**Long-term prognosis in adult patients with congenital myasthenic syndromes**

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# Abstract

Congenital myasthenic syndromes are clinically and genetically heterogeneous diseases caused by mutations affecting the neuromuscular transmission. Even if first symptoms mainly occurred during the neonatal period or during childhood, adult neurologists have to face this challenging diagnosis and have to manage these patients throughout their lives. However, long-term follow-up data from large cohorts of CMS patients are still missing and the fate of these patients is largely unknown.

Herein, we aimed to determine the long-term prognosis in a French cohort of 235 genetically confirmed adult patients followed in 23 specialized neuromuscular centers. Their genetical and clinical data were retrospectively analyzed.

Of the 235 patients, 123 were female (52.3%). The clinical diagnosis was made before 18 years in 82 patients (35.0%). In patients in whom the clinical diagnosis was made after 18 years, 110 had first symptoms before 18 years (46.8%), and 29 had first symptoms after 18 years (12.3%). The mean follow-up time between first symptoms and last visit was 34.0 years (SD=15.1). Pathogenic variants were found in 19 disease-related genes (*AGRN, CHAT, SLC18A3, CHRNA1, CHRNB1, CHRND, CHRNE, COL13A1, COLQ, DOK7, DPAGT1, GFPT1, GMPPB, LRP4, MUSK, RAPSN, SCNA4, SLC5A7, TOR1AIP1*). There was no significant difference in the frequency of the different symptoms between the initial presentation and the last visit. Only four genetic groups had a proportion of patients requiring ICU admission that exceeded 20%; *RAPSN* (54.8%), *MUSK* (50%), *DOK7* (38.6%), and AGRN (25.0%). 90.4% and 100% of ICU admissions in *RAPSN* and *MUSK* patients occurred before 18 years whereas this occurs after the age of 18 in *DOK7* and *AGRN* patients respectively in 52.3% and 100% of them. The proportion of patients requiring ventilation at the last follow-up reached 55.0% and 36.3% in the SCCMS and *DOK7* groups respectively. 36.3% of *DOK7* patients, 25% of *GMPPB* patients, and 25.0% of *GFPT1* patients were wheelchair-bound at the last visit. Only six patients died in this cohort (mean = 53.5 years, SD = 17.5).

In conclusion, the long-term prognosis of most CMS patients is favorable, with no need for ventilation or wheelchair. SCCMS patients often require ventilatory assistance and thus their respiratory function needs to be monitored all along their lives. *DOK7* patients also frequently require respiratory assistance at last follow-up but are more prone to be wheelchair-bound.

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# Introduction

Congenital myasthenic syndromes (CMS) are clinically and genetically heterogeneous diseases characterized by a neuromuscular transmission defect caused by mutations disturbing the synaptic structure or function.1 In the past decades, molecular basis of CMS have expanded, and more than 35 genes have been associated with the disease.2–4 CMS usually present at birth or during early childhood.5–8 However, first symptoms can occur in adulthood.9 Its prevalence, around 2.8-14.8/1000000, is probably underestimated, given the complexity of the diagnostic process, especially for mild or late-onset forms and those presenting with atypical or complex phenotypes.10–14 Clinical symptoms and severity range from mild ocular or bulbar symptoms to severe limb weakness leading to loss of ambulation.5 Such heterogeneity, also observed in the response to treatment, depends on the underlying molecular mechanism and thus the precise genetic defect.1 Decrement or increment evidenced on repetitive nerve stimulation (RNS) supports the neuromuscular transmission defect in these diseases. Although the clinical spectrum of the different CMS subtypes keeps expanding 5–8, follow-up data are scarce. When present, the median follow-up time is frequently short (maximum median of 12.8 years), and/or the cohort size is small, without precise evaluation criteria.9,15,16 Clinical data from large cohorts of adult CMS patients with long-term follow-up and detailed clinical characteristics are missing. These data are important to better define the long-term prognosis of CMS according to the genetic background, and help neurologists to improve their management of adult patients.

We retrospectively analyzed the long-term follow-up data of 235 patients with genetically confirmed CMS followed in all French specialized neuromuscular centers, thus informing diagnosis, management, and long-term prognosis, also paving the way for clinical trial readiness.

# Methods

## Study design and population

This retrospective, observational, and multicenter study included all adult patients followed for genetically confirmed CMS until July 2023 in the specialized neuromuscular centers of 23 University Hospitals in France (Lyon, Paris, Marseille, Strasbourg, Saint-Etienne, Lille, Nantes, Bordeaux, Toulouse, Amiens, Rouen, Rennes, Nantes, Caen, Créteil, Grenoble, Nancy, Limoges, Clermont, Angers, Montpellier, Nîmes, and Nice). All clinical data were collected anonymously from the study units’ medical files. All patients provided written informed consent for genetic tests and the use of their data for research purposes. All procedures involving patients performed in this study were carried out in accordance with the ethical standards of the Assistance Publique des Hôpitaux de Paris (ethics approval #20230524134437) and with the 1964 Helsinki declaration.

## Clinical, laboratory, and electrophysiological data

## The demographic data collected encompassed sex, ethnic origin, family history of CMS, mode of inheritance, parents’ consanguinity, age at first symptoms, age at diagnosis, and last follow-up visit. The patients were further classified according to the age of onset of their symptoms in six sub-groups: neonatal period, infancy (1-3 years), childhood (3-10 years), teenage (10-18 years), adulthood (18-40 years) and late onset (more than 40 years). The clinical data of interest included the presence of limb weakness, either proximal or distal, axial muscle deficit, facial weakness, fatigability, bulbar symptoms (including dysphonia, swallowing disturbances), ptosis, oculomotor disturbances, arthrogryposis, intellectual disability, delayed motor milestones, scoliosis, dyspnea, need for ventilation, need for tube feeding, need for a wheelchair, and need for intensive care unit (ICU) admission. Myasthenia Gravis Foundation of America (MGFA) score was collected when available. All these data were collected at disease onset and at the last follow-up visit. Electromyography (EMG) exams were performed in each specialized neuromuscular center by trained neurologists. We collected the presence of an RNS decrement or increment and repeated compound muscle action potential (R-CMAP). CK values were also recorded, and considered elevated above 200 UI/L. Regarding treatment, the type of treatment (acetylcholinesterase (AChE) inhibitors, 3,4-diaminopyridine (3,4-DAP) quinidine, fluoxetine, ephedrine, and salbutamol) and its efficacy were collected.

## Genetic analyses

Until 2016, PCR and Sanger sequencing of CMS genes was used in a gene-after-gene approach. All exons and flanking intronic sequences of genes were PCR-amplified using genomic DNA of patients and sequenced using the BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems®, Life TechnologiesTM). From year 2016, next-generation sequencing (NGS) of CMS gene panels was used. Two panels (v2 2016-2017 and v3 2017-2020) were designed and successively used). NGS panel v2 targeted 25 CMS genes (*AGRN, ALG14, ALG2, CHAT, CHRNA1, CHRNB1, CHRND, CHRNE, CHRNG, COLQ, DOK7, DPAGT1, GFPT1, LAMB2, LRP4, MUSK, PLEC, PREPL, RAPSN, SCN4A, SLC18A3, SLC25A1, SLC5A7, SNAP25, SYT2*). NGS panel v3 targeted 31 CMS genes (genes of panel v2 and five additional genes *COL13A1, GMPPB, LAMB2, MYO9A, UNC13A, VAMP1*). NGS-based screening of CMS panel genes was performed using a SeqCapEZ capture design (Nimblegen), and a MiSeq sequencer (Illumina). Variants were identified through a bioinformatics pipeline (Genodiag, Paris, France). Copy number variations (CNVs) in targeted regions were searched for by a dedicated algorithm based on comparison of normalized number of reads of each region among the 12 samples of the sequence run.

The genetic diagnosis in affected relatives was performed by direct PCR and Sanger sequencing of gene regions encompassing the site of pathogenic variants identified in index cases.

## Statistical analyses

All data were analyzed with R.4.0. Associations between neuromuscular symptoms and implicated genes were evaluated using Chi-squared tests. Bonferroni correction was applied to adjust p-values for multiple comparisons, employing a significance threshold of 0.05. To identify symptoms that exhibited significant patterns in the heatmap, Z-scores were calculated to facilitated the interpretation of symptoms in relation to gene expression, normalizing the data around a mean of zero and a standard deviation of one. To visualize the relationship between neuromuscular symptoms and implicated genes, a heatmap was generated using the library Complexheatmap41. The hierarchical clustering of rows was conducted using the Ward.D2 method, and the distance between rows was computed using the maximum method on the percentage-based data matrix. The heatmap was color-coded to represent the range of proportion of symptoms, and an accompanying metadata panel was incorporated to display the mean age at the onset of the first symptom.

## Data availability

The anonymized data that support the findings of this study are available from the corresponding author, upon reasonable request.

# Results

## Demographic, genetic, and diagnostic characteristics

235 patients were included in this study. 123 were female (52.3%). These patients belong to 195 unrelated families. A positive family history was reported in 107 patients (45.6%), and consanguinity was formed in 55 cases (23.4%). 177 patients were Caucasian (75.3%), 32 originated from North Africa (13.6%), ten from Middle East (4.2%), seven from Sub-Saharan Africa (3.0%), while five came from Gypsy families (2.1%) and five (2.1%) had diverse other origins (Asia and South America). Pathogenic variants were found in 19 disease-related genes (*AGRN, CHAT, SLC18A3, CHRNA1, CHRNB1, CHRND, CHRNE, COL13A1, COLQ, DOK7, DPAGT1, GFPT1, GMPPB, LRP4, MUSK, RAPSN, SCNA4, SLC5A7, TOR1AIP1*). These variants either were described as likely pathogenic or pathogenic in the literature or were novel and retained as probably disease-causing according to the ACMG guidelines (Supplementary Table 1). All patients had genetically confirmed CMS: 56 in *CHRNE* characterized as low-expressor (LE) variants (23.8%), 44 in *DOK7* (18.7%), 33 in *RAPSN* (14.0%), 20 slow-channel congenital myasthenic syndromes (SCCMS, 8.5%) due to variants in *CHRNA1* for 14 and in *CHRNE* for six, 19 in *COLQ* (8.1%), 15 in *GFPT1* (6.4%), 12 in *AGRN* (5.1%), eight in *MUSK* (3.4%), four in *CHRND* and four in *GMPPB* (1.7% for each), four fast-channel congenital myasthenic syndromes (FCCMS) due to *CHRNE* variants (1.7%), and 16 in others genes (*CHAT, SLC18A3, CHRNA1* low-expressor, *CHRNB1, COL13A1, LRP4, SCNA4, SLC5A7, TOR1AIP1*; 6.8%; (Figure 1). The inheritance was recessive in 215 patients (91.5%), dominant in 16 patients (6.8%), and *de novo* in four patients (1.7%). Only patients with SCCMS had dominant or *de novo* inheritance. Symptoms onset occurred in the neonatal period in 81 patients (34.4%), in infancy in 55 patients (23.4%), in childhood in 44 patients (18.7%), and in teenage in 18 patients (7.7%). 25 patients had first symptoms between 18 and 40 years (10.6%), while only five patients had first symptoms after 40 years (2.1%). The age at first symptoms could not be clearly determined in seven patients (3.0%). 138 patients were previously misdiagnosed (58.7%). Among them, the main misdiagnoses previously evoked were congenital myopathy (50%), myasthenia gravis (29.0%), muscular dystrophy (15.9%), and mitochondriopathy (8.7%) (Table 1). The mean time between first symptoms and clinical diagnosis was 17.2 years (SD=15.3), while the mean time between first symptoms and molecular diagnosis was 22.0 years (SD=15.2). The clinical diagnosis was made before 18 years in 82 patients (35.0%). In patients in whom the clinical diagnosis was made after the age of 18, 110 had first symptoms before 18 years (46.8%), and 29 had first symptoms after 18 years (12.3%, Table 1). Age at first symptoms or clinical diagnosis was not available in 14 patients. The mean follow-up time between first symptoms and last visit was 34.0 years (SD=15.1). The mean age at last visit was 40.5 years (SD=15.1). There was no significant difference in age at last visit according to the genetic group (p=0.11).

## Genotype-Phenotype correlation along life

The proportion of symptoms per genotype at initial presentation is represented in Figure 2A. Genes were clustered according to these proportions, defining different groups. The first one included *CHRNE-*LE, *CHRND,* and *FCCMS*. In this group, ptosis was found in 53/54 *CHRNE* patients (98.1%), 4/4 *CHRND* patients (100%), and 4/4 FCCMS patients (100%). Moreover, ophthalmoparesis was reported in 46/54 CHRNE patients (85.2%), in 4/4 FCCMS patients (100%), and in 2/4 *CHRND* patients (50%). The second group was composed of SCCMS, with *AGRN* and *MUSK*. However, SCCMS remained quite isolated, with a high proportion of distal weakness at initial presentation in this group compared to the others (16/20 patients, 80%). The last group included *RAPSN*, *COLQ*, *DOK7*, *GMPPB* and *GFPT1*. *GMPPB* (4/4, 100%), *GFPT1* (15/15, 100%), COLQ (18/19, 94.7%), and DOK7 (40/44, 90.9%) patients presented with proximal weakness. While axial muscle weakness was frequently found in *GFPT1* patients (10/10, 100%) in our cohort, *GMPPB* patients could have intellectual disability (1/4, 25.0%). 23/24 *DOK7* patients (52.2%) were found to have scoliosis, and 7/32 *RAPSN* arthrogryposis (21.9%). The genetic groups that were associated with symptoms with a Z-score > 1 are represented in Figure 3. Only four groups had a mean age at first symptoms older than 10 years, represented by *AGRN* (14.75, SD = 12.8), SCCMS (14.4, SD = 14.7), *GMPPB* (11, SD = 10.5), and *CHRND* (10.25, SD = 11.8). The proportion of symptoms per genotype at last follow-up is represented in Figure 2B. There was no significant difference in any proportion of symptoms per genotype between the initial presentation and the last follow-up.

Regarding electrophysiological data, 213/220 patients (96.8%) with available electroneuromyography (ENMG) data had a decrement superior to 10% on repetitive nerve stimulation at 3 Hz in at least one nerve/muscle couple. Five patients had an increment on post-exercise CMAP, three in the *AGRN* group, one in the *COLQ* group, and one *TOR1AIP1* patient. An R-CMAP was found in 15/19 COLQ patients (78.9%) and in 15/20 SCCMS patients (75.0%) and was significantly more frequent in these groups compared to the others (p<0.01).Values of CK levels were available in 134 patients. 34 of them (25.4%) had elevated CK levels. The proportion of patients with elevated CK was significantly increased in the GMPPB group (4/4 patients, p<0.01) compared to the others. In this group, the mean CK level was 2035.3 UI/L (SD=1291.8). The second group associated with elevated CK was the GFPT1 group, with 7/12 patients with available CK having an elevating level (mean of 311.5 UI/L, SD=238.0). The three *MUSK* patients with available CK levels had elevated values with a mean of (339, 57, and 4558 UI/L). Regarding the two most represented genes, 3/30 CHRNE patients (10%) and 7/28 DOK7 patients (25%) had elevated CK values.

## Long-term prognosis

The type of disease course according to the genotype is represented in Figure 4A. Most patients in *CHRNE-*LE (40/56, 71.4%), *CHRND* (4/4, 100%), and FCCMS (3/4, 75.0%) groups had stable disease courses. A progressive improvement was reported in 16/33 *RAPSN* patients (48.5%), and in 3/8 *MUSK* patients (37.5%). On the opposite, *GMPPB* (2/4, 50.0%) and *GFPT1* (6/15, 40.0%) patients had frequently progressive worsening courses. A proportion of *DOK7* (11/44, 25.0%), *COLQ* (5/19, 26.3%), and *AGRN* (3/12, 25.0%) patients had multiphasic disease courses. An example of multiphasic disease is given in Supplementary Figure 1. Regarding the exacerbations, the proportion of patients who experienced them reached 20% in most of the genetic groups (Figure 4B). *RAPSN* patients required significantly more ICU admissions during their disease course compared to the others (17/31, 54.8%, p<0.01). Three of them required non-invasive ventilation, 11 were intubated, and three required tracheostomies. Three other genetic groups had a proportion of patients requiring ICU admission that exceeded 20% (Figure 4C); *MUSK* (4/8, 50%), *DOK7* (17/44, 38.6%), and AGRN (3/12, 25.0%). 19/21 (90.4%) and 4/4 (100%) ICU admissions in *RAPSN* and *MUSK* patients occurred before 18 years. 11/21 (52.3%) and 4/4 (100%) ICU admissions in *DOK7* and *AGRN* patients occurred after 18 years. In 74 women patients who had a pregnancy, 24 (32.4%) reported symptoms worsening during pregnancy. 20/123 women patients (16.2%) reported symptoms worsening during menstruation. Other triggers for symptoms worsening were infection in 35 patients (14.9%), warm temperatures in 19 patients (8%), cold temperatures in 15 patients (6.3%), anesthesia in 9 patients (3.8%), and psychological stress in five patients (2.1%). The proportion of patients requiring ventilation at the last follow-up was significantly elevated in SCCMS (p<0.01) and *DOK7* patients (p=0.04), and reached 11/20 patients (55.0%) and 16/44 patients (36.3%) patients respectively in thesegroups (Figure 4D). This proportion did not exceed 25% in the other genetic groups (Figure 4D). Six patients were tracheotomized at last follow-up: one SCCMS, one *CHRNE*, three *DOK7*, and one *SLC5A7* patients. Only two patients (one *CHRND* and one SCCMS) required a tube feeding at last visit. Regarding the motor long-term prognosis, *DOK7* patients were significantly more wheelchair-bound compared to the others (16/44, 36.3%, p<0.01). One *GMPPB* patients (25.0%) and 3/12 *GFPT1* patients (25.0%) were wheelchair-bound at the last visit (Figure 4E). The proportion of patients per MGFA category according to the genetic group is given in Figure 4F. The highest proportion of category 4 patients was found in the *DOK7* group (12/44, 27.2%) (Figure 4F). Patients who were both wheelchair-bound and ventilated were found mainly in the *DOK7* group (9/44, 20.5%). This proportion did not exceed 10% in the other groups (SCCMS; 2/20, 10%).

Only six patients died in our cohort (2.6%). An *AGRN* patient died at 50 from respiratory insufficiency (Patient 1 in Jacquier et al.).17 One *COLQ* patient died at 52 from cancer. Two patients with *DOK7* variants died. The first at 41 from acute vocal cord paresis causing respiratory distress, and the second at 56 after falling down the stairs (Supplementary Figure 1). A *DPAGT1* patient died at 36 from pneumonia secondary to swallowing disorders. Finally, a patient with *RAPSN* variants died at 86, but the cause of the death was not available. Moreover, a family history of early death during infancy was reported in 15 patients (three *RAPSN*, three *COLQ*, two *DOK7,* and one patient for *CHRNE-*LE, *MUSK*, *GMPPB*, *GFPT1*, *COL13A1*, *SCNA4*, *SCL5A7*).

## Treatment

25 patients received immunomodulatory treatments before the diagnosis of CMS (10.6%). These treatments included corticosteroids, intravenous immunoglobulin, plasma exchange, and immunosuppressive treatments (aziothioprine, mycophelonate mofetil). Moreover, eight of them had a thymectomy. Any of these patients reported a long-term improvement with these therapies. 224 patients received non-immunomodulatory CMS treatments (95.3%). These treatments included AChE inhibitors, 3,4-DAP, salbutamol, ephedrine, salbutamol, and quinidine. 138 patients received more than one of these treatments (58.8%). Response to non-immunomudulatory therapy in all patients are summarized in Figure 4. All SCCMS, *COLQ*, and *DOK7* patients reported either no effect or worsening with AChE inhibitors. Only 1/5 patients (20.0%) in the MUSK group and 1/10 patients (10.0%) in the *AGRN* group claimed they have been improved by these treatments, the remaining patients reported no effect or worsening symptoms. AChE inhibitors were efficient in more than 75% of the patients in the other genetic groups (*CHRNE-*LE, FCCMS, *CHRND*, *RAPSN*, *GFPT1*, and *GMPPB*). 3,4-DAP was reported as efficient in more than half of the patients in the *CHRNE-*LE, *CHRND*, *RAPSN*, *DOK7,* and *GFPT1* groups. 50% of FCCMS, SCCMS, and *COL*Q patients had their symptoms improved with AChE inhibitors, while it was not efficient in 3/4 *MUSK* patients (75.0%), in 6/7 *AGRN* patients (85.7%), and even led to symptoms worsening in 1/7 *AGRN* patients (14.3%). Salbutamol improved the symptoms of more than 75% of *CHRNE-*LE, *CHRND*, *MUSK*, *AGRN*, *RAPSN*, *COLQ*, *DOK7*, *GFPT1*, and *GMPPB* patients. One of two FCCMS patients (50.0%) treated with salbutamol reported symptom improvement, while the other reported no effect. Two of five SCCMS patients (40.0%) treated with salbutamol reported treatment efficacy, while the three others reported no effect. Ephedrine was reported to improve to symptoms in the solely FCCMS patient treated, in 5/7 *AGRN* patients (71.4%), 7/8 *COLQ* patients (87.5%), 22/23 *DOK7* patients (95.7%), and 3/3 *GFPT1* patients (100%). No effect was reported with this therapy in the singles *CHRND* and *RAPSN*-treated patients, and in the remaining patients: *AGRN* (2/7, 28.6%), *COLQ* (1/8, 12.5%), and *DOK7* (1/23, 4.3%). Fluoxetine was found efficient in 7/13 SCCMS patients (53.8%), with the resting 6/13 patients (46.1%) reporting no benefit. In the other groups, one FCCMS patient, one *AGRN* patient, and three *RAPSN* patients were inefficiently treated with fluoxetine. Finally, all five SCCMS patients treated with Quinidine reported an improvement of motor weakness.

## Prognostic data for genes with a small number of patients

### *TOR1AIP1* gene

Three patients with *TOR1AIP1* variants were included in our cohort (Table 2). One of them (patient 1) had been previously published (main proband of Malfatti et al.).18 We report herein two other patients who are brothers, harboring the same c.63dupC (p.Arg22Glnfs\*88) and c.72dupC (p.Ile25Hisfs\*85) heterozygous variants (patients 2 and 3). They were born from consanguineous Algerian healthy parents. The first variant was detected in the mother, and the second in the father. Their motor milestones were normal, but they had difficulties in sports activities during their teenage years. The first one (patient 2) developed progressive walking and respiratory difficulties at 35 years old, leading to the need for a banister to climb stairs at 40 years. At 47, an acute coronary syndrome revealed alveolar hypoventilation requiring non-invasive ventilation. Neurological examination found a proximal muscle and finger extensor muscle weakness in the upper limbs, associated with distal muscle weakness in the lower limbs, and cervical spine, fingers, wrists, and Achilles tendons’ contractures. Repetitive nerve stimulation at 3 Hz performed at age 48 showed a 48% decrement from *tibialis anterior* and *anconeus* muscles, and 39% from *trapezius* muscle. The CK level was mildly elevated at 227 UI/L (N<200 U/L). He was mildly improved by AChE inhibitors with an increased walking distance. At the last follow-up (51 years), he was still ambulant without walking aid but required nocturnal non-invasive ventilation. The second patient (patient 3) was admitted in the ICU at age 48 for an acute respiratory insufficiency revealing an alveolar hypoventilation and requiring intubation. His evolution was favorable, and he had a non-invasive ventilation at discharge. His neurological examination showed elbows and finger contractures, and mild deltoid muscle weakness. He walked unaided. His CK level was normal (189 UI/L). Repetitive nerve stimulation at 3 Hz showed a 19% decrement on from *tibialis anterior* muscle and 15% from *trapezius* muscle. Data regarding treatment efficacy are not available for this recently diagnosed patient.

### *DPGAT1* gene

Three patients in our cohort had *DPAGT1* variants (Table 2, patients 4, 5 and 6). They all presented at birth with hypotonia and contractures. Two had associated central nervous system (CNS) signs characterized by delayed motor milestones, intellectual disability, optic disk atrophy, and epilepsy, associated with deafness and cerebellar ataxia for one of these two (patients 5 and 6). AChE inhibitors were reported to improve their symptoms in two of them and were considered not efficient in one. At last follow-up, patient 4 (49 years) was still ambulant but required a banister to climb stairs, while patients 5 and 6 were wheelchair-bound (34 and 21 years). None of them required respiratory assistance. Patient 5 at 36 from pneumonia secondary to swallowing disorders.

### *SLC5A7* gene

Two patients had biallelic variants in the *SLC5A7* gene (Table 2, patients 7 and 8). They both presented with hypotonia and respiratory insufficiency at birth, requiring ICU admissions. One of them (patient 8) had arthrogryposis (*equinovarus*) and developed epileptic seizures. A mild efficacy was reported for both with AChE inhibitors. However, their final prognosis was different. While patient 7 improved and was asymptomatic at 20 years old, patient 8 progressively became wheelchair-bound and required nocturnal non-invasive ventilation at last visit (22 years).

### *CHAT/SLC18A3* genes

Two brothers had compound heterozygous variants in the *CHAT* gene (Table 2, patients 9 and 10). Their first symptoms occurred in infancy with episodic apnea and mild limb muscle weakness. They had delayed motor milestones and learning difficulties. However, their disease course was benign and characterized by an improvement, and their symptoms were limited to fatigability at 20. AChE and 3,4-DAP were reported as efficient. They were both ambulant and ventilation-free. One patient with a *SLC18A3* heterozygous variant, associated with a *SLC18A3/CHAT* deletion was included (Table 2, patient 11). After neonatal hypotonia, she developed episodic apnea, feeding difficulties, ptosis, and ophthalmoparesis. However, she improved during childhood with AChE inhibitors and could walk unaided at 18. She was ventilation-free, and her symptoms were restricted to ptosis and bulbar symptoms.

The remaining patients are described in the Table 2. Of note, the LRP4 and SCN4A patients have already been published.19,20

# Discussion

We reported herein a French multicentric nationwide cohort of 235 adult patients to better describe CMS patients' phenotype and long-term prognosis, according to their genotype. *CHRNE-*LE variants were the most common ones and considered as the main cause of CMS worldwide.6,21 As in an Australian cohort, *DOK7* was herein the second most commonly involved gene.22 *RAPSN* variants were also frequent, as in previously published cohorts.6,8,23 *COLQ* was only the fifth gene involved in our cohort, while it was one of the three main genes in several previous studies in different populations.7,8,15

Adult neurologists can face a CMS patient in three different conditions. First, there are simple cases when the diagnosis has already been made by a pediatrician, and the adult neurologist continues the patient’s follow-up (35% of patients in our cohort). In the second situation, symptoms have already been present in childhood or infancy, but the diagnosis has not been made. This situation was the most common in our study (46.8% of patients herein), as previously observed.9 Third and rarely, the first symptoms occur in adulthood, as noticed in our cohort (12.3%). These last two situation are more challenging, especially for adult neurologist non-specialized in the neuromuscular field.

Misdiagnoses were frequent in our cohort (58.7%), and the diagnostic delay was long, in accordance with previously published cohorts.21 Congenital myopathy was the most common misdiagnosis due to a mainly neonatal or early childhood onset. Myasthenia gravis was the second most frequent misdiagnosis, leading to an immunosuppressive treatment in 25 patients. With the development of new immunosuppressive treatments in the last decade, we recommend considering the diagnosis of CMS in patients with seronegative myasthenia gravis before starting such treatments which could cause serious adverse events.24 A careful search for respiratory distress, bulbar symptoms and motor symptoms in infancy or childhood, are key points to orient the diagnosis towards CMS.

We clustered patients’ genotypes according to their initial phenotypes. The clustering method we applied led to the formation of groups of phenotypes, that could be split into four different categories. The first group was composed of *CHRNE*, *CHRND*, and FCCMS patients and was characterized by predominating ocular symptoms such as ptosis and ophthalmoparesis. Acetylcholine receptor endplate deficiencies are known to cause predominant ocular symptoms, and FCCMS patients have essentially the same phenotype.4,8,23 Then, the second group could be considered as SCCMS isolated. Indeed, these patients presented a particular phenotype with predominantly distal weakness, especially affecting finger extensors.15 The third group was composed of *AGR*N and *MUSK* patients. Patients of this group developed variable symptoms such as ocular symptoms, bulbar symptoms, respiratory involvement, and muscle weakness, which led us to consider it as a generalized phenotype. These genes have already been associated with such diverse symptoms.25,26 Interestingly, *AGRN* patients had frequently distal weakness, but contrary to SCCMS patients, they had rarely axial muscle weakness. Finally, the last group of our cohort composed of *GMPPB*, *GFPT1*, *DOK7*, *COLQ,* and *RAPSN* could be gathered as limb-girdle muscle dystrophy (LGMD)-like phenotype, associated with some additional characteristic features for some of these genes. As previously described, GMPPB and GFPT1 patients presented with relatively pure proximal weakness.27,28 However, *GMPPB* patients also frequently had CNS involvement with delayed motor milestones and intellectual disability. *DOK7* and *COLQ* patients also presented with a proximal weakness dominating the phenotype but were more prone to have associated symptoms such as bulbar or ocular symptoms.29,30 *DOK7* was associated with a high rate of scoliosis, as previously observed.29 *RAPSN* was included by the clustering method in this group due to frequent proximal and axial muscle weakness. However, the *RAPSN* patients included developed more ocular, bulbar, and respiratory symptoms than the other genes of this group and we consider that they fall into a “generalized” phenotype. *RAPSN* has already been associated with such clinically diverse symptoms.8 In our cohort, *RAPSN* patients had more arthrogryposis, hypotonia at birth, and sudden respiratory insufficiency during childhood than other patients. Together these hallmarks are evocative of *RAPSN*-related CMS.31 Regarding electrophysiological data, we confirmed that an R-CMAP is a hallmark of *COLQ* and *SCCMS* patients resulting from a neuromuscular junction gain-of-function.32 A highly elevated CK level is suggestive of *GMPPB* gene mutation, as this gene have also been reported in LGMD or overlapping LGMD-CMS phenotype.33

Our main objective was to describe the long-term prognosis of adult CMS patients. Firstly, we noticed that CMS patients did not switch from one phenotype group to another along their disease course. *CHRNE*, *CHRND*, and FCCMS patients were prone to have a stable disease course. Moreover, they remained mainly ambulant at the end of the follow-up and did not require ventilation. Even if they could experience symptoms’ exacerbations, these exacerbations were relatively moderate since they were infrequently admitted to the ICU. This relatively good prognosis was suggested by previous cohorts.15,16 SCCMS patients were frequently stable regarding their disease course but could worsen in approximately one-third of patients. Although they remain ambulant, more than half of them required respiratory support at the end of follow-up. This proportion was higher than in previous cohorts, leading us to recommend monitoring the respiratory functions of these patients through regular pulmonary functional tests. *RAPSN*, *DOK7*, *MUSK*, *COLQ*, and *AGRN* patients had various disease courses, represented either by stability, worsening, or improvement. Moreover, *DOK7*, *COLQ*, and *AGRN* patients frequently presented several types of disease courses throughout their lives. For the latter ones, clinicians should be aware that phases of worsening and improvement can succeed each other, and cautions should be taken when defining the long-term prognosis of one patient, even if the patient is worsening. Patients with *RAPSN*, *DOK7*, *MUSK*, and *AGRN* mutations were more prone to have severe exacerbations requiring ICU admissions. While most ICU admissions occurred in childhood for *RAPSN* and *MUSK* patients, most of them occurred in adulthood for *DOK7* and *AGRN* ones. Thus, adult neurologists should be aware that severe exacerbations are possible in their *DOK7* and *AGRN* patients. *DOK7* had the more severe motor prognosis among these genes. Indeed, while most *RAPSN*, *MUSK*, *COLQ*, and *AGRN* patients were ambulant at last follow-up, *DOK7* patients were wheelchair-bound in approximately one-third of cases. This proportion was higher than in a previously published cohort of adult CMS patients.9 Regarding respiratory functions, ventilation was also more frequent in *DOK7* patients. It is interesting to note that despite severe initial phenotypes characterized by hypotonia and respiratory distress during childhood requiring ICU, the overall final phenotype of *RAPSN* patients is favorable. Finally, *GMPPB* and *GFPT1* patients were prone to have worsening disease course. Approximately 20% of these patients were non-ambulatory at last follow-up, but they were ventilation-free in most cases. In these patients with glycosylation defects, myopathic changes can be observed in muscle biopsies and MRI, giving a possible explanation for the worsening course.28 Pregnancy seems to be a risk period for symptom exacerbations. Indeed, 32.4% of our female patients with at least one pregnancy experienced a symptom exacerbation during their pregnancy. This frequency was inferior to previous data.9,34 This could be explained by the retrospective design of this study, which was not specifically designed to assess this question. Only six patients died in our adult cohort. Thus, the vital prognosis of adult CMS patients seems to be quite favorable. However, we found a family history of early death in infancy in 15 patients, with most of them in *RAPSN* and *COLQ*, suggesting a possible life-threatening condition for these genes in some cases during childhood.

It was more difficult to draw conclusions regarding the long-term prognosis of rare CMS genes present in our cohort due to the small number of patients per gene. However, patients with mutations in pre-synaptic genes implied in acetylcholine production and transport (*CHAT*, *SLC5A7*, *SLC18A3*) seem to have a favorable long-term motor and respiratory prognosis, despite severe symptoms in infancy such as hypotonia, feeding difficulties, and episodic apnea, even if one *SLC5A7* patients was wheelchair-bound and ventilated at last visit. *DPAGT1* patients were prone to develop CNS signs such as intellectual disability as previously found.35 They seem to have a poor motor prognosis with the need for a wheelchair. *CHRNA1* and *CHRNB* patients in our cohort had ocular symptoms such as *CHRNE-*LE patients. While the prognosis of the *CHRNB* patient resembles the *CHRNE-*LE group and seems favorable with a preserved ability to walk, the *CHRNA1* patient was wheelchair-bound.

This cohort gives important information regarding CMS treatment. As previously found, AChE inhibitors should be avoided in SCCMS, COLQ, and DOK7 patients, in whom they could lead to symptoms worsening.15,25,36,37 This treatment was often not effective in *AGRN* patients.38 Furthermore, 3,4-DAP was frequently inefficient in *AGRN* and *MUSK* patients, raising the question of early treatment with salbutamol. SCCMS patients’ symptoms were difficult to improve because 3,4-DAP, salbutamol, and fluoxetine were not effective in about half of these patients. Thus, quinidine seems to be an interesting option in these patients. Apart from these cases, most patients favorably respond to AChE inhibitors and other treatments regularly administrated as second-line therapies. Notably, *CHRNE-*LE patients were treated only with three molecules (AChE inhibitors, 3,4-DAP, and salbutamol), suggesting that the symptoms’ control was satisfying simply with these three therapies.

Our study provides two new cases of *TOR1AIP1*-related CMS. To our knowledge, this is only the third published family for this phenotype, with one of the variants (c.63dupC; p.Arg22Glnfs\*88) being already published. This frameshift variant is localized between the two first alternative start codons for LAP1B and LAPC isoforms and was associated with a selectively decreased level of LAP1B isoform in patients' fibroblasts, when present in a homozygous state. The second frameshift variant (c.72dupC; p.Ile25Hisfs\*85) is newly described and is also present between the two first start codons and is thus predicted to selectively impact LAP1B, as the first one. It was absent from the gnomAD database. Moreover, these variants were heterozygous in the parents, confirming the familial segregation. These patients shared common features with the previously published patients: normal developmental milestones, a late-onset disease, contractures, and a predominant proximal muscle weakness associated with mild distal weakness, such as finger extensors.18,39 However, contrary to previous cases, ours developed severe acute respiratory insufficiency requiring admission to ICU, and required non-invasive ventilation at discharge. Thus, we provide data indicating that respiratory involvement can be a major feature of *TOR1AIP1*-related CMS, which should lead to respiratory function monitoring in these patients. On the opposite, the motor prognosis seems favorable because all published patients were still ambulant at last visit.18,39

This study has some limitations. First, due to its retrospective design, some clinical data on the initial phenotype could be missed. The size of the groups of patients was not equal between genes, due to the variable prevalence of the different CMS genotypes, and this could lead to difficulties in comparing genetic groups. Finally, treatment efficacy was determined retrospectively according to the patients’ feedback, and not on objective and repeated validated scales. However, the large size of this cohort and the mean follow-up of 34.0 years allowed us to obtain reliable prognosis data.

In conclusion, the long-term prognosis of most CMS patients is favorable, most of them do not require ventilation and/or wheelchair at last follow-up. Nevertheless, two subtypes of CMS have a more unfavorable prognosis: SCCMS patients remain ambulatory at last visit but often require ventilation and *DOK7* patients were more prone to be wheelchair-bound and ventilated at last follow-up.

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**Table 1: Misdiagnoses in the adult CMS cohort.**

|  |  |
| --- | --- |
| **Misdiagnoses** | **Number of patients (% in patients with misdiagnosis / % of all patients)** |
| Congenital myopathy | 69 (50 / 29.4) |
| Myasthenia gravis | 40 (29.0 / 17.0) |
| Muscular dystrophy | 22 (15.9 / 9.4) |
| Mitochondriopathy | 12 (8.7 / 5.1) |
| Distal myopathy | 4 (2.9 / 1.7) |
| Spinal muscular atrophy | 4 (2.9 / 1.7) |
| Metabolic myopathy | 3 (2.2 / 1.3) |
| Channelopathies / Periodic paralysis | 3 (2.2 / 1.3) |
| Myositis | 2 (1.5 / 0.9) |
| Amyotrophic lateral sclerosis | 1 (0.7 / 0.4) |
| Moebius syndrome | 1 (0.7 / 0.4) |
| Lambert-Eaton syndrome | 1 (0.7 / 0.4) |
| Fibromyalgia | 1 (0.7 / 0.4) |
| Lyme disease | 1 (0.7 / 0.4) |

CMS: congenital myasthenic syndrome

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Patient** | **Gene** | **Sex** | **Age at first symptoms** | **First symptoms** | **Disease course** | **ICU admission (age)** | **Wheelchair at last visit** | **Respiratory assistance at last visit (type)** | **Treatment response** | **Other features** |
| 1 | TOR1AIP1 | F | 25 | Gowers’ sign, axial muscle weakness, fatigability | Stable | No | No | No | AChE inhibitors (+) | Small size |
| 2 | TOR1AIP1 | M | 10 | Fatigability, difficulties in sports activities | Worsening | Yes (47) | No | Yes (NIV) | AChE inhibitors (+) | Contractures |
| 3 | TOR1AIP1 | M | 10 | Fatigability, difficulties in sports activities | Stable | Yes (48) | No | Yes (NIV) | NA | Contractures |
| 4 | DPAGT1 | F | 0 | Neonatal hypotonia and respiratory insufficiency | Stable | No | No | No | AChE inhibitors (-) | Contractures |
| 5 | DPAGT1 | F | 0 | Neonatal hypotonia | Worsening | No | Yes | No | AChE inhibitors (+) | Contractures, delayed motor milestones, intellectual disability, optic disk atrophy, epilepsy, deafness, cerebellar ataxia |
| 6 | DPAGT1 | F | 0 | Neonatal hypotonia | Worsening | Yes (18) | Yes | No | AChE inhibitors (+) | Contractures, delayed motor milestones, intellectual disability, optic disk atrophy, epilepsy |
| 7 | SLC5A7 | M | 0 | Neonatal hypotonia and respiratory insufficiency | Improvement | Yes (0) | No | No | AChE inhibitors (+) | / |
| 8 | SLC5A7 | M | 0 | Neonatal hypotonia and respiratory insufficiency | Worsening | Yes (0) | Yes | Yes (tracheotomy) | AChE inhibitors (+) | Arthrogryposis (equinovarus), epilepsy |
| 9 | CHAT | M | 4 | Episodic apnea, limb muscle weakness | Improvement | No | No | No | AChE inhibitors, 3,4-DAP (+) | / |
| 10 | CHAT | M | 2 | Episodic apnea, limb muscle weakness | Improvement | No | No | No | AChE inhibitors, 3,4-DAP (+) | / |
| 11 | SLC18A3/CHAT | F | 0 | Neonatal hypotonia, episodic apnea, feeding difficulties, ptosis, ophthalmoparesis | Improvement | Yes (0) | No | No | AChE inhibitors (+) | / |
| 12 | CHRNA1 | H | 0 | Feeding difficulties, proximal muscle weakness | Worsening | No | Yes | No | AChE inhibitors (+) | Jaw malformation, and foot arthrogryposis |
| 13 | CHRNB1 | F | 0 | Neonatal respiratory insufficiency, ptosis, ophthalmoparesis | Stable | Yes (0) | No | No | AChE inhibitors, 3,4-DAP (+) | / |
| 14 | COL13A1 | F | 10 | Axial muscle weakness, scoliosis, fatigability, bulbar symptoms | Stable | No | No | No | AChE inhibitors, 3,4-DAP (/) | Retrognathia and low-set ears |
| 15 | LRP4 | F | 19 | Fatigability, muscle weakness | Worsening | No | No | No | AChE inhibitors (/) | Cenani-Lenz syndrom |
| 16 | SCNA4 | F | 0 | Neonatal hypotonia, muscle weakness, bulbar symptoms | Stable | No | No | No | AChE inhibitors, 3,4-DAP (/) | Small size |

**Table 2: clinical characteristics and long-term prognosis genetic groups with a small number of patients.**

Treatment response: (+) = improvement, (/) = No effect, (-) = worsening.

F: female, M: male, ICU: intensive care unit, AChE: acetylcholinesterase, 3,4-DAP: 3,4-diaminopyridine.

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Description générée automatiquement

**Figure 1: Genetic and diagnostic characteristics of the cohort.**

(**A**): Proportion of genetic groups present in the cohort. (**B**) Diagnostic categories according to age at first symptoms and age at diagnosis. CMS: congenital myasthenic syndromes, FCCMS: fast-channel congenital myasthenic syndrome, SCCMS: slow-channel congenital myasthenic syndrome, y: years, LE: low-expressor.

**Une image contenant carré, ligne, diagramme, Rectangle

Description générée automatiquement**

**Figure 2: Genotype-Phenotype correlation**

Heatmap and clustering of genetic groups according to symptoms at initial presentation (**A**) and at last follow-up (**B**), and age at first symptoms. LE: low-expressor.

**Une image contenant squelette, diagramme, capture d’écran

Description générée automatiquement**

**Figure 3: Characteristic symptoms per genetic group.**

"Human body showing genetic groups with high prevalence of symptoms (Z-score > 1) in specific categories of symptoms”

LE: low-expressor

Une image contenant texte, capture d’écran, diagramme, Police

Description générée automatiquement

**Figure 4: Long-term data of CMS patients.**

(**A**) Disease course category and (**B**) proportion of patients with exacerbations according to the genetic group. (**C**) proportion of patients requiring ICU admission along their disease courses, (**D**) ventilation at last follow-up, (**E**) and wheelchair at last follow-up according to the genetic group. (**F**) MGFA category at last follow-up according to the genetic group.

CMS: congenital myasthenic syndrome, ICU: intensive care unit, LE: low-expressor, MGFA: myasthenia gravis foundation of America.

Une image contenant texte, capture d’écran, diagramme, Police

Description générée automatiquement

**Figure 5: Treatment efficacy according to the genetic group.**

Green: improvement, Grey: inefficiency, Red: worsening. LE: low-expressor.

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Description générée automatiquement  
**Supplementary Figure 1: A representative DOK7 patient with a multiphasic disease course.**

(**A**) After respiratory distress at birth requiring ICU admission, the patient had stable proximal muscle weakness, ptosis, ophthalmoparesis and bulbar symptoms during childhood. (**B**) His proximal muscle weakness progressively improved during his teenage years. (**C**) Since 24 years, he reported a progressive worsening of its symptoms, leading to a tracheostomy with nocturnal ventilation at 29, and he became wheelchair-bound at 30. (**D**): In his forties, his upper limb weakness progressively worsened. (**E**) At 46, the start of 3,4-DAP and ephedrine after the genetic diagnosis allowed an improvement in arm strength and in proximal lower limb strength allowing him to walk 30 meters. (**F**) He died at 56 after falling down the stairs.

**Supplementary Table 1: List of patients and variants identified in the cohort.**

A faire.