Vrije Universiteit Brussel, Universitair Ziekenhuis Brussel, Brussels, Belgium

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ABSTRACT

Background: Congenital myasthenic syndromes (CMS) are a group of genetic disorders characterized by impaired neuromuscular transmission. CMS typically present at a young age with fatigable muscle weakness, often with an abnormal response after repetitive nerve stimulation (RNS). Pharmacologic treatment can improve symptoms, depending on the underlying defect. Prevalence is likely underestimated. This study reports on patients with CMS followed in Belgium in 2022.
Methods: Data were gathered retrospectively from the medical charts. Only likely pathogenic and pathogenic variants were included in the analysis.
Results: We identified 37 patients, resulting in an estimated prevalence of 3.19 per 1,000,000. The patients harbored pathogenic variants in *CHRNE*, *RAPSN*, *DOK7*, *PREPL*, *CHRNB1*, *CHRNG*, *COLQ*, *MUSK*, *CHRND*, *GFPT1*, and *GMPPB*. *CHRNE* was the most commonly affected gene. Most patients showed disease onset at birth, during infancy, or during childhood. Symptom onset was at adult age in seven patients, caused by variants in *CHRNE*, *DOK7*, *MUSK*, *CHRND*, and *GMPPB*. Severity and distribution of weakness varied, as did the presence of respiratory involvement, feeding problems, and extraneuromuscular manifestations. RNS was performed in 23 patients of whom 18 demonstrated a pathologic decrement. Most treatment responses were predictable based on the genotype.

* Communications should be addressed to: Dr. Smeets; UZ Brussel; Laarbeeklaan 101; Jette 1090, Belgium.
E-mail address: Nathalie.smeets@uzbrussel.be (N. Smeets).

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Conclusions: This is the first pooled characterization of patients with CMS in Belgium. We broaden the phenotypical spectrum of pathogenic variants in *CHRNE* with adult-onset CMS. Systematically documenting larger cohorts of patients with CMS can aid in better clinical characterization and earlier recognition of this rare disease. We emphasize the importance of establishing a molecular genetic diagnosis to tailor treatment choices.

Introduction

Congenital myasthenic syndromes (CMS) are a group of genetic disorders characterized by impaired neuromuscular transmission.[1] To date, pathogenic variants are described in more than 30 CMS-related genes.[2,3] CMS can be categorized according to the location of the mutant protein at the neuromuscular junction (NMJ) into presynaptic, synaptic, and postsynaptic subgroups. Postsynaptic CMS can result from acetylcholine receptor (AChR) deficiency or kinetic defects, where in fast channel syndromes there is a reduction of AChR channel opening time and in slow channel syndromes a prolonged opening time. A fourth subgroup consists of different glycosylation defects causing combination defects of the NMJ.[4-6] Most of the CMS-causing variants have a recessive inheritance pattern; a dominant pattern is seen in slow channel syndrome and in pathogenic variants in the presynaptic *SNAP25* and *SYT2* genes.[6]

The prevalence of CMS varies considerably in different reports, ranging between 2.8 and 22.2 per million in the pediatric population[7-9] and 1.8 and 3.1 per million in the general population,[9-11] and is likely underestimated.[12]

Recognition of CMS is important, as pharmacologic treatment is available. Drugs effective in one subgroup can be ineffective or harmful in another subgroup, depending on the underlying pathologic mechanism. Hence, identification of the affected gene is of paramount importance.[1,13]

Clinically, CMS manifest with fatigable weakness of variable severity of ocular, facial, bulbar, respiratory, truncal, and/or limb muscles. Symptoms are often present at birth or arise during early childhood. An abnormal response on repetitive nerve stimulation (RNS) or an abnormal single-fiber electromyography supports the clinical suspicion.[1,14] Late-onset presentation, extraneuromuscular manifestations, and a normal neurophysiological examination are associated with a missed or delayed diagnosis.[14,15]

This study describes the clinical and molecular spectrum of patients with CMS in Belgium.

Materials and Methods

We collected data from all seven Belgian Neuromuscular Reference Centers (NMRCs) on pediatric and adult patients with CMS followed in 2022. Only patients considered by the treating physician to have genetic confirmation were included. Physicians working at the NMRCs retrospectively gathered data from the medical charts and completed a pseudonymized case report form including detailed data on age of onset, current age, clinical presentation, disease course, molecular genetics, electrophysiological findings and other relevant diagnostic investigations, treatment and response to pharmacologic treatment. A decrement larger than 10% after RNS was defined as pathologic.[16]

All identified genetic variants were evaluated according to the standards of the American College of Medical Genetics and Genomics (ACMG).[17] Likely pathogenic and pathogenic variants in accordance with the expected mode of inheritance were included in the analysis. Variants of unknown significance (VUS) were reported separately.

The Medical Ethics Committee of UZ Brussel/VUB approved the study protocol. All the collaborating centers confirmed compliance with their local regulatory guidelines.

Results

Genetic data

Pathogenic and likely pathogenic variants

We identified 37 patients with (likely) pathogenic variants in CMS-related genes. Supplementary Table 1 provides the list of variants and their classification according to the ACMG criteria. Parental segregation analysis was available for 20 of 37 patients. In most of the patients carrying class 4 variants, pathogenicity was supported by segregation analysis, a decremental response on RNS, and/or a positive response to therapy. Molecular diagnosis was ascertained through a targeted gene panel in nine patients, single gene testing in 10, whole exome sequencing (WES) in nine, mendeliome sequencing in three, and single nucleotide polymorphism microarray with single gene testing for the other variant in one other patient (*PREPL*). For five patients, the information on the type of analysis was not available. Different laboratories were implicated in the molecular genetic analyses.

The patients harbored causative variants in *CHRNE, RAPSN*, *DOK7, PREPL, CHRNB1, CHRNG*, *COLQ, MUSK*, *CHRND*, *GFPT1,* or *GMPPB* (in order of prevalence, see Fig 1).

Variants of unknown significance (VUS)

In six other patients a VUS was found in CMS-related genes. The list of VUS and their classification according to the ACMG criteria, as well as detailed clinical information, are available as Supplementary Information (Supplementary Tables 2 and 3). These patients were not included in the following analysis.

Demographic data

Based on the identification of 37 patients with CMS and the Belgian population data reported in Statbel (https://statbel.fgov.be) in 2022, the prevalence of CMS was calculated at 3.19 per million. At the time of inclusion, 23 patients were adults (age ranging between 21 and 73 years with a median at 37 years), 13 were aged between one and 18 years (ranging between 5 and 17 years with a median at 13 years), and one patient had not yet reached age one year. Most prevalent ethnic origin was Caucasian; (North)African origin was the second most frequent. Consanguinity was reported in 10 patients.

Age at onset versus age at diagnosis

In 19 patients (51%) the first symptoms appeared at birth or during the first year of life. In 11 patients (30%), symptoms

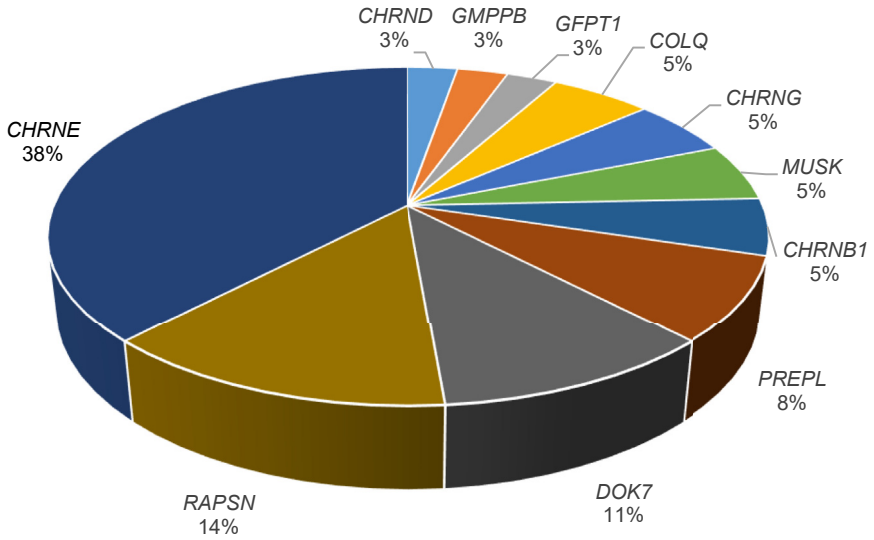

FIGURE 1. Percentage distribution of affected congenital myasthenic syndromes genes identified in the Belgian population. The color version of this figure is available in the online edition.

manifested at a later age (between one and 18 years). Seven patients (19%) had symptom onset after age 18 years. Figure 2 illustrates the age range at symptom onset in relation to the affected gene. The age of onset and the age of diagnosis were available in 34 of 37 studied patients. Time between symptom onset and diagnosis ranged from 0 (no delay) to 50 years (median 6 years).

Diagnostic evaluation

RNS with 3 Hz at rest was performed in 23 patients with pathogenic variants in *CHRNE*, *RAPSN*, *DOK7*, *MUSK*, *CHRNB1*, *CHRND*, *GFPT1*, and *GMPPB*. Five patients had a normal response, harboring pathogenic variants in *CHRNE* (two), *RAPSN* (two), and *CHRNB1* (one). Three of the patients with a normal response on RNS were under age two years at the time of the examination. In one neonatal patient, it was not considered safe to discontinue pyridostigmine for the examination (*CHRND1*). In the patients with normal RNS, single-fiber electromyography was not performed.

In some patients, alternative diagnoses were considered before arriving at the final diagnosis of CMS, leading to the performance of ancillary examinations (see Supplementary Information). Unspecific histologic abnormalities or a myopathic EMG was sometimes reported.

Disease course and treatment

For 33 patients, time between onset of symptoms and study inclusion was available and ranged from 10 months to 55 years, with a median follow-up period of 17 years. In the other four patients, all presenting symptoms since infancy or childhood, follow-up information is available until adult age. The disease course, stratified by the affected gene, is illustrated in Fig 3. The pharmacologic treatment and dosages of the different drugs that were used in our cohort are listed in Table 1 and compared with the dosages suggested in the literature.[13,18-21]

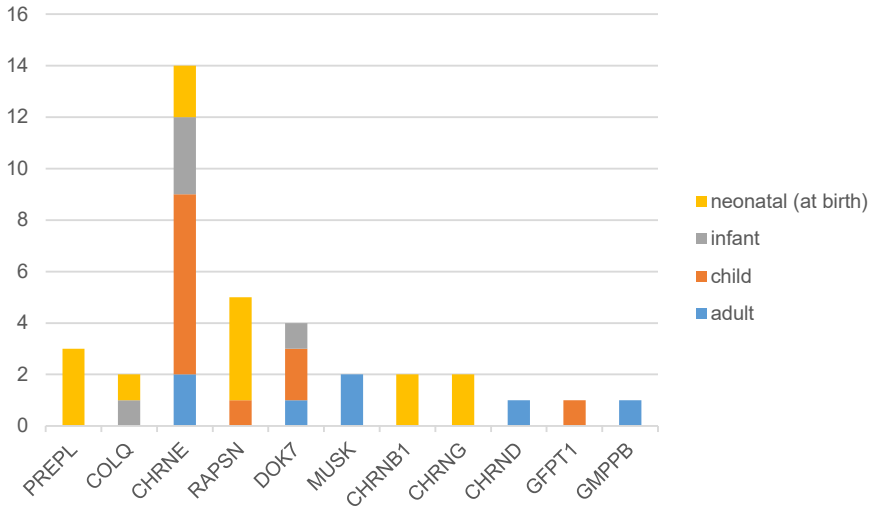

FIGURE 2. Age range of symptom onset in relation to the affected gene. The color version of this figure is available in the online edition.

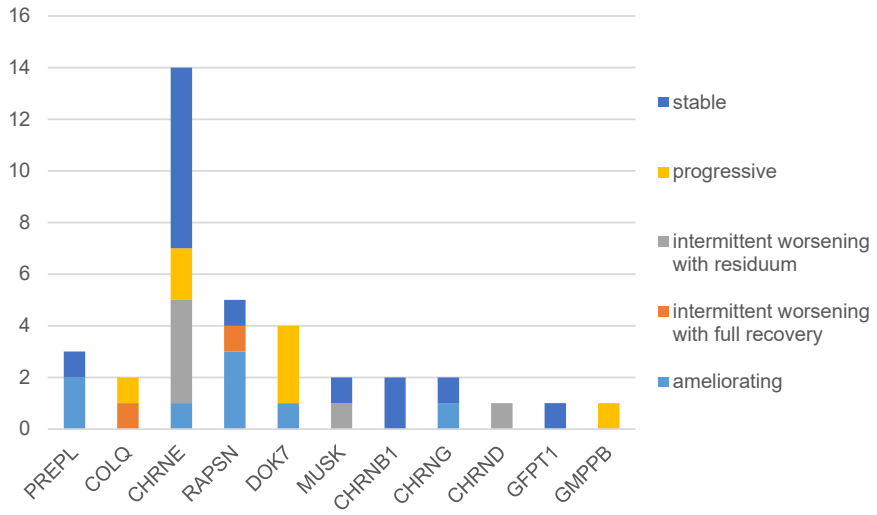

FIGURE 3. Disease course in relation to the affected gene. The color version of this figure is available in the online edition.

Clinical features and response to therapy in relation to the affected gene

The phenotypic features in relation to the affected gene are presented in Tables 2 and 3.

Presynaptic CMS

PREPL (n = 3)

Next to the myasthenic symptoms, all patients showed symptoms that are not related to a defect in neuromuscular transmission. In one patient pyridostigmine resulted in improvement of the muscle weakness. The medication could be stopped at age one year. The respiratory support, started at infancy, could be weaned off during childhood without medication. Two patients needed tube feeding, which could be stopped in the neonatal period.

Synaptic CMS

COLQ (n = 2)

Ophthalmoparesis and respiratory distress were consistent findings at onset in both patients. The use of salbutamol was reported in one patient, resulting in a positive effect on respiratory and muscle function, but not on ocular weakness. In one child, a moderate intellectual disability was reported.

Postsynaptic CMS

CHRNE (n = 14)

We identified 14 patients with pathogenic recessive variants in *CHRNE*. Six patients carried the NM_000080.4 (*CHRNE*):c.1353dup, p.(Asn452Glufs*4) variant in homozygosity. Table 4 compares the genotypic and phenotypic characteristics of these patients with those of the patients with CMS carrying other *CHRNE* variants. Ophthalmoparesis and ptosis were very common in the first group of patients, distinguishing them clinically from the other group of *CHRNE*-related CMS. In two patients with disease onset at birth, respiratory distress was the only presenting sign. Two patients presented symptom onset at adult age (at 26 and 43 years). One patient, homozygous for the c.1353dup variant, presented with ptosis, facial weakness, ophthalmoparesis, and limb muscle weakness. Disease course was stable with a good response to pyridostigmine on mobility. The other patient, homozygous for the variant c.1327del, presented with ptosis, ophthalmoparesis, dysphagia, and fatigable weakness. There was the need for a wheelchair for long distances. Initially, the patient responded well to pyridostigmine, but in time the muscle weakness and ocular symptoms progressed gradually. Treatment with 3,4-diaminopyridine (3,4-DAP) was combined, which resulted in improvement of the symptoms. Next to myasthenic symptoms, one patient also presented with a sensorineural hearing loss with dysmorphic features from an unknown cause to date, based on microarray, gene panel, and gene sequencing studies for variants in genes causing hearing loss. WES was not

TABLE 1.
Pharmacologic Treatment and Dosages Used in Our Cohort Compared With the Dosages Suggested in the Literature

Treatment	Dosages as Suggested in the Literature	Dosages Used in Our Cohort
Pyridostigmine	Pediatric: 4-6 (rarely 7-9) mg/kg/d Adult: up to 500 mg/d divided in 4-6 doses	Pediatric: 1.8-10 mg/kg/d (mean 5.1 mg/kg/d) Adult: 90-720 mg/d (mean 333 mg/d) *(data available from 25 of 28 treated patients)*
3,4-Diaminopyridine	Pediatric: 0.25-1 mg/kg/d divided in 4 doses Adult: 15-80 mg/d divided in 3-4 doses	Pediatric: 1 mg/kg/d Adult: 10-60 mg/d (mean 45 mg/d) *(data available from 6 of 11 treated patients)*
Salbutamol	Pediatric: 0.05-0.2 mg/kg/d divided in 2-3 doses Adult: 4-12 mg/d divided in 1-3 doses	Pediatric: 0.14-0.2 mg/kg/d (mean 0.17 mg/kg/d) Adult: 2-15 mg/d (mean 7.3 mg/d) *(data available from 10 of 12 treated patients)*
Ephedrine	Pediatric: 1-3 mg/kg/d divided in 3 doses Adult: 2-3 times 25-50 mg/d	Pediatric: none Adult: 50 mg/d *(1 patient)*
Fluoxetine	Pediatric: no reports in literature Adult: 80-100 mg/d	Pediatric: none Adult: 20 - 40 mg/d (mean 30 mg/d) *(data available from 2 of 3 treated patients)*
Quinidine	Pediatric:15-60 mg/kg/d divided in 4-6 doses Adult: 200 mg/d titrated up, to reach a serum level of 1 to 2.5 μg/mL	Not administered

TABLE 2.
Symptoms at Onset in Relation to the Affected Gene

Symptoms at Onset	*PREPL* (n = 3)	*COLQ* (n = 2)	*CHRNE* (n = 14)	*RAPSN* (*n* = 5)	*DOK7* (n = 4)	*MUSK* (n = 2)	*CHRNG* (n = 2)	*CHRNB1* (n = 2)	*CHRND* (n = 1)	*GFPT1* (n = 1)	*GMPPB* (n = 1)
Ptosis	0 (0%)	1 (50%)	8 (57%)	2 (40%)	2 (50%)	1 (50%)	1 (50%)	1 (50%)	1 (100%)	0 (0%)	0 (0%)
Facial weakness	0 (0%)	0 (0%)	2 (14%)	3 (60%)	2 (50%)	0 (0%)	0 (0%)	2 (100%)	1 (100%)	0 (0%)	0 (0%)
Ophthalmoparesis	0 (0%)	2 (100%)	5 (35%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (100%)	1 (100%)	0 (0%)	0 (0%)
Dysphagia	3 (100%)	1 (50%)	3 (21%)	4 (80%)	0 (0%)	0 (0%)	1 (50%)	2 (100%)	0 (0%)	0 (0%)	0 (0%)
Stridor	0 (0%)	0 (0%)	0 (0%)	2 (40%)	1 (25%)	0 (0%)	0 (0%)	2 (100%)	0 (0%)	0 (0%)	0 (0%)
Vocal cord paralysis	0 (0%)	0 (0%)	4 (28%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Respiratory distress	0 (0%)	2 (100%)	2 (14%)	1 (20%)	0 (0%)	0 (0%)	1 (50%)	2 (100%)	0 (0%)	0 (0%)	0 (0%)
Limb weakness	1 (33%)	1 (50%)	6 (42%)	3 (60%)	4 (100%)	2 (100%)	1 (50%)	2 (100%)	0 (0%)	1 (100%)	1 (100%)
Neck extensor weakness	0 (0%)	1 (50%)	0 (0%)	2 (50%)	0 (0%)	0 (0%)	1 (50%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Hypotonia (axial)	1 (33%)	1 (50%)	0 (0%)	4 (80%)	1 (25%)	0 (0%)	1 (50%)	2 (100%)	0 (0%)	0 (0%)	0 (0%)
Delayed motor development	0 (0%)	0 (0%)	1 (7%)	0 (0%)	1 (25%)	0 (0%)	2 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Fatigability	0 (0%)	1 (50%)	7 (50%)	1 (20%)	2 (50%)	1 (50%)	0 (0%)	0 (0%)	1 (100%)	1 (100%)	1 (100%)
High-arched palate	0 (0%)	0 (0%)	1 (7%)	1 (20%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Arthrogryposis	0 (0%)	0 (0%)	0 (0%)	2 (40%)	0 (0%)	0 (0%)	2 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

performed. Another patient was diagnosed with autism and intellectual disability. Ancillary studies, like a brain magnetic resonance imaging or WES, were not performed.

All but one patient were treated with pyridostigmine. Nine of them (69%) had a positive response on variable symptoms. In four patients with childhood onset, pyridostigmine was only started at adult age, resulting in amelioration of symptoms in two of them. In three patients, ephedrine or salbutamol was added to pyridostigmine; in two of them this resulted in additional improvement, and the third patient discontinued the medication due to side effects. In seven patients pyridostigmine was combined with 3,4-DAP with positive results in five of them. Two patients were also treated with fluoxetine. In one patient this was an empirical treatment choice. As soon as the genetic diagnosis was confirmed, fluoxetine was discontinued. In the other patient fluoxetine was administered as an antidepressant. No obvious beneficial effects were noticed on the myasthenic symptoms in both patients. The patient who received no medication showed an intermittent disease course with recovery between relapses and without the need for supportive therapy or mobility aids.

TABLE 3.
Overall Clinical Manifestations and Need for Supportive Therapy in Relation to the Affected Gene

Phenotypic Features	*PREPL* (n = 3)	*COLQ* (n = 2)	*CHRNE* (n = 14)	*RAPSN* (*n* = 5)	*DOK7* (n = 4)	*MUSK* (n = 2)	*CHRNG* (n = 2)	*CHRNB1* (n = 2)	*CHRND* (n = 1)	*GFPT1* (n = 1)	*GMPPB* (n = 1)
Symptoms appearing later in the disease course	NR	Fatig. (1) LW (1)	Fatig. (1) LW (5) OP (3) Ptosis (2) Dysphagia (1)	NR	Ptosis (1) Vocal cord paralysis (1)	NR	NR	NR	NR	NR	NR
Muscle contractures	0 (0%)	1 (50%)	1 (7%)	2 (40%) (Arthrogr.)	0 (0%)	0 (0%)	2 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Scoliosis	0 (0%)	1 (50%)	0 (0%)	1 (20%)	1 (25%)	0 (0%)	2 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Symptom fluctuations	0 (0%)	1 (50%)	12 (85%)	4 (80%)	3 (75%)	1 (50%)	0 (0%)	2 (100%)	1 (100%)	1 (100%)	0 (0%)
Respiratory support	2 (66%)	2 (100%)	0 (0%)	0 (0%)	1 (25%)	0 (0%)	2 (100%)	1 (50%)	0 (0%)	0 (0%)	0 (0%)
Tube feeding	1 (33%)	0 (0%)	0 (0%)	1 (20%)	0 (0%)	0 (0%)	1 (50%)	2 (100%)	0 (0%)	0 (0%)	0 (0%)
Wheelchair use	0 (0%)	0 (0%)	1 (7%)	0 (0%)	1 (25%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)
Other clinical features	ID (3) Dysm.(2) GHdef (1)	ID (1)	ASD with ID (1) Dysm. with SNHL (1)	EA (2) Dysm. (2, arthrogr.) Retro- and micrognathia (1)	EA (1, stridor)	NR	Dysm. (2, AMC)	EA (1)	NR	NR	NR

Abbreviations:
AMC = Arthrogryposis multiplex congenita
Arthrogr. = Arthrogryposis
ASD = Autism spectrum disorder
Dysm. = Dysmorphisms
EA = Episodic apnea
Fatig. = Fatigability
GHdef = Growth hormone deficiency
ID = Intellectual disability
LW = Limb weakness
NR = Not reported
OP = Ophthalmoparesis
OSA = Obstructive sleep apnea
SNHL = Sensorineural hearing loss

TABLE 4.
Phenotypic-Genotypic Correlations in *CHRNE* Patients

Phenotypic Features	*CHRNE* c.1353dup Variant (n = 6)	Other *CHRNE* Variants (n = 8)
Ptosis	6 (100%)	4 (50%)
Facial weakness	0 (0%)	1 (12%
Ophthalmoparesis	5 (83%)	3 (37%)
Dysphagia	1 (16%)	2 (25%)
Vocal cord paralysis	1 (16%)	3 (37%)
Respiratory distress	1 (16%)	1 (12%)
Limb weakness	5 (83%)	6 (75%)
Delayed motor development	1 (16%)	0 (0%)
Fatigability	3 (50%)	4 (50%)
High-arched palate	1 (16%)	0 (0%)
Other clinical features	SNHL (1)	ASD with ID (1)
Age range at symptom onset	Neonatal (1)	Neonatal (1)
	Infant (2)	Infant (1)
	Child (2)	Child (5)
	Adult (1)	Adult (1)
Disease course	Stable (4)	Stable (3)
	Progressive (1)	Ameliorating (1)
	Intermittent with residuum (1)	Progressive (1)
		Intermittent with residuum (3)
Need for respiratory, feeding, or mobility support	None	None
Positive response to acetylcholinesterase inhibitors	4/6	5/7

Abbreviations:
ASD = Autism spectrum disorder
ID = Intellectual disability
SNHL = Sensorineural hearing loss

RAPSN (n = 5)

In all patients the NM_005055.5(*RAPSN*):c.264C>A, p.(Asn88-Lys) variant was present, homozygous in one patient and heterozygous in the other patients. All were in their midteens at study inclusion and ambulatory. One of the patients was compound heterozygous for the c.264C>A variant in trans with the c.-210A>G E-box variant. This patient only presented with fluctuating ptosis with a mild disease course. Of the three other patients with compound heterozygosity, two had arthrogryposis (one generalized and one distal), whereas the other patient's disease course was complicated by scoliosis, severe respiratory distress during infections, and the need for tube feeding until childhood. The patient homozygous for the c.264C>A variant showed first symptoms at birth and presented with dysphagia, episodic apnea, and fluctuating weakness with a positive evolution over time. All five patients were treated with pyridostigmine since childhood with a positive effect on facial, bulbar, and limb weakness.

DOK7 (n = 4)

The NM_173660.5(*DOK7*):c.1124_1127dup, p.(Ala378Serfs*30) variant was found in heterozygosity in two of four patients and in homozygosity in one patient. All patients had proximal muscle weakness at presentation. In three patients, treatment with salbutamol was started: two experienced a positive effect on weakness and one stopped because of side effects. In two patients treated with pyridostigmine and one patient treated with 3,4-DAP, the medication was stopped due to a lack of effect. Three patients experienced a progressive disease course. One of these patients, who had had myasthenic symptoms since childhood, lost ambulation and developed a need for invasive continuous ventilation at age 40 years.

MUSK (n = 2)

In both patients the first disease manifestations were in the third decade of life. Both patients were treated with pyridostigmine. In one patient the symptoms worsened, especially when taking a higher dosage of pyridostigmine. In the other patient there was no worsening but just no improvement when taking pyridostigmine. In both patients pyridostigmine was discontinued. In one patient salbutamol ameliorated the limb weakness.

CHRNB1 (n = 2)

Both patients required tube feeding at neonatal age, which could be discontinued during infancy. One patient needed respiratory support, with the placement of a tracheal cannula because of recurrent respiratory crises with apnea. At age four years, the tracheal cannula was removed successfully. Pyridostigmine was administrated since infancy and improved ocular, facial, and limb weakness as well as feeding and respiratory difficulties. 3,4-DAP was added at age 12 years with additional amelioration on mobility.

CHRNG (n = 2)

Both patients presented with generalized arthrogryposis at birth with complications of bone deformations. No pharmacologic treatment was used.

CHRND (n = 1)

Previously reported functional studies of this variant showed evidence of a slow channel syndrome. Treatment with fluoxetine was started at a low dose and did not improve the symptoms. There was a positive effect of salbutamol.

CMS with glycosylation defects

GFPT1 (n = 1)

This patient showed proximal fatigable weakness since childhood. Pyridostigmine was started at adult age, resulting in an amelioration of the symptoms, but was discontinued due to side effects. There were good results with the use of salbutamol. A trial of 3,4-DAP was not successful.

GMPPB (n = 1)

The first signs of proximal fatigable weakness appeared in adulthood. There was a positive effect of treatment with pyridostigmine.

Discussion

We identified 37 patients with (likely) pathogenic variants in one of the genes related to CMS. Pathogenic variants in the *CHRNE* gene were the most prevalent, followed by the *RAPSN* and *DOK7* genes, which is consistent with large series reported in the literature.[2,12] Thirteen patients harbored variants that were not previously reported in the literature.

All the patients in our cohort affected by *PREPL* gene variants showed intellectual disability, which is a common finding in presynaptic CMS, attributed to central nervous system functions of presynaptic genes.[22,23] Growth hormone deficiency, hypogonadism, dry mouth, obesity, and dysmorphic features are regularly reported in *PREPL*-related patients.[22-24] The neuromuscular manifestations often ameliorate with age.[23,24] *COLQ*-CMS typically present with generalized muscle weakness or hypotonia. Ocular weakness and respiratory distress are common findings. Most patients have an early disease onset with progressive or relapsing disease course.[10,25] Slow pupillary reaction to light is a specific feature in *COLQ*-CMS but was not reported in our patients.[5] Recessive pathogenic variants in *CHRNE* cause end plate AChR deficiency and/or fast channel syndrome. These variants show

clinical features similar to myasthenia gravis, except with an early onset.[3] The disease course is often mild, but a progressive evolution is described in some cases even while under treatment with pyridostigmine.[20,26] We report two patients with pathogenic variants in *CHRNE* with onset of symptoms in adulthood. Adult-onset CMS are increasingly described in the literature, and reports of adult onset are available in patients harboring pathogenic variants in *RAPSN*,[27] *DOK7*,[28,29] *MUSK*,[30] *PLEC*,[31] *COLQ*,[32] *GMPPB*,[9] and *GFPT1*[33] and in slow channel syndrome.[34] Adult-onset CMS have not yet been reported in autosomal recessive pathogenic variants in *CHRNE*. We describe two patients with extraneuromuscular symptoms from an unknown cause; this is not typically reported in patients with postsynaptic CMS. In our cohort of patients with *RAPSN*-related CMS, we report a large variability in disease severity from mild fluctuating ptosis to arthrogryposis and severe respiratory distress. This variation is acknowledged in previous case series. An ameliorating disease course is often observed with age.[27,35,36] Both DOK7 and MUSK share a role in the AChR clustering pathway. Pathogenic variants in these genes show similar clinical features, wherein proximal weakness is a consistent finding with or without ptosis. A fluctuating weakness is not always present. Stridor has been described in the literature, as has vocal cord paralysis.[37,38] In our cohort, some of these features only appeared later in the disease course. Srour et al. confirm a considerable intrafamilial phenotypic variability in terms of age of onset and disease severity in patients harboring the *DOK7* c.1124_1127dup variant in homozygosity, with one individual who was still asymptomatic at age 50 years.[39]

We describe two patients with pathogenic variants in *CHRNB1*. Limited reports are available; however, respiratory problems are common in these variants.[40-42] We report one patient with slow-channel CMS due to a heterozygous pathogenic variant in the *CHRND* gene. Two patients were affected with *CHRNG* gene variants fitting the phenotype of a fetal defective NMJ.[43] Pathogenic variants in the *GFPT1* and *GMPPB* genes cause CMS with limb-girdle weakness.[3] High creatine kinase levels have been reported in both *GFPT1*- and *GMPPB*-related CMS.[3] High creatine kinase was not present in our patient with *GFPT1* gene variants; in our patient with *GMPPB* CMS creatine kinase levels were not analyzed. Tubular aggregates, which are typically seen in muscle biopsy in *GFPT1*,[3] were not observed in our case.

Differential diagnosis

A differential diagnosis of congenital myopathy is often made because of the shared clinical features of early-onset muscle weakness, especially in case of a lack of fluctuating weakness or typical fatigability. In CMS, fluctuating weakness or fatigability is not always present or only becomes clear later in the disease course. In muscle biopsies of patients with CMS myopathic changes can be seen.[44,45] Also on EMG, myopathic potentials are regularly observed.[10,16,44,46] In this case, RNS demonstrating significant decrement (>10%) can distinguish CMS from myopathy. Normal RNS, however, does not exclude the disease, making early diagnosis challenging.[15,16,47] The influence of pyridostigmine on the decremental response was examined in patients with myasthenia gravis.[48,49] It is advised to withhold the medication 12 hours before the examination, if this can be done safely.[50] In the case of late-manifesting fluctuating myasthenic signs, seronegative myasthenia gravis is sometimes diagnosed. CMS should be considered, especially when the expected treatment response is lacking. When cognitive impairment or other extraneuromuscular manifestations are present, CMS are generally not the first diagnosis considered. However, especially the presynaptic forms should be taken into consideration.

Treatment

The choice of pharmacologic treatment is dependent on the underlying defect. Cholinesterase inhibitors like pyridostigmine and the potassium channel blocker 3,4-DAP act by increasing the availability of acetylcholine in the NMJ. Cholinesterase inhibitors are generally effective in AChR deficiency, fast channel syndrome, rapsyn deficiency, some presynaptic forms, and glycosylation defects. 3,4-DAP can be useful as an additional agent. The open-channel blockers fluoxetine and quinidine are only indicated in slow-channel CMS.[13] Adrenergic agonists like salbutamol and ephedrine improve neuromuscular transmission by stabilizing the NMJ.[51,52] In *DOK7, MUSK,* and *COLQ* CMS salbutamol is the treatment of choice, whereas pyridostigmine is not effective and can aggravate symptoms.[13,53] In other CMS, salbutamol can give an additional improvement of symptoms.[53] There are reports of positive results of salbutamol monotherapy in CMS related to *CHRNE* variants.[54,55] In our cohort most treatment responses could be predicted based on the above-described treatment mechanisms. Even when started later in the disease course, a positive effect was seen in several patients. In two of 14 patients affected with pathogenic variants in *CHRNE,* antagonizing drugs (fluoxetine and pyridostigmine) were used. There was no beneficial effect of fluoxetine in these patients. There is a need of CMS-specific outcome measure to evaluate treatment response.[40,56]

Strengths and limitations

More than half of the patients in our cohort had lived a long disease duration at the time of inclusion, which gives valuable insights into the disease course. However, there is a risk of bias due to the retrospective collection of data. By including all the Belgian NMRCs we assume that all known cases are included in the study, and cases were ascertained by applying the ACMG guidelines in the evaluation of the genetic variants. Patients with CMS without genetic confirmation were not included, probably underestimating the true number of patients in our country. Symptoms of PREPL deficiency are not restricted to the NMJ, and neuromuscular symptoms usually improve considerably with age. Therefore, it is likely that not all patients with PREPL deficiency are followed in the NMRCs and therefore probably not all Belgian patients with PREPL deficiency have been included in this study. Pathogenic variants in *CHAT* were not detected in our cohort. According to the literature, they account for 4%-5% of all CMS cases and are the most common form of presynaptic CMS; this could suggest underdiagnosis in our population. Intellectual disability is often reported. A decrement is only detected after prolonged high-frequency RNS. RNS at low-frequency stimulation is often normal, which increases the risk of underdiagnosis.[2,3]

For six patients with a VUS detailed clinical information is presented. Functional studies are indicated to confirm the pathogenicity. Owing to the retrospective nature of the study, we could not include this in the current project.

Conclusion

This is the first pooled characterization of patients with CMS in Belgium. The wide phenotypic spectrum of CMS and the variability in age at onset and disease course is confirmed by our findings. We identified 13 causative variants not previously reported in the literature. We broaden the phenotypical spectrum of pathogenic variants in *CHRNE* by reporting two patients presenting with adult-onset CMS. Systematically documenting larger cohorts of patients with CMS can aid in better clinical characterization and earlier recognition

of this rare disease. We emphasize the importance of establishing a molecular genetic diagnosis to tailor treatment choices.

CRediT authorship contribution statement

Nathalie Smeets: Writing – original draft, Visualization, Validation, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Alexander Gheldof:** Writing – review & editing, Validation, Methodology, Conceptualization. **Bart Dequeker:** Writing – review & editing, Validation, Methodology. **Margaux Poleur:** Writing – review & editing, Investigation. **Sofia Maldonado Slootjes:** Writing – review & editing, Investigation. **Vinciane Van Parijs:** Writing – review & editing, Investigation. **Nicolas Deconinck:** Writing – review & editing, Investigation. **Pauline Dontaine:** Writing – review & editing, Investigation. **Alicia Alonso-Jimenez:** Writing – review & editing, Investigation. **Jan De Bleecker:** Writing – review & editing, Investigation. **Willem De Ridder:** Writing – review & editing, Investigation. **Sarah Herdewyn:** Writing – review & editing, Investigation. **Stéphanie Paquay:** Writing – review & editing, Investigation. **Arnaud Vanlander:** Writing – review & editing, Investigation. **Liesbeth De Waele:** Writing – review & editing, Investigation. **Geertrui Peirens:** Writing – review & editing, Investigation. **Diane Beysen:** Writing – review & editing, Investigation. **Kristl G. Claeys:** Writing – review & editing, Investigation. **Nicolas Dubuisson:** Writing – review & editing, Investigation. **Isabelle Hansen:** Writing – review & editing, Investigation. **Gauthier Remiche:** Writing – review & editing, Investigation. **Sara Seneca:** Writing – review & editing, Validation. **Véronique Bissay:** Writing – review & editing, Validation, Methodology, Investigation, Conceptualization. **Luc Régal:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization.

Declaration of competing interest

There has been no commercial involvement in the study design or manuscript preparation, and none of the authors report any other conflicts of interest.

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Supplementary Data

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